1st Edition

Appendices on management measure assessments

A manual for the management of vertebrate invasive alien species of Union concern, incorporating animal welfare

Feedback: Comments which could support improvement of this document are welcome. Please send your comments by e-mail to ENV-IAS@ec.europa.eu

Recommended citation: Smith, K.G., Nunes, A.L., Aegerter, J., Baker, S.E., Di Silvestre, I., Ferreira, C.C., Griffith, M., Lane, J., Muir, A., Binding, S., Broadway, M., Robertson, P., Scalera, R., Adriaens, T., Åhlén, P-A., Aliaga, A., Baert, K., Bakaloudis, D.E., Bertolino, S., Briggs, L., Cartuyvels, E., Dahl, F., D'hondt, B., Eckert, M., Gethöffer, F., Gojdičová, E., Huysentruyt, F., Jelić, D., Lešová, A., Lužnik, M., Moreno, L., Nagy, G., Poledník, L., Preda, C., Skorupski, J., Telnov, D., Trichkova, T., Verreycken, H. and Vucić, M. (2022). A manual for the management of vertebrate invasive alien species of Union concern, incorporating animal welfare. 1st Edition. Technical report prepared for the European Commission within the framework of the contract no. 07.027746/2019/812504/SER/ENV.D.2.

Front cover photos: © Stanze CC BY-SA 2.0 (top), Dennis Irrgang CC BY 2.0 (bottom left), © Seotaro CC BY-SA 3.0 (bottom centre), © Budak CC BY-NC-ND 2.0 (bottom right)

Design and layout: Imre Sebestyén, Unit Graphics

This document presents appendices (#1-33) for *A manual for the management of vertebrate invasive alien species of Union concern, incorporating animal welfare. 1st Edition*. It contains information on the feasible management measures for the 22 invasive alien vertebrate species of Union concern listed as of December 2021 with a view to eradicating, controlling and/or containing their populations in order to minimise their impact on biodiversity and related ecosystem services. These measures have been assessed in terms of their costs and effectiveness, welfare impacts (e.g. sparing any avoidable pain, distress or suffering) and other possible positive or negative side-effects (e.g. on other invasive alien species, on non-targeted native species, on the environment or on human health).

Appendices 1 - 32 present the detailed 'management measure assessments', starting with the measures used to restrain, capture and/or kill animals in the field (Appendices 1-21), followed by measures used to dispatch or remove an individual once captured (Appendices 22-32). It is important to note that the information collated for the assessments, especially in relation to the costs and effectiveness of case studies, is not based on a comprehensive literature search.

Appendix 33 provides the impact categories retrieved from Sharp and Saunders (2011), used to guide the assessments of humaneness of each of the management measures.

Table of Contents

Appendix 1. Native Predators

1. Measure name	
1.1. English:	Native predators
1.2. Lethal or non-lethal:	Lethal

1.3. Other languages (if available):

Bulgarian	Въвеждане на местни хищни видове	Italian	Predatori nativi
Croatian	Zavičajni predatori	Latvian	Dabiskie plēsēji
Czech	Původní predátoři	Lithuanian	Natūralūs priešai
Danish	Hjemmehørende rovdyr	Maltese	
Dutch	Beheer met inheemse predatoren	Polish	Rodzime drapieżniki
Estonian	Kohalikud kiskjad	Portuguese	Predadores naturais
Finnish	Luontaiset viholliset	Romanian	Utilizarea prădătorilor nativi pentru ținerea sub control a speciilor străine invazive
French	Prédateurs indigènes	Slovak	Pôvodní /domáci predátori
German	Management mit natürlichen Prädatoren	Slovenian	Domorodni plenilci
Greek	Γηγενείς θηρευτές	Spanish	Depredadores naturales
Hungarian	Őshonos ragadozók	Swedish	Inhemska predatorer
Irish			

2. Technical details of measure

2.1.a. Measure description

The intentional use of native predators to reduce the abundance and/or spread of an IAS (deliberate management activity to promote predation).

The measure might include introducing predators where they have recently been absent, or enhancing the predatory activity of existing native predators. Unequivocal or direct examples of the exploitation of predators to successfully and sustainably achieve specific management objectives against IAS are limited as most examples which demonstrate the potential of the measure against IAS are drawn from either short-lived experimental studies which may require continual intervention in order to meet management objectives (e.g. the use of predatory fish in lentic freshwaters; Case studies 1-4), or alternatively is based on inference drawn from correlative studies made where native predators return to areas from which they had previously been removed; either through translocation as part of conservation action, or natural range expansion (e.g. Case study 6). In part this general reluctance to explore the use of predators as bio-control agents may stem from a shared culture informed by a series of historical bio-control attempts (using vertebrate predators against vertebrate prey; e.g. [21]) which failed disastrously and which have been taught to generations of young ecologists as examples of the unintended consequences of changing food webs or ecosystems with introduced predators. One case example, including a number of listed IAS is presented here to reinforce this principle (Case study 5).

In principle, this measure includes;

- local planned introduction/re-introduction of native predators
- Interventions to promote increases in predator density (numerical response) which might include provision of resources to increase the abundance of predator species [21] or regulating activities which may remove predators (e.g. reducing or prohibiting the killing of predator species)
- management action to promote predation on IAS (functional response). This might include attempts to manage the densities of predators or IAS prey (e.g. around multi-species feeding stations), or managing behaviors and habitats to promote the interaction between predators and prey [21].

Excludes;

- introduction of non-native predators
- Incidental/unintended consequences of activity intended to achieve other goals or natural processes. The ethical issues and effectiveness of this measure can only be assessed where it is implemented for the stated purpose of achieving control, containment or eradication of IAS.

Recognizing that predation produces a hierarchy of effects on populations of IAS prey

- Regulation being the long-term reduction in populations of prey species to a natural equilibrium
- Control being the reduction in populations to a predefined threshold. Maintenance of this effect may require continual intervention
- Control and regulation may not be synonymous (i.e. regulation may be insufficient to produce control or any effective benefit)
- Containment requiring the effect of predation to include the prevention of spread
- An ecosystem or local food-web being the current inter-relationship between species across a range of trophic levels

Many IAS have demonstrated their potential to establish and spread despite the presence of a suite of native predators known to take adults, young or eggs. Descriptions of IAS often list known predators, either from their natural range, or within their introduced range in Europe, which include; red fox (*Vulpes vulpes*), carrion crow (*Corvus corone*) and the domestic cat (*Felis catus*) and dog (*Canis lupus familiaris*). The ubiquity of these generalist predators across most Member States indicates the scale of effect that predators managed to specifically target IAS populations must exceed in order to control, contain or eradicate IAS.

Here we do not assume: that populations of re-introduced predators necessarily have to become self-sustaining in order to maintain an effect (though this is often assumed when discussing the effectiveness of the approach in principle); that predatory pressure will necessarily develop within the lifetime of any given project period (it may take many years for a sufficient density of predators to develop to begin to produce useful effects); or that there are any significant ethical or welfare burdens produced on the predators themselves. Such burdens might include the welfare costs of translocation on predators (i.e. capture, captivity/treatment and release [22,23]), or the consequences of modifying predator ecology and behavior where attempts may be made to increase the density or predatory effectiveness of predator species, e.g. agonistic interaction between ordinarily solitary animals [23], esp. at feeding stations.

2.1.b. Integration with other measures

Can be integrated with many other non-lethal measures, or lethal measures which select strongly against non-target effects. Used alone, unlikely to result in eradication, and may even be insufficient to produce reliable or sustained control.
May be useful in some very restricted scenarios (e.g. freshwater aquatic species in small lentic ponds and lakes), and is unsuitable in others (e.g. habitats avoided by predators, urban or sub-urban settings) or where the degree of intervention would provoke dramatic (deleterious) changes to native natural ecosystems or food-webs.

2.2.a. Availability - species and objectives										
Objective	**Unknown objective**		**Rapid Eradication**		**Management**					
					Eradication		**Control**		**Containment**	
Species	Avail.	Ref(s).	Avail.	Ref(s).	Avail.	Ref(s).	Avail.	Ref(s).	Avail.	Ref(s).
Acridotheres tristis										
Alopochen aegyptiaca										
Callosciurus erythraeus										
Corvus splendens										
Herpestes javanicus										
Lepomis gibbosus							P	[2,4]	P	
Lithobates catesbeianus							A	[4]	A	[4]
Muntiacus reevesi										
Myocastor coypus										
Nasua nasua										
Nyctereutes procyonoides										
Ondatra zibethicus										
Oxyura jamaicensis										
Perccottus glenii							A	[1]	A	[1]
Plotosus lineatus										
Procyon lotor										
Pseudorasbora parva							A	[2,3,4]	A	[2,3,4]
Sciurus carolinensis									P	[6-9,18]
Sciurus niger										
Tamias sibiricus										
Threskiornis aethiopicus										
Trachemys scripta							P	[24]		

2.2.b. Application – EU Member States and objectives										
Objective	**Unknown objective**		**Rapid Eradication**		**Management**					
					Eradication		**Control**		**Containment**	
Country	Avail.	Ref(s).	Avail.	Ref(s).	Avail.	Ref(s).	Avail.	Ref(s).	Avail.	Ref(s).
Austria										
Belgium										
Bulgaria										
Croatia										
Cyprus										
Czech Republic										
Denmark										

Estonia										
Finland										
France										
Germany										
Greece										
Hungary										
Ireland										
Italy										
Latvia										
Lithuania					X	[1]	X	[1]		
Luxembourg										
Malta										
Netherlands										
Poland										
Portugal										
Romania										
Slovakia										
Slovenia										
Spain										
Sweden										
United Kingdom*										

* Not an EU Member State

3. Humaneness of the measure

3.1. Welfare for all measures

Measure type (if applicable):	**Humaneness impact categories**		
Domain	**No impact**	**Mild-Moderate**	**Severe - Extreme**
1: Water deprivation, food deprivation, malnutrition	IAS removed in its free-living state, with no restriction of access to resources		

2: Environmental challenge	IAS removed in its free-living state, with no change to environmental conditions		
3: Injury, disease, functional impairment		IAS escaping predation attempts may be injured – some of these injuries will be moderate	IAS escaping predation attempts may be injured – some of these injuries will be severe. IAS taken by a predator may not die swiftly. Whilst suffering may not be prolonged, death will be produced by extreme injuries and the duration of suffering is not controlled.
4: Behavioural, interactive restriction	Free-living IAS may be exposed to fear and stress, but are able to relieve these by expressing natural responses; avoiding distress		
5: Anxiety, fear, pain, distress, thirst, hunger etc.		Threat of predation does provoke changes to the behavior of animals; commonly referred to as fear. In principle, the freedom/opportunity to vary their behavior to mitigate this state avoids this being considered distress, but may produce chronic mild stress throughout the life of the prey. In contexts where measures result in reduced mitigation of fear (e.g. loss of predator free spaces) this stress may become long-lasting distress.	As noted above, some animals may experience severe pain during predation.

3.2. Mode of death (if relevant)

Measure type (if applicable):	Immediate death (i.e. no suffering)	Not immediate death (mild - moderate suffering)	Not immediate death (severe - extreme suffering)
Rationale:	Most predation of IAS in most contexts will produce a very short period of extreme fear (pursuit) and a short period of suffering (seconds). However, it is unlikely that there will be no suffering.	Most predation of IAS in most contexts will produce a very short period of extreme fear (pursuit) and a short period of suffering (seconds).	An unknown proportion of predation events will result in injury producing severe to extreme suffering. This is likely to be a smaller proportion than for mild and moderate suffering. Dependent young deprived of maternal support are unlikely to die in a humane manner.

3.3. Humaneness summary	The use of predators to kill IAS prey cedes responsibility for the quality of the death or production of injuries to an animal without our moral inhibitions. It will select prey (including those with dependent young or threatened native species) and hunt according to their own requirements and capabilities, without any reference to human expectation. Kills may not be swift, and hunts may injure prey without always killing them. Sufficient is known of the 'inefficiency' of large predators to suggest this should be recognized as a characteristic of predation, applicable to the predation of IAS by native predators despite the absence of specific evidence about their efficiency of their kills. This argument either recognizes the potentially inhumane character of natural predation, or emphasizes the critical uncertainty about specific interactions, which should lead to extreme caution in considerations of use. Predators should not be considered humane by any use of the word (*sensu* [17]). The fear of predation is a natural state for prey species, mitigated by behaviours to avoid predators. In stable ecosystems this will include predator free spaces (spatial or temporal). Where man intentionally changes the natural equilibrium between predator and prey, the responsibility for the consequences of that intervention fall on man.

4. Costs and effectiveness of the measure	
General effectiveness of the measure	The assessment of the effectiveness of using predators to manage IAS requires an explicit list of criteria against which the measure should be assessed as well as statistically and scientifically robust observations (describing its effectiveness) independent of the operation of the measure. The absence of both in any case-study precludes a worthwhile assessment of the effectiveness of the use of predators. Agreeing such a list of criteria itself will be dependent on each case, and as the measure only appears to have been attempted once in the EU but without robust post-treatment observation, it is impossible to identify a fair list of expectations for this measure. However, criteria might include the speed and geographical scale at which IAS target populations are required to suppressed, and whether these meet the expectations of control,

	containment or eradication. Unusually, this measure might also require the sustainability of the effect to be considered, as it is usually discussed in the context of long-term and geographically significant projects. Sustainability might include the degree to which predator populations become self-sustaining, or alternatively require continual intervention to maintain their effectiveness (e.g. maintain artificially high densities of predators by supplementary feeding, continual translocation, captive breeding). Finally, unlike many other measures, predators have agency and may produce socially or ecologically problematic effects which cannot simply be managed by no-longer applying the measure. Effectiveness therefore requires a measure of the harms the measure might produce or the costs and effectiveness of its mitigation (a separate program to humanely manage predators). Unlike many other measures assessed in the toolbox where the metric of effect is 'self-reported' in well-organized management campaigns (animals presented in traps, animals shot, toxic baits chewed etc.), the effect of predators is difficult to identify and disentangle from other environmental drivers. Neither can the size or distribution of the residual post-measure population of target species (description of success) be inferred through the analysis of operational metrics such as site-specific effort per removal. Workers need to acquire independent evidence (descriptions of the densities and distributions of both predator and IAS prey) to quantify the suppression/eradication of the target IAS as well as evidence that these metrics are related. Robust estimates of these descriptions are notoriously hard to acquire, especially at extensive geographic scales. Similarly the period across which the effect must be produced or sustained is likely to be substantially different for this measure compared to others, where substantial immediate effects within the life-time of a 5-10 year project may not be sustainable (Case studies 1-4 might all follow that pattern), or conversely where useful effects only begin to occur after 5-10 years as populations of translocated predators move beyond the establishment phase and begin to achieve the densities or geographic spread to achieve management objectives (Case study 6 might follow this pattern).

4.1. Case studies

CASE STUDY #1	
Measure type (if relevant):	Introduction of a native top predator to freshwater systems
Species:	*Pseudorasbora parva, Lepomis gibbosus*
Objective:	Research to support control/population regulation
Use of measure	
Combined with other measure(s):	
Country(ies) of application:	Belgium
Geographic scale (km^2) and/or population size measure applied to:	Ponds and small lakes
Time period:	1.5 years

Effort:	unavailable
Costs:	Overall costs: unavailable Personnel costs: unavailable Equipment and infrastructure: unavailable Other, including overheads: unavailable
Effectiveness:	Lemmens et al. [2] undertook a study by adding pike (*Esox lucius*) to ponds in a replicated randomized trial and found they reduced substantially the biomass of *P. parva* compared to ponds where pike were not added. Presence of the top predator may have limited the re-establishment of *P. parva* in treated ponds, and/or subsequently regulated the growth of *P. parva* populations in the ponds. The composition and biomass of a community of native species (of assorted trophic levels) also added to the experimental ponds was largely unaffected by pike though this may have been an artefact of the study (stocking dates, breeding dates and brief duration). However, the presence of pike did not systematically inhibit the natural colonization of experimental ponds by *L. gibbosus*. This suggests still freshwater systems lacking a ubiquitous top predator might be made more resilient by their re-introduction. Study authors are cautious in suggesting that this result is general to all still freshwater systems as the experimental system did not provide predator free spaces commonly found in larger lakes and relied on a specific and unusual predator demographic (pike were only represented by a single age class).

CASE STUDY #2	
Measure type (if relevant):	Introduction of a native predator to freshwater systems
Species:	*Pseudorasbora parva*
Objective:	Control/regulation
Use of measure:	A replicated experiment compared a systematic program of netting *P. parva* as a tool to remove them from a network of ponds, with the introduction of native perch (*Perca fluviatilis*). In addition, the work sought direct evidence of the direct predation of *P. parva* by perch and the relative contribution of the IAS prey in its diet.
Combined with other measure(s):	
Country(ies) of application:	UK
Geographic scale (km^2) and/or population size measure applied to:	Ponds and small lakes
Time period:	2 years
Effort:	unavailable

Costs:	Overall costs:	
	unavailable	
	Personnel costs:	
	unavailable	
	Equipment and infrastructure:	
	unavailable	
	Other, including overheads:	
	unavailable	
Effectiveness:	The effectiveness of both approaches was measured at a range of intervals across 2 years of study [3]. Populations of *P. parva* in netted ponds recovered quickly after every removal intervention (compensatory growth), whilst populations of *P. parva* in those ponds where perch were added declined and were maintained at a much lower density until the end of the experiment. Whilst perch are not top predators in all freshwaters their effects on changing the simple food-web in the study were noted and the authors noted the importance of the choice of introduced predator and how predation by its different life-stages (on prey of differing types and sizes) might affect ecosystems. Neither perch nor netting eradicated *P. parva*.	

CASE STUDY #3	
Measure type (if relevant):	Introduction of a native top predator to freshwater systems
Species:	*Lithobates catesbeianus, Pseudorasbora parva, Lepomis gibbosus*
Objective:	Control and Containment
Use of measure:	Louette [4] undertook a replicated randomized study using a factorial design exploring the effects of introducing pike and drawdown on the abundance of *L. catesbeianus* and other fish in an aquaculture pond system.
Combined with other measure(s):	Experiment included drawdown (habitat management) but inference can be made exclusive of this additional treatment.
Country(ies) of application:	Belgium
Geographic scale (km^2) and/or population size measure applied to:	Ponds and small lakes
Time period:	2.5 years
Effort:	Unavailable
Costs:	Overall costs:
	Unavailable but authors cite other evidence suggest it is practical and inexpensive.
	Personnel costs:
	Unavailable

	Equipment and infrastructure:
	Unavailable
	Other, including overheads:
	Unavailable
Effectiveness:	Whilst an annual drawdown had no effect on the biomass of *L. catesbeianus* adults or tadpoles, the presence of pike did substantially reduce the abundance (biomass) of *L. catesbeianus* tadpoles by the end of the study though the biomass of adult frogs was unaffected [4]. Similarly, ponds without pike produced thriving fish communities including *P. parva* and *L. gibbosus* whilst these species were less abundant in ponds with pike. The mechanisms for the observed effects on *L. catesbeianus* are unknown but may have included direct predation of tadpoles, the effect of the top predator on the community of other predatory fish (e.g. enhancing native meso-predators of tadpoles), and indirect effects on the habitat produced by the suppression of planktivorous species (including *P. parva* and *L. gibbosus*) which may have made ponds with pike less attractive as breeding sites to dispersing adult frogs. However, it was not clear if the observed effects would play a significant role in regulation or control of the bullfrog population as tadpole mortality and the recruitment and productivity of adult frogs are not linked. Authors [4] note the specificities of their study system (small uniform shallow ponds) and how natural bodies of water with sensitive ecosystems (developed in the absence of local top predators) may be harmed by the introduction of a naturally absent top predator. They also note that the predation they observed required continual (annual) re-introductions of pike. The presence of pike did not stop ponds being exploited by adult bullfrogs, or the spread of adults between ponds in this system. Whilst the addition of pike did substantially change the abundance of two listed IAS (fish) and tadpoles of the bullfrog, it neither eradicated any of the species, nor stopped their spread. Pike were identified as producing a series of positive effects making the pond ecosystem more resilient to the presence of the IAS, but did not directly or indirectly result in the removal of these species.

CASE STUDY #4	
Measure type (if relevant):	Introduction of native predators to freshwater systems
Species:	*Perccottus glenii*
Objective:	Control
Use of measure:	Four freshwater ponds and natural lakes dominated by *P. glenii*, were stocked and restocked with pike (*Esox lucius*) or pike with European perch (*P. fluviatilis*) over 3 years [1]. The predators were apparently absent from these waters. Both native predators of *P. glenii* were stocked/restocked at a range of densities. The

	abundance and size of all fish in the lakes was monitored using short electro-fishing transects throughout the work as well as in the following year.
Combined with other measure(s):	
Country(ies) of application:	Lithuania
Geographic scale (km^2) and/or population size measure applied to:	Ponds and small lakes
Time period:	5 years
Effort:	Unavailable
Costs:	Overall costs:
	Unavailable but authors cite other evidence suggest it is practical and inexpensive.
	Personnel costs:
	Unavailable
	Equipment and infrastructure:
	Unavailable
	Other, including overheads:
	Unavailable
Effectiveness:	Generally, the abundance and size of *P. glenii* declined substantially in all lakes, and became too difficult to detect by the end of the work in the largest three waters [1]. However the diversity and abundance of the community of native prey species also declined dramatically over the study and became trophically unbalanced. Evidence suggests pike, as top predator, ate everything available in open water habitats as prey abundances declined, though they may have left small *P. glenii* in shallow dense macrophyte beds, where they were available to smaller perch. The single lake maintaining a residual population of small (immature) *P. glenii* was stocked with pike at the lowest density and was not stocked with perch. The authors suggest eradication of *P. glenii* occurred in some lakes but do not present data to support this. The authors state, that in comparison with other control measures "…..the reintroduction of native piscivorous fish species is a feasible, cost-effective, uncontroversial, and sustainable management approach." However, the effectiveness of this approach may be limited to small shallow eutrophic still waters as *P. glenii* is known to have spread and co-exists with pike and perch in a natural equilibrium throughout more complex natural hydrological systems [14,15].

CASE STUDY #5	
Measure type (if relevant):	Meso-predator suppression by a top predator causes release of IAS populations

Species:	*Lithobates catesbeianus*
Objective:	Control
Use of measure:	
Combined with other measure(s):	
Country(ies) of application:	United States of America
Geographic scale (km^2) and/or population size measure applied to:	Ponds and small lakes
Time period:	n/a
Effort:	Unavailable
Costs:	Overall costs: Unavailable Personnel costs: Unavailable Equipment and infrastructure: Unavailable Other, including overheads: Unavailable
Effectiveness:	Adams et al. [5] explored factors explaining the presence and success of *L. catesbeianus* in ponds and small lakes in Oregon, USA. This included a survey of many ponds (for frogs, fish and various environmental predictors) as well as field-based predation experiments. Natural waters in this region are often devoid of fish or host a sparse native fauna mostly unable to take bullfrog tadpoles. An extensive suite of non-native fish occurs in many waters, including *Lepomis gibbosus* and *Lepomis macrochirus*. The work identified that the presence of nonnative fish was a key factor promoting bullfrog presence and abundance. Experimental studies identified that L*epomis macrochirus* (bluegill sunfish) preferentially foraged on macro-invertebrates known to consume bullfrog tadpoles. Suppression of these invertebrate meso-predators appears to enable the establishment of *L. catesbeianus* as adults move between waters. Whilst the example is not European, and the top fish predator is not a native to either Europe or the study area, it does demonstrate the fragility of freshwater food webs and the unexpected effects of introducing predators to these systems. In this case, the addition of a top predator inadvertently promoted the abundance and spread of a listed IAS.

CASE STUDY #6	
Measure type (if relevant):	Predation of grey squirrels (*Sciurus carolinensis*) by pine martens (*Martes martes*)
Species:	*Sciurus carolinensis*
Objective:	Control
Use of measure:	Regional scale declines in the abundance of grey squirrels in the Irish midland counties were initially associated with the natural spread and increase in abundance of pine martens across the same landscapes following their protection from persecution [6]. Explanations were also sought to explain the unusual distribution of the grey squirrel in Ireland, which a century after their establishment appeared to be unable to support populations west of the River Shannon despite rapid spread elsewhere; this western region represents the core range for the remnant pine marten population [20]. The suppression of grey squirrel populations was also associated with a recovery of red squirrel populations in the same areas, and research indicated that martens preferentially predated grey squirrels over red squirrels, a finding strengthened by recent work [7, 8].
Combined with other measure(s):	Studies conducted in landscapes historically supporting shooting and trapping of grey squirrels.
Country(ies) of application:	UK and Ireland
Geographic scale (km^2) and/or population size measure applied to:	Regional areas
Time period:	n/a
Effort:	n/a
Costs:	Overall costs:
	Unavailable
	Personnel costs:
	Unavailable
	Equipment and infrastructure:
	Unavailable
	Other, including overheads:
	Unavailable
Effectiveness:	Subsequent work has identified that the presence of martens *per se* is insufficient to affect populations of the grey squirrel [18,20], as the southerly expansion of grey squirrels in Ireland continues despite the presence of martens [20]. However, the ongoing suppression of grey squirrel spread, establishment or population development in western and central regions of Ireland may be explained by a combination of factors including landscape composition, configuration and connectivity (affecting both the spatial dynamics of martens and the availability of non-squirrel prey) and an unusual local abundance of pine martens [20]. However, grey squirrels have not been eradicated from the largely rural regions where their populations have been suppressed [20].

	Further work in the UK (in three contrasting study sites in Scotland) have characterized relationships between landscape connectivity (for martens as well as red and gray and squirrels), their population densities, as well as indicating the importance of the longevity of the marten/grey squirrel interaction in describing the potential regulation of grey squirrel populations [19]. Again the scale of the predatory effect on grey squirrels is related to a range of factors including one associated with marten density and movement [19], though it is unclear from this work if martens alone could locally or regionally extirpate grey squirrels. Inference from both the Irish and Scottish work is currently limited to correlations describing existing dynamics, with poor historical records limiting inference about the speed at which population level effects might occur or a planned comparison between contrasting landscapes, though both studies tangentially suggest that the suppression of grey squirrels takes decades to achieve. Subsequently, studies of the relationships between grey squirrels and pine martens are beginning to directly describe the response of both populations to marten re-introduction programs in Wales and England, where martens are of conservation concern following their extirpation from much of the country. Here an experimental 'before/after' comparison is available for populations in the same landscapes which allows for strong inference. Current indications are that the predatory pressure on grey squirrel populations necessary to suppress their growth or spread will require marten populations to develop substantially from their initial post-establishment densities [18], possibly over decades, despite immediate effects on grey squirrel behavior or social dynamics being noted [18]. Further, modelling work suggests that grey squirrels may remain in urban refugia or other areas of the landscape unsuitable to martens, and that reliance on predation to achieve containment or control may not be feasible [9].

4.2. Costs effectiveness summary	All case examples directly measuring the responses of IAS to known/controlled interventions were limited to aquatic IAS in small lentic ponds and lakes, with three undertaken as experiments in aquaculture ponds. In none of the examples was a listed IAS shown to have been eradicated by the management (introduction) of the predator; one study claims eradication but did not provide robust and extensive / prolonged monitoring to support the claim. In all cases where the deliberate introduction of predators was intended to reduce the abundance of a target IAS species, reductions were observed; this was also true for some incidental IAS species measured in the same studies. Whether the character of the response was maintained cannot be judged as experiments were relatively short; many studies noted that the initial and final fish communities were 'un-natural' in that balanced or sustainable trophic communities and demographic profiles for key species were not achieved. Occasionally the depression of target IAS populations was substantial and introduced predators appeared to either inhibit the re-establishment of IAS, or limit their population growth. In other cases the effects were more variable, affecting one life-stage but not another, and in all cases, predators alone were ineffective at reliably inhibiting spread or establishment of IAS.

	Most case examples here relied on continual intervention in order to produce what may have been a short-lived effect with no information available about the costs or resource necessary to produce the effect. Most cases were also conducted as experiments in relatively unusual contexts (aquaculture facilities). The case study exploring marten predation on squirrels highlights the potential constraints on success for this measure, requiring extensive scientific research to justify the translocation of martens, and substantial monitoring effort to identify the effectiveness and geographic scale of the predator effect. Its effect may only be sufficient in landscapes or regions combining specific habitat compositions or configurations favorable to successful predation. This is also likely to require decades of monitoring in order to unequivocally identify the role the predator has in the population dynamics of the IAS prey and describe it relative effectiveness.

5. Side effects	
Non-target native species, their habitats and the broader environment:	Positive:
	Potential side effects may include the release of competitive suppression of native species, as suggested by work exploring the effects of the pine marten (*Martes martes*) on the grey squirrel (listed IAS) and the subsequent positive effects on the release of the red squirrel (*Sciurus vulgaris*) which is conservation concern in the UK [6-8, 19, 20]. However, the scope and scale of this positive effect may be limited to areas of the landscape available to martens [9] or where marten abundance produces sufficient predator pressure [18-20].
	Negative:
	The profound effects the introduction or promotion of top predators may have on ecosystems cannot be overstated, effectively articulated in a recent article [9] where the authors state "Confounding effects of indirect interactions between animal species and habitat factors, however, make predicting the consequences of species invasion interactions challenging, especially where a landscape is modified greatly by human activity [25]. This is exemplified by the unforeseen, adverse consequences of predator introductions for biological control of an invasive prey species [26], which can result in disastrous, unintended impacts on native species ill-equipped to deal with the presence of a novel predator [27,28]." A similar statement is made by Sheehy et al. [19].
Other invasive alien species:	Positive:
	Negative:
	Case study 5 exemplifies the principle of meso-predator release by an introduced predator potentiating the establishment and development of a population of a listed IAS (*Lithobates catesbeianus*). Although the introduced predator was a fish that was not native to the non-European ecosystem (incidentally a *Lepomis sp.*) it preferentially predated on invertebrate meso-predators which suppressed the establishment and development of populations of *Lithobates*, permitting them to increase and spread where otherwise they might not [5].

Public health and well-being:	Positive:	
	Negative:	
	Predator re-introduction programs, or those schemes supporting the natural return of top predator species to landscapes from which they have historically been absent are renowned for creating conflict with a variety of stakeholders [10-13].	
Economic:	Positive:	
	Introduced predators, especially top predators large enough to take larger IAS, will be generalist predators and produce a threat to livestock. Candidate predators such as lynx may take poultry from backyard holdings, or lambs from open fields, whilst wolf or bear may take larger animals (sheep, goats, calves and foals) [29]. This may cause damage for which compensation will be required, e.g. [11,13].	
	Negative:	

6. Conclusion

Overall assessment of the measure (qualitative)

The use of native predators as a measure to intentionally manage IAS appears superficially attractive. For potentially little outlay (catching and translocating some animals) native predators might give the appearance of a benign bio-control agent, whilst also contributing to the restoration of degraded ecosystems and rewilding landscapes often considered to be in need of conservation action. By bringing these iconic and often charismatic species geographically and metaphorically closer to people there is a chance of using them to engage citizens in wider ecological/conservation discourse, as well as benefit from the free ecological service they would provide in consuming listed IAS to the benefit of society. However, the use of bio-control agents imported into ecosystems has a very long history of disaster, with at least four of the listed IAS having established their threat to Europe by repeatedly having been imported and released to control weeds/pests in other parts of the world, and subsequently damaging ecosystems at national or even continental scales (e.g. common myna). It should be remembered that the modern productive anthropogenic landscapes of Europe, often occupied by home-owners as frequently as they are farmers or foresters (e.g. spread of urban and urbanized land-uses), are so different to the unaltered natural ecosystems previously occupied by the former native predators, as to make the re-introduced marten, lynx, wolf or bear a novel presence in these anthropogenic ecosystems. The repeated failures of bio-control should teach us to be extremely cautious about introducing novel species even if they are native.

To maintain some focus, this assessment considers mainly studies or examples where predators have been introduced with the primary intended purpose of managing IAS; predation as a utilitarian tool. In this context predation is either known to work, in a reliable or repeatable way with a predictable cost-benefit (a measure in terms comparable with other measures considered in this project), or it does not. If predation cannot be seen in terms comparable with other measures it might be considered as an adjunct or supportive act, where other tangential benefits may accrue (i.e. conservation, public engagement), though we suggest that this might be conditional only if cheap to deploy and with few disadvantages. However, experience suggests that the re-introduction of top predators into European ecosystems or landscapes is rarely without complication, financial cost or social discord. These are unlikely to be off-set by the value predators may bring in helping to suppress populations of IAS. The cost/benefit equation is different if the predators arrive in their new landscapes through natural spread, though in this case the accrual of benefits is likely to take very many years.

Another reason for the specific focus here is a consequence of the limited number of quantified case examples of predators native in Europe, having been shown to suppress, control, contain or eradicate listed IAS. It is worth noting that many of the listed IAS have successfully established and spread in landscapes replete with top predators; both those present in their ecosystem of introduction, or often introduced European predators (e.g. red fox, cat) also present as wild or feral predators in other locations around the world. Whilst evidence that European predators in non-European ecosystems have failed to control the establishment and spread of listed IAS is not definitive (the non-European ecosystem differs from those in Europe and many reasons may exist for failures of control), the more significant evidence comes from the number of listed IAS that have established, spread and become abundant in Europe despite the presence of a large suite of native terrestrial or avian predators. One exception to this observation may be emerging example of a native predator appearing to show some potential to suppress populations of a listed IAS comes from the UK, where pine martens, extirpated from much of the UK have been shown to have some effect on the populations of grey squirrel (Case Study 6). Much of the evidence is correlative or study specific and it is not yet clear how general the effect is or whether any useful control of grey squirrel populations would be possible, whilst the re-introduction of the pine marten across much of the UK would be resisted by many landowning stakeholders (owners of poultry and water fowl, as well as the game-hunting industry). Further it is worth noting that despite the general absence of lynx, wolf and bear from European landscapes, it is likely that even these top-predator species would be unlikely to have any useful effect on some of the larger IAS. For example, a recent study examining the relative contribution of hunting and predators in the population dynamics of Tuscan (Italy) roe deer (a close analogue for Muntjac deer), found that uncoordinated sports hunting was responsible for removing 8-9 times the biomass compared to wolves [16]. Native predators may be suitable to provide temporary regulation of populations of IAS in specific limited circumstances (i.e. small lentic fresh-water bodies with fish or amphibian IAS). It is unknown if the reductions in IAS populations produced are sustained without continual intervention and presence of predators has been demonstrated not to have prevented the spread of freshwater fish IAS per se, so it may be of limited use as a long-lasting means of control or containment.

Assessor:	James Aegerter
Reviewer 1:	Riccardo Scalera
Reviewer 2:	Sandro Bertolino

7. References

[1] Rakauskas, V., et al. (2019). "The use of native piscivorous fishes for the eradication of the invasive Chinese Sleeper, *Perccottus glenii*." Knowl. Manag. Aquat. Ecosyst.(420): 21.

[2] Lemmens, P., et al. (2015). "Suppression of invasive topmouth gudgeon *Pseudorasbora parva* by native pike *Esox lucius* in ponds." Aquatic Conservation **25**(1): 41-48.

[3] Davies, G. D. and J. R. Britton (2015). "Assessing the efficacy and ecology of biocontrol and biomanipulation for managing invasive pest fish." Journal of Applied Ecology **52**(5): 1264-1273.

[4] Louette, G. (2012). "Use of a native predator for the control of an invasive amphibian." Wildlife Research **39**(3): 271-278, 278.

[5] Adams, M. J., et al. (2003). "Indirect facilitation of an anuran invasion by non-native fishes." Ecology Letters **6**(4): 343-351.

[6] Sheehy, E., & Lawton, C. (2014). Population crash in an invasive species following the recovery of a native predator: the case of the American grey squirrel and the European pine marten in Ireland. *Biodiversity and Conservation, 23*(3), 753-774. doi:10.1007/s10531-014-0632-7

[7] Twining, J. P., Montgomery, W. I., & Tosh, D. G. (2020). The dynamics of pine marten predation on red and grey squirrels. *Mammalian Biology, 100*(3), 285-293. doi:10.1007/s42991-020-00031-z

[8] Twining, J. P., Montgomery, W. I., Price, L., Kunc, H. P., & Tosh, D. G. (2020). Native and invasive squirrels show different behavioural responses to scent of a shared native predator. *Royal Society Open Science, 7*(2), 191841. doi:doi:10.1098/rsos.191841

[9] Twining, J. P., Montgomery, W. I., & Tosh, D. G. Declining invasive grey squirrel populations may persist in refugia as native predator recovery reverses squirrel species replacement. *Journal of Applied Ecology, n/a*(n/a). doi:10.1111/1365-2664.13660

Citations referenced in [9]:

[25] Colautti, R. I., Ricciardi, A., Grigorovich, I. A., & MacIsaac, H. J. (2004). Is invasion success explained by the enemy release hypothesis?. *Ecology letters*, *7*(8), 721-733.

[26] Dickman, C. R. (1986). Niche compression: two tests of an hypothesis using narrowly sympatric predator species. *Australian Journal of Ecology*, *11*(2), 121-134.

[27] Kovacs, E. K., Crowther, M. S., Webb, J. K., & Dickman, C. R. (2012). Population and behavioural responses of native prey to alien predation. *Oecologia*, *168*(4), 947-957.

[28] Salo, P., Korpimäki, E., Banks, P. B., Nordström, M., & Dickman, C. R. (2007). Alien predators are more dangerous than native predators to prey populations. *Proceedings of the Royal Society B: Biological Sciences*, *274*(1615), 1237-1243.

[10] Arts, K., Fischer, A., & van der Wal, R. (2016). Boundaries of the wolf and the wild: a conceptual examination of the relationship between rewilding and animal reintroduction. *Restoration Ecology*, *24*(1), 27-34.

[11] Hawkins, S. A., Brady, D., Mayhew, M., Smith, D., Lipscombe, S., White, C., ... & Convery, I. (2020). Community perspectives on the reintroduction of Eurasian lynx (Lynx lynx) to the UK. *Restoration Ecology*.

[12] Van Heel, B. F., Boerboom, A. M., Fliervoet, J. M., Lenders, H. J. R., & Van den Born, R. J. G. (2017). Analysing stakeholders' perceptions of wolf, lynx and fox in a Dutch riverine area. *Biodiversity and Conservation*, *26*(7), 1723-1743.

[13] Bautista, C., Revilla, E., Naves, J., Albrecht, J., Fernández, N., Olszańska, A., ... & Härkönen, S. (2019). Large carnivore damage in Europe: Analysis of compensation and prevention programs. *Biological Conservation*, *235*, 308-316.

[14] Terlecki, J. and R. Pałka (1999). "Occurrence of *Perccottus glenii* Dybowski 1877 (Perciformes, Odontobutidae) in the middle stretch of the Vistula River, Poland." Fisheries & Aquatic Life **7**(1): 141-150.

[15] Reshetnikov, A. N. and G. F. Ficetola (2011). "Potential range of the invasive fish rotan (*Perccottus glenii*) in the Holarctic." Biological Invasions **13**(12): 2967-2980.

[16] Bassi, E., Gazzola, A., Bongi, P., Scandura, M., & Apollonio, M. (2020). Relative impact of human harvest and wolf predation on two ungulate species in Central Italy. *Ecological Research, 35*(4), 662-674. doi:10.1111/1440-1703.12130

[17] Hampton, J.O., Fisher, P.M. and Warburton, B. (2020), Reconsidering humaneness. Conservation Biology. Accepted Author Manuscript. doi:10.1111/cobi.13489

[18] McNicol, C. M., Bavin, D., Bearhop, S., Ferryman, M., Gill, R., Goodwin, C. E., ... & McDonald, R. A. (2020). Translocated native pine martens Martes martes alter short-term space use by invasive non-native grey squirrels *Sciurus carolinensis*. Journal of Applied Ecology.

[19] Sheehy, E., Sutherland, C., O'Reilly, C., & Lambin, X. (2018). The enemy of my enemy is my friend: native pine marten recovery reverses the decline of the red squirrel by suppressing grey squirrel populations. *Proceedings of the Royal Society B: Biological Sciences, 285*(1874), 20172603. doi:doi:10.1098/rspb.2017.2603

[20] Flaherty, M., & Lawton, C. (2019). The regional demise of a non-native invasive species: the decline of grey squirrels in Ireland. *Biological Invasions, 21*(7), 2401-2416. doi:10.1007/s10530-019-01987-x

[21] Hickling, G. J. (2000). Success in Biological Control of Vertebrate Pests. In G. Gurr & S. Wratten (Eds.), *Biological Control: Measures of Success* (pp. 341-368). Dordrecht: Springer Netherlands.

[22] Massei, G., Quy, R. J., Gurney, J., & Cowan, D. P. (2010). Can translocations be used to mitigate human wildlife conflicts? *Wildlife Research, 37*(5), 428-439. doi:https://doi.org/10.1071/WR08179
[23] Monk, C. T., Chéret, B., Czapla, P., Hühn, D., Klefoth, T., Eschbach, E., . . . Arlinghaus, R. Behavioural and fitness effects of translocation to a novel environment: Whole-lake experiments in two aquatic top predators. *Journal of Animal Ecology, n/a*(n/a). doi:10.1111/1365-2656.13298
[24] Aldridge, D., Ockendon, N., Rocha, R., Smith, R. K., & Sutherland, W. J. (2019). Action: Red-eared terrapin: Biological control using native predators in Some aspects of control of freshwater invasive species. In W. J. Sutherland, L. V. Dicks, N. Ockendon, S. O. Petrovan, & R. K. Smith (Eds.), *What Works in Conservation 2019* (pp. 569-602). Cambridge, UK: Open Book Publishers.
[29] Linnell, J. D. C., & Cretois, B. (2018). *The revival of wolves and other large predators and its impact on farmers and their livelihood in rural regions of Europe*. Retrieved from European Parliament, Brussels

Appendix 2. Aquatic barriers

1. Measure name			
1.1. English:	Aquatic barriers		
1.2. Lethal or non-lethal:	Non-lethal and lethal		
1.3. Other languages (if available):			
Bulgarian	Водни прегради (бариери)	Italian	Barriere acquatiche
Croatian	Vodne pregrade	Latvian	Ūdens barjeras
Czech	Vodní překážky	Lithuanian	Vandens užtvaros
Danish	Akvatiske barrierer	Maltese	
Dutch	Aquatische barrières	Polish	Bariery wodne
Estonian	Veetõkked	Portuguese	Barreiras aquáticas
Finnish	Vesi esteet	Romanian	Bariere acvatice
French	Barrières aquatiques	Slovak	Vodné bariéry/prekážky – fyzické & iné
German	Aquatische barrieren, wanderhindernisse	Slovenian	Vodne ovire – fizične in druge
Greek	Υδάτινοι φραγμοί	Spanish	Barreras acuáticas
Hungarian	Vízi akadályok	Swedish	
Irish			

2. Technical details of measure
2.1.a. Measure description
There are two distinct groups of aquatic barriers used in aquatic invasive alien species (IAS) management, physical and non-physical. **Physical barriers** include those used to prevent spread of species ‘in stream’, and include weirs and other physical structures which can be temporary to target migration periods, or permanent for example the ‘low-head’ barriers used to prevent sea lamprey (*Petromyzon marinus*) accessing spawning grounds in the Great Lakes Basin [1]. However, the containment of aquatic IAS is the unintended consequence of the vast majority of in-stream physical barriers (e.g. dams), and trade-offs arise when barrier removal intended to benefit native species interfere with management decisions intended to control the unwanted spread of IAS [2]. Research is ongoing to develop technologies that allow for native species upstream passage past existing barriers whilst reducing the risk of IAS spread. An example is the EU LIFE project on the Po River in Italy (LIFE11NAT/IT/188 Con.Flu.Po) who developed experimental metal cages with two funnel openings (upstream and downstream) with a 15cm mesh to capture larger specimens of the IAS Wells catfish (*Silurus glanis*) (c. >10kg) while allowing passage of native migratory species including the Adriatic strurgeon *Acipenser naccarii* [3,4]. Permeable barriers on lentic habitat outflows could also be used to contain the spread of aquatic IAS such as ***P. parva***, including larvae and young-of-year (<20mm) whilst allowing for water to drain from the site [5]. Permeable barriers (e.g. fine mesh screens) can also be used to prevent the introduction of aquatic IAS including ***P. glenii*** from infested fish farms that are connected to natural water systems or canals [6]. Physical mesh screens have been used to prevent spread of ***L. catesbeianus*** tadpoles during pond drainage actions in Belgium, and could also be potentially be used to support other similar eradication efforts (pond drainage) for the fish species.

Non-physical barriers include a variety of different technical applications, and can be considered as non-permanent as ‘treatments’ can be timed to meet and repel aquatic IAS but be deactivated to allow passage of native species and be removed or repositioned as management goals change [7]. They include the following methods, which have mostly been developed and applied to deter or repel fish from entering hydropower dams or power plants intakes, or steer or attract into traps, however their use for in stream containment of spread of aquatic IAS has been applied or tested in some cases.

Acoustic barriers. Sound travels efficiently though water as pressure waves and is used by fish for many life cycle functions including avoidance [8], and several studies have shown that specific sounds can deter fish movement in a species specific and directional manner [1]. No evidence could be found its application to aquatic IAS of Union concern, however there is evidence of bio-acoustic behavioural fish guidance system being used to restrict bighead carp (*Hypophthalmichthys nobilis*) movement in mid-western USA [9,10].

Electrical. An electrical current can be passed through water from an anode to a cathode, creating an electric field that deters fish. Fish inside the electrical field become part of the electrical circuit, and they can experience a reaction, such as avoidance, electrotaxis (forced swimming), electrotetanus (muscle contraction), electronarcosis (muscle relaxation or stunning) or death [11]. The effects vary due to many factors including the species of fish, size of fish (larger fish require less power to immobilze), water conductivity, design and placement of electrodes, type of electrical current used, and direction of current [7]. Electrodes can be laid horizontally across the bottom of the water (though sediment deposits can render them ineffective) or vertically along the sides of a geographical formation around the barrier. While they can use alternating current (AC), direct current (DC), or pulsed DC, most recent applications use pulsed DC, as AC has been found to be injurious to fish and is prohibited for use on fish under the Bern Convention. A graduated-field fish barrier (GFFB) uses pulsed DC and has parallel electrodes placed perpendicular to the flow with each electrode being more powerful, and are are designed to improve effectiveness but also to cause less harm to the fish [11]. No evidence could be found of the use of electrical barriers to the aquatic IAS of Union concern, however there is no reason why they couldn’t be applied as electric fields are non-selective and have been recommended to support containment of ***P. glennii*** [12,13]. The largest electrical dispersal barrier system (EDBS, 3 separate barriers) has been installed on the Chicago Sanitary and Ship Canal to prevent the invasion of bigheaded carps (*Hypophthalmichthys nobilis*), and silver carp (*H. molitrix*) into Lake Michigan, and GFFB have been used in the Great Lakes region to prevent invasive sea lamprey (*Petromyzon marinus)* reaching spawning areas [1].

Bubble curtain. A fence or curtain of bubbles are emitted from air diffusers placed along the bottom perpendicular to the channel, resulting in a continuous “screen” of bubbles in the water column that provides an unnatural visual cue for fish to avoid [7]. No evidence could be found of this measures application to aquatic IAS of Union concern, however Kang and Kim [14] found that a bubble curtain was effective at preventing the movement of bluegill *Lepomis macrochirus* in trials using an experimental channel at 0.7m deep.

Strobe light. The response of many fish eyes to light level changes is often slow, and strobe lights introduce unnatural light levels relative to the ambient environment, having the ability to negatively impact fish behaviors and induce an avoidance response [7]. The effects of strobe lights have been tested on a number of species as a deterrent, for example Kim and Mandrak [14] found through trials that strobe lights were effective as a deterrent for common carp (*Cyprinus carpio*) and brown bullhead (*Ameiurus nebulosus*), but not largemouth bass (*Micropterus salmoides*), and conclude that the measure may be species specific. No evidence could be found for any of the aquatic IAS of Union concern.

Carbon dioxide (dissolved oxygen levels). Fish can detect and avoid low oxygen gradients (or high CO2) which would affect their respiration (hypoxia or hypercapnia), therefore a potential barrier could be created by reducing dissolved oxygen by increasing levels of CO2 in ambient water [7]. A CO2 barrier could also be designed to either just deter fish movement, at which the CO2 concentration to be targeted may be the slightly above the threshold that induces avoidance behaviors, or block fish passage, at which the CO2 concentration to be targeted may be the greatest CO2 concentration that induces avoidance responses and allow the contact with elevated CO2 be sufficiently long enough to cause fishes to lose equilibrium [15]. No evidence could be found for the application or testing of this measure on any of the aquatic IAS of Union concern, though research has been undertaken to identify CO2 concentrations that elicit behavioral avoidance and equilibrium loss for a number of other species including *H. molitrix*, *H. nobilis*, *L. macrochirus*, and *M. salmoides* [15], and *P. marinus* [16].

Ozone. Ozone is used in low doses in aquaculture to control diseases, and in higher doses for ballast water treatments as a sterilization method and has shown promise as a non-physical barrier as it is lethal to a wide range of aquatic taxa [17]. Ozone barriers would need to be constantly operated at high concentration levels if it is to be effective (i.e. lethal) to aquatic IAS fishes. Ozone can be generated using several techniques including with commercially available generators, and once formulated it can be efficiently dissolved into water either by a simple injection system (e.g., O3 added through a ceramic diffuser), or by a more complex method (e.g., Venturi injector). The most efficient way to mass transfer O3 into water is to create an O3-liquid mix with high surface area to volume ratio (i.e., many tiny bubbles) [17]. No evidence could not be found for any of the aquatic IAS of Union concern, however few aquatic IAS have been specifically tested for mortality when exposed to O3 [17].

Velocity barriers. Natural or artificial areas of high water velocity are known to function as boundaries to the upstream movements of riverine fishes. By constricting water flow through a culvert, chute, or flume flow regimes can be artificially modified so that water velocities exceed the swimming ability of a targeted species [7]. While no evidence of this measure application to aquatic IAS of Union concern can be found, it may be a potential measure for *P. parva* that prefers lower stream reaches with moderate current speed and low flow rates [18], ***P. glenni*** which is a not a 'successful' swimmer and is expected to colonise mainly downstream of its introduced locations [12] and avoids river stretches with fast and even slow current [19], and ***L. gibbosus*** which avoids swift waters [19].

Pheromones. Pheromones could be collected or synthesized and released into the water column to act as a potential chemical barrier, or be used to exclude fish from a particular location, or aggregate fish away from a source of danger or closed passageway [7]. Their use should supplement and increase the efficiency of other control strategies such as trapping [9]. Pheromones have been used to support the control of *P. marinus* through pheromone baited traps but research is still ongoing [1].

Although no cases of non-physical barriers being applied to aquatic vertebrate species of Union concern could be mobilized, there is no reason why they could not be applied to support their containment following research into their potential effectiveness for the different species. However it must be stressed that their impacts to non-target species, especially migratory native species, needs to be considered. Reshetnikov & Karyagina [13] recommend the establishment of electric migration barriers or other technical solutions for preventing potential ***P. glenii*** moving through West-Ukranian canals, and Nehring & Steinhof [12] also recommend the installation of migration barriers (e.g. electrical deterrent systems, air bubble curtains, etc.) in the Main-Danube Canal and other key canals to prevent dispersal along German inland waters.

2.1.b. Integration with other measures

Aquatic barriers are often used alongside eradication measures such as dewatering canals, reservoirs, stream stretches, and ponds so that re-entry into the treated area [or escape from] does not take place [20]. Barriers are also used to support eradication campaigns to prevent reinvasion from hydrologically connected areas, for example in Italy where an electrical barrier was installed prior to the eradication (using electrofishing) of introgressed brown trout in Sardinia [21].

2.2.a. Availability - species and objectives										
Objective	**Unknown objective**		**Rapid Eradication**		**Management**					
					Eradication		**Control**		**Containment**	
Species	Avail.	Ref(s).	Avail.	Ref(s).	Avail.	Ref(s).	Avail.	Ref(s).	Avail.	Ref(s).
Acridotheres tristis										
Alopochen aegyptiaca										
Callosciurus erythraeus										
Corvus splendens										
Herpestes javanicus										
Lepomis gibbosus			P (physi cal)				P (physical)		P (physical)	
Lithobates catesbeianus			A (physi cal)	Expert opinion			A (physical)	Expert opinion	A (physical)	Expert opinion
Muntiacus reevesi										
Myocastor coypus										
Nasua nasua										
Nyctereutes procyonoides										
Ondatra zibethicus										
Oxyura jamaicensis										
Perccottus glenii			P (physi cal)				P (physical)		A (physical) P (non-physical)	Physical - [6] Non-physical - [12]
Plotosus lineatus										
Procyon lotor										
Pseudorasbora parva			P (physi cal)				P (physical)		A (physical)	[5]
Sciurus carolinensis										
Sciurus niger										
Tamias sibiricus										
Threskiornis aethiopicus										
Trachemys scripta										

2.2.b. Application – EU Member States and objectives										
Objective	Unknown objective		Rapid Eradication		Management					
					Eradication		Control		Containment	
Country	Avail.	Ref(s).	Avail.	Ref(s).	Avail.	Ref(s).	Avail.	Ref(s).	Avail.	Ref(s).
Austria										
Belgium										
Bulgaria										
Croatia										
Cyprus										
Czech Republic										
Denmark										
Estonia										
Finland										
France										
Germany										
Greece										
Hungary										
Ireland										
Italy	X	[21]								
Latvia										
Lithuania										
Luxembourg										
Malta										
Netherlands										
Poland										
Portugal										
Romania										
Slovakia										
Slovenia										
Spain										
Sweden										
United Kingdom*										

* Not an EU Member State

3. Humaneness of the measure			
3.1. Welfare for all measures			
Measure type (if applicable): Physical barriers	**Humaneness impact categories**		
Domain	**No impact**	**Mild-Moderate**	**Severe - Extreme**
1: Water deprivation, food deprivation, malnutrition	Physical barriers will have no effect on food/water intake.		
2: Environmental challenge	In general, physical barrier for the prevention of movement in streams (not dams for other purposes) will not expose animals to environmental conditions outside their normal range.	In some situations, as physical barriers can change water chemistry, temperature, flow regimes, and substrate etc., the measure could potentially deprive individuals from optimal habitat.	
3: Injury, disease, functional impairment	Physical barriers in wild will not lead to injury or functional impairment.		
4: Behavioural, interactive restriction	Physical barriers in the wild will have no interference with the behavioural needs of an animal. However, if the IAS was a migratory species the impact could be moderate as it would prevent the animal from accessing environments required to complete its life cycle, e.g. breeding (Note: none of the aquatic IAS of concern are migratory).		
5: Anxiety, fear, pain, distress, thirst, hunger etc.	In stream barriers will not lead to increased levels of anxiety, fear, pain, sickness, breathlessness, nausea, lethargy/ weakness etc., dizziness, greater than normal thirst and/or hunger or other negative affective experiences causing distress.		

Measure type (if applicable): Non-physical	**Humaneness impact categories**		
Domain	**No impact**	**Mild-Moderate**	**Severe - Extreme**
1: Water deprivation, food deprivation, malnutrition	All non-physical barriers should not lead to restrictions in the availability of food or water. However, there is the possibility that they may restrict the availability of food if their prey species is also affected by the barrier.		
2: Environmental challenge	All non-physical barriers that work as a deterrent stimuli (i.e. bubbles, light, acoustic) will not expose the animal to environmental conditions which are outside the normal range.	For chemical, electrical and velocity barriers fish will be exposed to short-term environmental conditions which are outside the normal range encountered by the animal.	
3: Injury, disease, functional impairment	All non-physical barriers that work as a deterrent stimuli will not expose the animal to disease, injury or functional impairment.	**Electric**. The over-exposure to the electric current causes fish to align perpendicular to the current flow (rheotaxis), allowing the current to sweep them downstream. However, the field produced by AC causes high levels of muscle contraction in the fish, resulting in immobilization [11]. Layhee et al. [22] found that passage through 30-Hz PDC voltage gradients (0.00-0.45 V cm) resulted in external bruising in 5 (7%) juvenile rainbow trout and 15 (21%) adult rainbow trout. Only three individual rainbow trout (two adults and one juvenile) were paralyzed within the barrier during experiments, and more adult rainbow trout with loss of equilibrium than juvenile rainbow trout. This suggests that acute exposure (20 s) may not elicit	**Ozone**. When the animal is exposed to non-lethal doses, it can be injurious to the peripheral tissues in adult and larval fish, and can cause gill lamellar clubbing, hypertrophy, and necrosis. High doses of O3 can also impair the oxygen binding capabilities of red blood cells [17].

		noticeable behavioral changes in Juveniles. **CO2**. CO2 exposure of *Lepomis macrochirus* to 30 mg/L-1 dissolved CO2 for 1 h, resulted in alterations to ionic– osmotic balance. Exposure of fish to 70 mg/L-1 CO2 caused a reduction in ventilation rates after 1 hour [15]. There is the potential of more severe impacts as fish can succumb to the anesthetic effect of hypercarbia exposure and lose equilibrium if they cannot avoid exposure to elevated CO2 over extended time periods [15].	
4: Behavioural, interactive restriction	All non-physical barriers that work as a deterrent stimuli will not interfere with the behavioural needs of the animal. In relation to strobe lights Kim and Mandrak [23] did not observe individual fish reacting strongly (e.g., jumping, turning swiftly) to avoid strobe lights, responses were more gradual and relatively slow.	**Electric**. See domain 3. **CO2**. Both sea lamprey adults and transformers displayed agitation (i.e. erratic swimming, elevated activity, and twitching) when concentrations of CO2 exceeded 40 mg/L and sea lamprey experienced loss of equilibrium at concentrations above 120 mg/L. [16]. CO2 exposure of *Lepomis macrochirus* to 30 mg/L-1 dissolved CO2 for 1 h, resulted in an elevated stress response[15]. However it is important to note that these may not be the conditions faced by the animals when this measures is applied in the field. **Ozone**. There is a sparsity of literature describing any behavioral or other minute physiological effects induced by elevated ozone concentrations [17].	

5: Anxiety, fear, pain, distress, thirst, hunger etc.	All non-physical barriers that work as a deterrent stimuli should not lead to anxiety, fear, pain etc.	**CO2** & **Electric** – CO2 and electric barriers have the potential to cause pain and distress if animals are exposed to high levels of CO2, i.e. if the animals do not, or cannot for some reason move away from areas of high CO2 or electric field.	**Ozone** - There is the potential for severe to extreme injury due to non-lethal exposure to high levels of ozone concentrations. However the exposure time and ozone concentrations that would result in this for IAS of Union concern is not known, and the possibility of this occurring in field application of the measures is not known.

3.2. Mode of death (if relevant)			
Measure type (if applicable): Non-physical barrier Ozone	**Immediate death (i.e. no suffering)**	**Not immediate death (mild - moderate suffering)**	**Not immediate death (severe - extreme suffering)**
Rationale:			Ozone is the only barrier intended to be lethal and it is very much in the development phase. Lethal concentrations vary considerably from species to species, and time to death may also change based on O3 concentrations. For example, 0.5 min exposure at 1.5mg/L results in 99% mortality of *Perca fluviatilis* juveniles and larvae, and 30 minutes exposure at 0.7mg/L results in 100% mortality of *Salvelinus leucomaensis* due to gill lamella degeneration [assumed asphyxiation]. In general measure only requires a short contact time [17].

3.3. Humaneness summary	Most 'types' of aquatic barriers, in particular physical barriers or non-physical barriers that work as a deterrent stimuli, have little or no humaneness impact upon the aquatic IAS of Union concern. However, there would be at least moderate impacts within the behavior domain if the species were migratory as they would be blocked from reaching breeding grounds – though it needs to be stressed that this would be the point of such barriers for migratory aquatic IAS (e.g. those applied to *P. marinus* in Great Lakes basin). Electric and CO2 barriers have the potential for more serious humaneness impacts, as they can lead to injury, and distress with prolonged exposure.

	Ozone is the only barrier that is intentionally lethal, and exposure to non-lethal doses can lead to serious injury, however evidence could not be found of its application in the field.

4. Costs and effectiveness of the measure	
General effectiveness of the measure	No case studies could be found of the measures application to the aquatic IAS of Union concern. However the measures effectiveness and related costs in general is presented here. **Physical barriers** – Physical in stream barriers are effective at blocking upstream movement of many species, however they are much less effective at blocking downstream migration especially for aquatic IAS that have life stages that drift on water currents [24]. In addition, lentic habitats upstream of dams may provide conditions for multiple point-source colonization and subsequent downstream spread [24]. In terms of permeable barriers applied on lentic habitats outflows to prevent spread of *P. parva*, there are no known documented examples of where this has been enacted at large spatial scales and been effective. However it represents a potentially effective measure at preventing the dispersal of individuals, especially those of small body sizes (<20 mm) [5]. According to Verreycken [6], fine mesh screens will certainly diminish the number of aquatic IAS specimens that escape from fish farms to enter via outflow waters into open waters. However, reliable separation of larvae and small-sized individuals of *P. glenii* and *P. parva* from departing water flows is a difficult task, and often only coarse meshed screens are applied to aquaculture ponds to prevent the escape of farmed fish, which does not prevent small bodied fish and fish larvae escaping to adjacent waters. **Non-physical barriers** – Based on a review by Noatch and Suski in 2012 [7], the primary drawback to any non-solid fish barrier is the <100% long-term effectiveness associated with virtually every barrier - such gasps could be spatial, particularly during periods of high water, or temporal, such as during power outages. This makes non-physical barriers suitable for deterrents, for example to reduce fish mortality at hydroelectric plants. However, each type of non-physical barrier is discussed below with reference to more recent application and research where available. *Acoustic* – For sound barriers to be effective, fish must be able to detect the frequency, localize the sound source, and stop or move away from the source [10]. In addition, the effectiveness of acoustic deterrent systems can be affected by bottom morphology and hydrology for example, low frequency sounds can propagate poorly in shallow water and across hard substrate [7]. A variety of frequencies and ultrasound have been tested as a deterrent against a number of species, for example Vetter et al. [10] tested the effectiveness of pure tones (0.5-2 kHz) and complex sounds (outboard motor recording, 0-10 kHz) to deter *H. molitrix* in concrete ponds. They found that fish habituated quickly to the pure tones, but that the complex sounds consistently directed fish to the opposite ends of the pond. *Electric* – While electrical fields are non-selective, the amount of energy transferred to the fish is dependent upon the species, size of the fish (small fish receive less energy than large) and the orientation of the fish in the field [1]. Horizontal bottom mounted electrodes are also weaker at the surface, and during floods the upper water column

may not be sufficiently electrified to block fish [25]. Pulsed DC fields generated by vertical electrodes may be more effective as they do not weaken with depth, and more versatile as they can be quickly deployed without significant stream modification allowing rapid response to new invasions [25]. Electric barriers are also more effective at blocking upstream rather than downstream migration, as fish can be stunned by the barrier and will then be carried by the current. Small fish may also be unaffected by the barriers [7]. The EDBS on the Chicago Sanitary and Ship Canal has been successful in that only a single adult silver carp was found upstream of the barrier in 2017, however the efficacy of the EBDS in preventing the movement of small juvenile individuals and the potential for tow mediated fish passage is being investigated [26]. Sabatini et al. [21] also report that an electric barrier in Sardinia has been successful in preventing the re-invasion of introgressed brown trout, apart from when the barrier was damaged by a lightning strike in 2013 which allowed individuals to re-invade. In an electric fish barrier scoping study for the US Dept. for Interior, Little et al. [11] reports that the electric barriers are only effective at blocking fish movement upstream in water with velocities between 0.6-3.0 m/s, and blocking downstream movement with velocities under 0.5 m/s. The faster the velocity the less likely it is that a fish will be able to swim across the barrier. Little et al. [11] also reports that to avoid high-maintenance costs, the electrodes should not be made out of a highly-corrosive material, and that due to erosion and electrolytic buildup on the electrodes, electrodes will eventually lose their effectiveness. Power outages also need to be considered, during the construction of the Central Arizona Project canal an electric barrier installed on two distributary canals to prevent movement of non-indigenous fish power outages totaled 100 hours (between 1988-2000) which represents jut 0.001% downtime, however it is nearly certain that this allows the immigration of undesired fishes [27].

Ozone. According to a review by Buley et al. [17]the use of dissolved ozone has the potential to be used as an effective (lethal) barrier to prevent the movement of fishes, and other aquatic organisms as it is lethal to a wide range of aquatic taxa (for example it is currently used for sterilization of ballast waters). The effects of ozone on some fish species have been studied, including for *L. macrochirus* where 0.13-0.17 mg/L O3 for 0.25 minutes resulted in 50% mortality of larvae and eggs, *Perca flavescens* 1.2 mg/L O3 for 0.5 minutes results in 99% mortality of larvae and eggs, and *Pimaphales promelas* <0.1 mg/L O3 for 0.5 minutes results in 50% mortality of larvae and eggs. However, more research is needed on individual species responses to ozone and methods for generating substantial ozone in the field.

Strobe lights. Strobe-light deterrent systems effectiveness varies according to the target species, design and brightness of the lights, turbidity, and ambient light level, and as a stand-alone method of deterrence is unlikely to provide an effective deterrence system [7].

Bubbles. Bubble curtains on their own have limited potential as a barrier to movement, as they rely upon being seen by the fish and are therefore less effective in turbid water. However, there is potential for their application in combination with other measures, e.g. acoustic deterrents [7], velocity barriers [14].

Velocity barriers. To be effective velocity barriers must consistently provide water flow velocity in excess of the target fish aerobic swimming capacity or power output, and ideally a channel length greater than the distance it can cover in an anaerobic burst [7]. An experimental velocity barrier was tested in 1993 for *P. marinus* in Ontario and while initial reports indicated success, passage was observed within a year [1]. Kang et al. [14] tested a velocity

	barrier on *L. macrochirus*, in experimental conditions (0.7 m deep, three flow velocities 0.2 m/s, 0.1 m/s and 0.05 m/s) and found that the barrier elicited avoidance from most of the fish. *Carbon dioxide*. Whereas electrical barriers effectiveness is size (of the fish)dependent, gas barriers have the potential to be effective across all sizes once a particular threshold is reached, and the treatment zone can be extended so it is hypothetically impossible to cross [7]. The dissolved CO2 concentrations that result in the target species showing avoidance and losing equilibrium (inability to stay upright in the water column) needs to be identified. Kates et al. [28] found that *L. macrochirus* exposure to 30 mg/L dissolved CO^2 for 1 hour resulted in an elevated stress response, 70 mg/L dissolved CO^2 for 1 hour resulted in a reduction in ventilation rates, and that the species showed avoidance at approximately 100 mg/L dissolved CO^2. Dennis et al. (2015) found that for the same species showed avoidance at approximately 200 mg/L, and also that CO2 is effective at deterring the movement of juvenile and larval fishes. They conclude that A CO2 barrier should be effective at deterring fish movement across species and life-stage. *Pheromones*. In application to fish, it has only been applied as baited traps to attract and trap female sea lampreys and more research is needed into the measures effectiveness and application before it can be considered as a non-physical barrier [7].

4.1. Case studies	
CASE STUDY #1	
Measure type (if relevant):	
Species:	
Objective:	
Use of measure	
Combined with other measure(s):	
Country(ies) of application:	
Geographic scale (km^2) and/or population size measure applied to:	
Time period:	
Effort:	
Costs:	Overall costs:
	Personnel costs:

	Equipment and infrastructure:	
	Other, including overheads:	
Effectiveness:		
References:		

4.2. Costs effectiveness summary	As no case studies can be mobilized for the aquatic IAS of Union concern a cost effectiveness summary for the species cannot be undertaken. In general non-physical aquatic barriers are most effectively used as deterrents of fish away from infrastructure such as hydropower dams or power plants intakes, where 100% effectiveness is not a critical issue. However, their development and use for the containment of spread of aquatic IAS is growing (especially in North America), and while they may still not be 100% effective especially when used in isolation, ongoing research is improving their effectiveness and new technologies are being developed. They may also be used to support eradication measures, to reduce the likelihood of re-invasion.

5. Side effects	
Non-target native species, their habitats and the broader environment:	Positive:
	Non-physical barriers – Not necessarily a positive impact but rather neutral, is the fact that non-physical barriers do not affect water flow regimes, and some can be applied to target specific taxa.
	Negative:
	Physical barriers – Permanent in stream barriers will likely block the movement of native fishes or other aquatic species, this is particularly damaging for species that migrate or require natural dispersal. Barriers also lead to numerous physical and chemical changes to the river, including changing sediment loads, flow regimes, water temperature and dissolved oxygen levels. **Non-physical barriers** – Many types, such as electric barriers are non-species specific and will block native and alien species alike, and permanent installations are likely to be unsuitable for river systems that provide significant migratory routes for native species [9]. However, the impact of electric barriers on non-target, migratory native species is unknown [22]. Ozone creates bromides when added to seawater, so if ozone is used in any brackish or estuary area, even if leading to freshwater, the generation of bromides could be a problem [17]. Any non-physical barrier that alters abiotic variables (e.g. carbon dioxide, Ozone) will also affect all other aquatic species exposed including being lethal [7,15,17]. In addition, carbon dioxide introductions in

	water will reduce the pH of a target system, and changes in pH will likely alter the solubility of metals and other constituents in water [29]. However the use of these systems in containable areas with minimal connections to the surrounding ecosystem, such as in lock systems or shipping canals, may reduce their environmental impacts [17].
Other invasive alien species:	Positive:
	Physical barriers – Physical barriers will block most aquatic species, native and IAS.
	Negative:
Public health and well-being:	Positive:
	Negative:
	Non-physical barriers – In relation to ozone barriers, some O3 will off-gas during its addition to water, creating the potential for human (occupational and incidental) exposure, which could cause a human health hazard as ozone is a respiratory irritant [17]. Future users of CO2 must also carefully consider impacts to human health and safety during applications. The Occupational Safety and Health Administration lists CO2 as an indoor air contaminant [29]. The application of electricity barriers poses some potential risk to human safety; however, modern electrical barrier systems are designed for safe operation. Current barriers use direct current which is safer for humans and fish and there have been no reports of serious injury or fatalities in humans resulting from electric fish barriers [1].
Economic:	Positive:
	Non-physical barriers - Most non-physical barriers can be custom designed and effectively positioned within water ways without posing a restriction to navigation or impounding water flow [7].
	Negative:
	Physical barriers – A direct impact of physical barriers is the modification of water flow and interaction with debris and boats navigating in the water way [1]. Permeable mesh screens on the outlets of aquaculture ponds can be responsible for flooding the ponds when they get blocked by debris. In addition when fine mesh screens are used (which are more effective at preventing escape for larvae) for preventing escape during the draining of aquaculture ponds to harvest fish it can lead to higher fish mortality and increased predation rates by birds, increased labour costs and time loss leading to economic losses [6]. **Non-physical barriers** - An issue is the ongoing running and maintenance costs, for example for those barriers that require significant power (e.g. electrical barriers), or production of gases (e.g. ozone).

6. Conclusion

Overall assessment of the measure (qualitative)

Physical barriers, especially temporary ones, that are specifically constructed for the containment of aquatic IAS have a role to play in certain situations, for example to block migratory passage. However, while they may be effective in preventing spread of aquatic IAS, they have significant side effects upon hydrological regimes and water chemistry, native biodiversity especially migratory species, and can block navigation.

Non-physical barriers have fewer negative side effects particularly on the environment, and can be used without blocking flow and navigation, but are not yet 100% effective, especially when used in isolation. They are still mostly used as a deterrent to stop fish entering intakes for power plants etc., but may also be used to support eradication campaigns to prevent reinvasion. However new research, and the development of new methods (e.g. CO_2 and Ozone) show promise. The most successful of the established non-physical barriers is the electric barriers (PDC), such as EDB on the Chicago ship canal which has so far been successful at preventing the movement of silver carp, however its effectiveness at blocking juvenile individuals which have been recorded closer to the barriers, has yet to be tested. In addition, an electrical barrier has been shown to be effective at preventing re-invasion of introgressed brown trout in a stream in Sardinia (Sabitini et al. 2018). For most non-physical barriers, especially those that work as a deterrent stimuli (light, acoustic, and bubbles), there are little to no negative humane impacts, however electric and CO_2 can potentially lead to injury and distress if the fish cannot move away from the barrier. Ozone is the only measure that is intentionally lethal and it is assumed that the fish die through asphyxiation. There are no case studies that could be found for non-physical barrier use for the aquatic IAS of Union concern, but there is no reason why they could not be applied to them. In addition, non-physical barriers have ongoing running and maintenance costs, and malfunction, damage or human error can reduce their effectiveness.

Assessor:	Kevin Smith
Reviewer 1:	Riccardo Scalera
Reviewer 2:	Sandro Bertolino

7. References

[1] Zielinski, D. P., McLaughlin, R., Castro-Santos, T., Paudel, B., Hrodey, P., & Muir, A. (2019). Alternative Sea Lamprey Barrier Technologies: History as a Control Tool. Reviews in Fisheries Science & Aquaculture, 27(4), 438-457. DOI:10.1080/23308249.2019.1625300

[2] McLaughlin, R. L., Smyth, E. R. B., Castro-Santos, T., Jones, M. L., Koops, M. A., Pratt, T. C., & Velez-Espino, L.-A. (2012). Unintended consequences and trade-offs of fish passage. *Fish & Fisheries*, 14: 580–604. https://doi.org/10.1111/faf.12003

[3] Scalera, R., Cozzi, A., Caccamo, C., & Rossi, I. (2017). A catalogue of LIFE projects contributing to the management of alien species in the European Union. Platform Meeting on Invasive Alien Species (IAS).

[4] LIFE. Undated. Laymans report. LIFE11Nat/IT/188 Con.Flu.Po. Restoring connectivity in Po River Basin opening migratory route for *Acipenser naccarii* and 10 fish species in Annex II.

[5] Britton, R. (2019). Information on measures and related costs in relation to species included on the Union list: *Pseudorasbora parva*. Technical note prepared by IUCN for the European Commission.

[6] Verreycken, H. (2019). Information on measures and related costs in relation to the species included on the Union list: Perccottus glenii. Technical note prepared by IUCN for the European Commission.

[7] Noatch, M. R., & Suski, C. D. (2012). Non-physical barriers to deter fish movements. *Environmental Reviews*, 20(1), 71-82. https://doi.org/10.1139/a2012-001

[8] Zielinski, D. P., & Sorensen, P. W. (2017). Silver, bighead, and common carp orient to acoustic particle motion when avoiding a complex sound. *PLOS One*, 12(6): e0180110. https://doi.org/10.1371/journal.pone.0180110
[9] Britton, J. R., Gozlan, R. E., & Copp, G. H. (2011). Managing non-native fish in the environment. *Fish and Fisheries*, DOI: 10.1111/j.1467-2979.2010.00390.x
[10] Vetter, B. J., Cupp, A. R., Fredricks, K. T., Gaikowski, M. P., & Mensinger, A. F. (2015). Acoustical deterrence of Silver Carp (*Hypophthalmichthys molitrix*). Biological Invasions, 17:3383-3392
[11] Little, D. (2016). Effect if electric fish barriers on corrosion and cathodic protection. Research and Development Office Science and Technology Programme. U.S. Department of the Interior Bureau of Reclamation
[12] Nehring, S. & Steinhof, J. (2015). First records of the invasive Amur sleeper, *Perccottus glenii* Dybowski, 1877 in German freshwaters: a need for realization of effective management measures to stop the invasion. *BioInvasions Records*, 4(3),223–232 doi: http://dx.doi.org/10.3391/bir.2015.4.3.12
[13] Reshetnikov, A. N., & Karyagina, A. S. (2015). Further Evidence of Naturalisation of the Invasive Fish Perccottus glenii Dybowski, 1877 (Perciformes: Odontobutidae) in Germany and Necessity of Urgent Management Response. *Acta Zoologica Bulgarica*, 67(4), 553-556.
[14] Kang, J-G., & Kim, J-T. (2017). Analysis on the Bluegill Blocking Effects using Bubbles. *Journal of the Korea Academia-Industrial Cooperation Society*, 18(2), 390-397.
[15] Dennis III, C. E., Adhikari, S., & Suski, C. D., (2015). Molecular and behavioral responses of earlylife stage fishes to elevated carbon dioxide. Biological Invasions, 17, 3133–3151. DOI ttp://dx.doi.org/10.1007/s10530-015-0941-0.
[16] Dennis III, C. E., Wright, A. W., & Suski, C. D., (2016). Potential for carbon dioxide to act as a non-physical barrier for invasive sea lamprey movement. Journal of Great Lakes Research, 42, 150-155. DOI http://dx.doi.org/10.1016/j.jglr.2015.10.013
[17] Buley, R. P., Hasler, C. T., Tix, J. A., Suski, C. D., & Hubert, T.D. (2017). Can ozone be used to control the spread of freshwater Aquatic Invasive Species? *Management of Biological Invasions,* 8(1), 13-24. DOI: https://doi.org/10.3391/mbi.2017.8.1.02
[18] Carosi, A., Ghetti, L., & Lorenzoni, M. (2016). Status of *Pseudorasbora parva* in the Tiber River Basin (Umbria, central Italy) 20 years after its introduction. Knowledge and Management of Aquatic Ecosystems, 417, 22. DOI: 10.1051/kmae/2016009
[19] Kottelat, M. & J. Freyhof. (2007). Handbook of European freshwater fishes. Publications Kottelat, Cornol and Freyhof, Berlin. 646 pp.
[20] Zogaris, S. (2017c). Information on measures and related costs in relation to species considered for inclusion on the Union list: *Lepomis* spp. Technical note prepared by IUCN for the European Commission.
[21] Sabatini, A., Podda, C., Frau, G., Cani, M. V., Musu, A., Serra, M., & Palmas, F. (2018). Restoration of native Mediterranean brown trout *Salmo cettii* Rafinesque, 1810 (Actinopterygii: Salmonidae) populations using an electric barrier as a mitigation tool. *The European Zoological Journal*, 85(1), 137-149, DOI: 10.1080/24750263.2018.1453554
[22] Layhee, M. J., Sepulveda, A. J., Shaw, A., Smuckalla, M., Kapperman, K., & Reyes, A. (2016). Effects of electric barrier on passage and physical condition of juvenile and adult rainbow trout. *Journal of Fish and Wildlife Management,* 7(1), 28-35; e1944-687X. doi: 10.3996/042015-JFWM-039
[23] Kim, J., & Mandrak, N. E. (2017). Effects of vertical electric barrier on the behaviour of common carp. *Management of Biological Invasions,* 8 (4), 497–505.
[24] Tummers, J. S. & Lucas, M. C. (2019). T4.2.2. Role of barriers in managing aquatic invasive species. Amber International.
[25] Johnson, N. S., Thompson, H. T., Holbrook, C., & Tix, J. A. (2014). Blocking and guiding adult sea lamprey with pulsed direct current from vertical electrodes. *Fisheries Research*, 150, 38-48. http://dx.doi.org/10.1016/j.fishres.2013.10.006
[26] LeRoy, J. Z., Davis, J. J., Shanks, M. R., Jackson, P. R., Murphy, E. A., Baxter, C. L., Trovillon, J. C. and McInerney, M. (2019). Efficacy of increasing discharge to reduce tow-mediated fish passage across an electric dispersal barrier system in a confined channel. *Journal of Great Lakes Research,* 45, 1320–1331. https://doi.org/10.1016/j.jglr.2019.08.007
[27] Clarkson, R. W. (2002). Effectiveness of Electrical Fish Barriers Associated with the Central Arizona Project.North American Journal of Fisheries Mangement, 24(1),94-105. https://doi.org/10.1577/M02-146
[28] Kates, D., Dennis, C., Noatch, M. R., & Suski, C. D. (2012). Responses of native and invasive fishes to carbon dioxide: potential for a nonphysical barrier to fish dispersal. Canadian Journal of Fisheries and Aquatic Sciences, 69, 1748–1759.
[29] Treanor, H. B., Ray, A. M., Layhee, M., Watten, B. J., Gross, J. A., Gresswell, R. E., & Molly, A. H. Webb. (2017). Using Carbon Dioxide in Fisheries and Aquatic Invasive Species Management, *Fisheries*, 42(12), 621-628, DOI: 10.1080/03632415.2017.1383903

Appendix 3. Aquatic habitat management - Pond drying/draining

1. Measure name	
1.1. English	Aquatic habitat management - Pond drying/draining
1.2. Lethal or non-lethal:	Lethal or non-lethal, depending on how the measure is applied
1.3. Other languages (if available):	

Bulgarian	Регулиране на водните местообитания – пресушаване/ отводняване на стоящи водоеми	Italian	Gestione dell'habitat acquatico - Essiccazione / drenaggio dello stagno
Croatian	Upravljanje vodenim staništima – ispuštanje/isušivanje ribnjaka	Latvian	Ūdens dzīvotņu apsaimniekošana – dīķu nosusināšana / ūdens novadīšana
Czech	Management vodního prostředí – vypuštění/vysušení rybníka	Lithuanian	Vandens buveinių tvarkymas – tvenkinių vandens nuleidimas, sausinimas
Danish	Forvaltning af vandmiljøer – udtørring/dræning af søer	Maltese	
Dutch	Beheer van aquatisch habitat - droogleggen van vijvers	Polish	Zarządzanie siedliskami wodnymi – osuszanie/opróżnianie stawów
Estonian	Vee-elupaikade muutmine - tiikide kuivendamine	Portuguese	Gestão do habitat aquático - drenagem de corpos de água
Finnish	Vesiympäristön hoito – Lammen kuivatus	Romanian	Gestionarea habitatelor acvatice – uscare/drenare corpuri de apă
French	Gestion de l'habitat aquatique - drainage des étangs	Slovak	Manažment vodného prostredia – vypustenie/odvodnenie/vysušenie rybníka
German	Teichentleerung und - trockenlegung	Slovenian	Upravljanje z vodnim habitatom - izsuševanje vodnih teles
Greek	Διαχείριση υδάτινων ενδιαιτημάτων-Ξήρανση/αποστράγγιση	Spanish	Gestión del hábitat acuático – desecación, drenaje de zonas húmedas
Hungarian	Vizes élőhelyek kezelése – Tavak kiszárítása/lecsapolása	Swedish	Torrläggning av vattendrag
Irish			

2. Technical details of measure
2.1.a. Measure description
Pond draining or dewatering, which consists of completely emptying a water body by removing all its water, is a technique commonly used in the management of aquatic invasive alien species (IAS), both plants and animals (although this assessment is focused on animals). Drawdown is a similar

technique, by which the water is removed only to a given depth for an extended period of time, but is mainly used for the management of aquatic plants [3, 25] or for improving fisheries [24]. Before considering the use of pond draining, it is important that the hydrology of the water body is investigated, in order to confirm that there are no underground springs, land drainage issues or seepage that could cause rapid refilling [1, 2]. As such, draining is limited to water bodies that are isolated, that have no significant inflows or where existing ones can be diverted, namely ponds, reservoirs and man-made lakes.

Through this process, water is pumped out of a water body mechanically using anything from small portable pumps to large industrial pumps, and filtered with a variety of inlet and outlet screenings before being released into nature (preferably onto dry land well away from water bodies), to avoid animals spreading into other water bodies [1, 3, 4, 5]. After all, or the majority of, the water has been pumped out, all the animals remaining should be removed and different actions can follow, depending on the target species or habitat to be treated. For example, the entire bottom mud layer and all vegetation of the water body can be mechanically removed down to the mineral substrate, for instance by using an excavator with a flat shovel [1, 9], or the remaining mud can be covered with a layer of sand [10]. This is to avoid any risk of the target species (or their eggs) surviving, especially for turtle species such as the pond slider ***Trachemys scripta*** that can burrow deep into the silt at the bottom of the water body, and for fish species that can tolerate low oxygen levels and are resistant to desiccation, as is the case of the Amur sleeper ***Perccottus glenii***, which is able to survive in the mud of dried out or completely frozen water bodies [4, 6, 9]. In order to avoid the removed mud flowing back into the water body with rain or snowmelt, it should be placed and levelled far away from it. To ensure complete removal of the target species, the water body should stay empty in dry weather conditions for several weeks following the draining operations take place and be refilled naturally [4, 9]. However, operational delays such as inclement weather and other unforeseen circumstances, might complicate this or delay the effectiveness of the activities [1].

Ideally, non-target species should be removed from the water body prior to draining and kept elsewhere until the management actions are complete. Because of the potential strong negative impacts of this method on non-target aquatic and semi-aquatic species (see side effects section below), it is important to assess the overall consequences of this process on the whole species community before applying it to the water body [9].

Regarding the aquatic vertebrate IAS of Union concern, this measure has been applied in the management of five of those species:

The pumpkinseed ***Lepomis gibbosus*** has been successfully eradicated from a moorland in the Netherlands by draining the pools, removing all the fish with seine nets and subsequently covering the remaining mud with a layer of sand in order to kill the last fishes and reduce the depth of the water body, thereby changing it from a permanent into a temporary pool [10]. In Japan, pond managers have been using pond draining to eradicate non-native fish for many years, such as bluegill (*Lepomis macrochirus*), but the technique does not seem to be entirely effective in controlling this species [11].

Given that breeding populations of the American bullfrog, ***Lithobates catesbeianus***, have been found to disappear following natural pond drying [12], draining of water bodies at least every two years has been suggested as an effective management strategy for largely reducing populations of this species [13]. Although these operations are targeted at removing all life stages of the species, they are mostly effective for the stages dependent on permanent waters (eggs and tadpoles), given that almost 30% of bullfrog adults hibernate outside of the water, under leaves or in burrows [5]. Indeed, draining has been used to eradicate or control populations of ***L. catesbeianus*** in Germany [14, 15], France [5, 16], Belgium [8, 17], the Netherlands [18], the UK [19] and the USA [20].

In Valdayskiy National Park, Russia, an isolated population of ***P. glenii*** was successfully eradicated by drying the water body followed by processing of the substrate with chemicals [21]. In Belgium, draining followed by application of lime chloride to kill remaining life has been suggested for the eradication of ***P. glenii*** from two small isolated natural ponds [8].

Several cases of successful eradication of ***Pseudorasbora parva*** by dewatering water bodies have also been reported in the UK. ***Pseudorasbora parva*** were successfully eradicated from two adjacent small fishing lakes in West England through dewatering followed by quicklime application to the lake bed and from one pond close to London through draining followed by de-silting [22].

Draining invaded waterbodies can also be used for localised management of ***T. scripta*** populations. This has been successfully implemented in Australia, where a series of water bodies invaded with ***T. scripta*** were drained, de-silted using an excavator, filled and compacted; the muck was spread, turned and raked with a tractor, and all remaining animals were removed by hand [6, 23].

While pond drainage can be successfully used to eradicate fish populations from localised areas, in terms of management objectives as defined by the EU IAS Regulation the measure is unlikely to be effective for 'eradication' i.e. the complete and permanent removal of a widespread population from a Member State, but rather be applied for rapid eradication of a population at an early stage invasions (i.e. one or a few localities) or to support control or containment of a widespread population. However, the measures could be applied to support the eradication of ***T. scripta*** in areas where it can't breed.

2.1.b. Integration with other measures

For the control of amphibian and reptile species, prior to draining, barriers and pit fall traps need to be set up around the entire water body, in order to ensure that the targeted species cannot leave the area [6, 7, 8]. This measure has also been followed by seine netting to capture the remaining animals in the mud or by processing of the substrate with chemicals [1, 8, 21]. More generally, draining has also been used as part of integrated pest management protocols, together with a suite of other management techniques such as hand removal, trapping, netting, spearing, shooting and electrofishing [5, 6, 8, 20, 22].

Following the removal of the target aquatic IAS they will need to be dispatched humanely [1].

2.2.a. Availability - species and objectives

Objective	Unknown objective		Rapid Eradication		Management					
					Eradication		Control		Containment	
Species	Avail.	Ref(s).	Avail.	Ref(s).	Avail.	Ref(s).	Avail.	Ref(s).	Avail.	Ref(s).
Acridotheres tristis										
Alopochen aegyptiaca										
Callosciurus erythraeus										
Corvus splendens										
Herpestes javanicus										
Lepomis gibbosus	A		A	10, 11			A	37	P	
Lithobates catesbeianus	A		A	5, 8, 14, 15, 16, 18, 19, 20			A	17, 36	A	
Muntiacus reevesi										
Myocastor coypus										
Nasua nasua										

Nyctereutes procyonoides										
Ondatra zibethicus										
Oxyura jamaicensis										
Percottus glenii	P		A	8, 21			A		P	
Plotosus lineatus										
Procyon lotor										
Pseudorasbora parva	P		A	1, 8, 22			A	34	A	
Sciurus carolinensis										
Sciurus niger										
Tamias sibiricus										
Threskiornis aethiopicus										
Trachemys scripta	P		P		A	6	A	35	P	

2.2.b. Application – EU Member States and objectives										
Objective	**Unknown objective**		**Rapid Eradication**		**Management**					
					Eradication		**Control**		**Containment**	
Country	Avail.	Ref(s).	Avail.	Ref(s).	Avail.	Ref(s).	Avail.	Ref(s).	Avail.	Ref(s).
Austria										
Belgium							X	8, 17		
Bulgaria										
Croatia										
Cyprus										
Czech Republic										
Denmark										
Estonia										
Finland										
France							X	5, 16		
Germany							X	14, 15, 34, 35, 36		
Greece										
Hungary										
Ireland										
Italy										
Latvia										
Lithuania										
Luxembourg							X	37		
Malta										
Netherlands							X	10, 18		

Poland										
Portugal										
Romania										
Slovakia										
Slovenia										
Spain										
Sweden							X	33		
United Kingdom*			X	1			X	19, 22		

* Not an EU Member State

3. Humaneness of the measure			
3.1. Welfare for all measures			
Measure type (if applicable): Pond drying/draining	**Humaneness impact categories**		
Domain	**No impact**	**Mild-Moderate**	**Severe - Extreme**
1: Water deprivation, food deprivation, malnutrition		Depending on how long it takes for the target species to be removed from the water body during/after draining, animals can experience periods without water and food.	
2: Environmental challenge			Depending on how long it takes for the target species to be removed from the water body during/after draining, the potential acute exposure to air and lack of water places aquatic animals far outside of their normal environmental conditions, which can seriously compromise their health and even lead to un-intended death.
3: Injury, disease, functional impairment			Following pond draining, aquatic animals can experience severe stress responses induced by air exposure and lack of water, which can cause functional impairment

			and impact their growth and survival [26, 27].
4: Behavioural, interactive restriction		In the absence (or very low levels) of water, aquatic animals are not able to swim or hide anymore, which induces escape behaviours [26]. Freshwater turtles (***T. scripta***) respond to water drying by first displaying stress avoidance mechanisms such as migratory behaviours and increased secreted corticosterone, and later by digging into the mud [6, 28].	
5: Anxiety, fear, pain, distress, thirst, hunger etc.		If animals are removed quickly from water body (for dispatch/relocation) during drainage, the animals should experience moderate levels of stress and human contact with minimum physical handling.	In cases where the animals are not removed from drained ponds or left outside of the water for a considerable amount of time, the lack of water and extreme exposure to air can be highly aversive, ultimately inducing loss of reaction, unconsciousness, loss of movement and eventually death [26].

3.2. Mode of death (if relevant)			
Measure type (if applicable): Pond drying/draining	**Immediate death (i.e. no suffering)**	**Not immediate death (mild - moderate suffering)**	**Not immediate death (severe - extreme suffering)**
Rationale:			The measure is lethal in cases where the target species is left in the fully drained pond for an extended period of time. In this case, the lack of water and extreme exposure to air will be highly aversive and incur a slow death to aquatic animals.

3.3. Humaneness summary	Depending on how long after draining, and under which circumstances, the target species is removed from the water body, this measure can expose aquatic animals to extreme conditions, i.e. acute exposure to air and lack of water. If this is the case, this is highly aversive and can ultimately result in death. As such, when applying this measure, it is important to ensure that all target aquatic animals are removed from the water body before it is completely devoid of water and later euthanized appropriately, in order to guarantee that the measure is applied in a humane way.

4. Costs and effectiveness of the measure	
General effectiveness of the measure	Draining is the only known method that can effectively guarantee the complete eradication of aquatic species (present in a confined area) without resorting to the single use of toxins [29] (although chemicals are sometimes applied after draining to guarantee eradication). Especially if the water body is drained in several consecutive years, the success of the management operation is likely to be high [7]. However, draining can only be applied in limited circumstances, often with an increased investment of money and time than chemical application, and a possible decrease in eradication certainty [1, 4]. To date, its successful application has been limited to relatively small sites, such as a 1,000 m^2 water body in Flanders [32], whereas in large areas, such as wetlands, localised and periodical pond drying will have no real effect on populations of aquatic species [30].

4.1. Case studies	
CASE STUDY #1	
Measure type (if relevant):	Draining
Species:	*Pseudorasbora parva*
Objective:	A rapid eradication operation was designed to eliminate both the topmouth gudgeon and non-native parasites found at two adjacent small fishing lakes located in the West Midlands of England, which are adjacent to, and discharge via pipes into, a small tributary of the River Teme.
Use of measure	The lakes were dewatered using mechanical pumps, all water was passed through a series of fine nets and finally discharged over agricultural land. During dewatering, all fish were removed and humanely destroyed. An extensive layer of silt was mechanically removed from the bottom of the water bodies and the lake beds were allowed to dry out.
Combined with other measure(s):	The measure was combined with the application of quicklime to the dried lake bed to raise pH to lethal levels in the sediment to eliminate any remaining life stages of the parasites to be removed.
Country(ies) of application:	United Kingdom (West Midlands)
Geographic scale (km^2) and/or population size measure applied to:	6825 m^2 of area treated
Time period:	The operations took place from February to August 2006.

Effort:	Dewatering operations and quicklime application over the course of 7 months.
Costs:	Overall costs:
	The total cost of the operation was £50,800, with a cost of £7.9 per m^2.
	Personnel costs:
	Equipment and infrastructure:
	Other, including overheads:
Effectiveness:	The populations were eradicated and 96 km of river were protected from species dispersal.
Reference	1

CASE STUDY #2	
Measure type (if relevant):	Draining
Species:	*Lithobates catesbeianus*
Objective:	Eradication of the species from five ponds where it had successfully reproduced for several years.
Use of measure	The five ponds were electronically fished and pumped out twice each year, with no further details reported.
Combined with other measure(s):	The measure was combined with the use of electrofishing.
Country(ies) of application:	Germany (Karlsruhe)
Geographic scale (km^2) and/or population size measure applied to:	Not reported.
Time period:	From 2001 to 2004 eradication measures were carried out each year.
Effort:	From 2001 to 2004 the five ponds were electronically fished and pumped out twice each year, with the help of 20 volunteers and the local fire department.
Costs:	Overall costs:
	Annual cost of 53,000 EUR per pond per year, thus for five ponds 270,000 EUR annually, and a grand total of 1 million EUR.
	Personnel costs:
	20 volunteers, working occasionally over the course of a year, are roughly the equivalent of one full-time employee, hence 50,000 EUR.

	Equipment and infrastructure:	
	Costs for the draining were 500 EUR/day and to for the electrofishing 1,200 EUR/day.	
	Other, including overheads:	
Effectiveness:	More than 15,000 tadpoles, about 8,000 juveniles and 196 adults were captured and removed from the area. Spread of the population was prevented and the success of the eradication measures was still being discussed, but there have been no further reports of the species in the area.	
Reference	14, 15	

4.2. Costs effectiveness summary	The draining of water bodies is a costly operation, involving large amounts of resources, often logistically difficult and prone to unexpected delays. Depending on the scale of the area to be treated and the species targeted for management, activities can either be performed only once or need to be sustained over several years, which will influence the total costs of the operations. In any case, it is a very effective technique to manage aquatic IAS, which acquires special relevance when eradication of a species without resorting to the use of toxins is the ultimate goal.

5. Side effects	
Non-target native species, their habitats and the broader environment:	Positive:
	Negative:
	Pond draining can have significant environmental effects on non-target species that are intolerant of desiccation or low water levels. However, if all or the most susceptible animals present are retrieved from the water body prior to the activities, translocated and later released again, the negative effects can be largely minimised. For example, for rare amphibian populations that are usually small and reproduce annually in isolated water bodies, adult specimens can be retrieved from the water body, temporarily kept ex situ and returned after the operations are finalised [9]. Fish species present in the water body can also be caught and removed, e.g. using seine nets, prior to draining activities [2, 5]. If this is not possible, activities should be performed during the time of the year during which a lower number of native species are active or vulnerable (e.g. avoid amphibians' reproductive season) [8]. In any case, where meta-populations of native aquatic and semi-aquatic organisms occur, temporary unavailability of water is not expected to largely impact their populations and natural recolonization of the water body can take place after the management activities are finalised [9].
Other invasive alien species:	Positive:
	Draining may allow for several aquatic invasive alien species, including plants and animals, to be managed at the same time.

	Negative:	
	Draining can facilitate the colonisation of farm ponds by species such as the red swamp crayfish (*Procambarus clarkii*), which in turn may have detrimental effects on the biodiversity and water quality of ponds [11]. Drawdown may provide an opportunity for highly weedy or adventive plant species, particularly annuals, to spread further [3].	
Public health and well-being:	Positive:	
	Negative:	
	Draining can severely interfere with the recreational use of water bodies [3] and often raise public concern due to the unpleasant aesthetics of the water body, disruption of the aquatic habitat and potential destruction of non-target fauna and flora [29, 31].	
Economic:	Positive:	
	Negative:	
	Draining can cause significant economic problems, by severely interfering with the intended functions of a specific water body, such as electricity or power generation, drinking water supply or their use for recreational fisheries [1, 3].	

6. Conclusion	
Overall assessment of the measure (qualitative)	
In conclusion, draining invaded water bodies is an effective tool for the management of aquatic vertebrate IAS. Although more costly than only using chemical application, it is the only other method that can warrant the complete eradication of aquatic species from specific localised areas. Provided that good practices are implemented in order to avoid unnecessary suffering of the target animals and that negative impacts on non-target native species are carefully managed, this measure can prove very useful and effective for the management of aquatic vertebrate IAS. The measure is, however, only appropriate and effective in small enclosed waterbodies and in areas where it is acceptable to use highly disruptive methods (e.g. sites with low nature conservation value). As such, before the measure is undertaken, it is of utmost importance to take into consideration both the suitability and susceptibility of the area and of the aquatic ecosystem in general, to maximise effectiveness and minimise non-desired potential impacts in the community.	
Assessor:	Ana Nunes
Reviewer 1:	Pete Robertson
Reviewer 2:	Riccardo Scalera

7. References

[1] Britton J.R., Brazier M., Davies G.D., Chare S.I. (2008). Case studies on eradicating the Asiatic cyprinid *Pseudorasbora parva* from fishing lakes in England to prevent their riverine dispersal. Aquatic Conservation: Marine and Freshwater Ecosystems 18(6): 867-876.

[2] Britton J.R., Gozlan R.E., Copp G.H. (2011). Managing non-native fish in the environment. Fish and Fisheries 12(3): 256-274.

[3] Madsen J.D. (2000). Advantages and disadvantages of aquatic plant management techniques. ERDC/EL MP-00-1, U.S. Army Engineer Research and Development Center, Vicksburg, MS.

[4] Collier K.J., Grainger N.P.J. (eds) (2015). New Zealand Invasive Fish Management Handbook. Lake Ecosystem Restoration New Zealand (LERNZ; The University of Waikato) and Department of Conservation, Hamilton, New Zealand. 212 p.

[5] Sarat E., Mazaubert E., Dutartre A., Poulet N., Soubeyran Y. (2015). Invasive alien species in aquatic environments. Practical information and management insights. Volume 2. Management insights. Onema. Knowledge for action series. 252 pages.

[6] O'Keeffe S. (2005). Investing in conjecture: eradicating the red-eared slider in Queensland. Proceedings of the 13th Australasian Vertebrate Pest Conference, Wellington, New Zealand, 169-176.

[7] Sarat E., Mazaubert E., Dutartre A., Poulet N., Soubeyran Y. (2015). Invasive alien species in aquatic environments. Practical information and management insights. Volume 1. Practical information. Onema. Knowledge for action series. 252 pages.

[8] Adriaens T., Branquart E., Gosse D., Reniers J., Vanderhoeven S. (2019). Feasibility of eradication and spread limitation for species of Union concern sensu the EU IAS Regulation (EU 1143/2014) in Belgium. Report prepared in support of implementing the IAS Regulation in Belgium. Institute for Nature and Forest Research, Service Public de Wallonie, National Scientific Secretariat on Invasive Alien Species, Belgian Biodiversity Platform.

[9] De Vries W., Rannap R., Briggs L. (2012). Guidelines for eradication of invasive alien aquatic species. Project Report "Securing *Leucorrhinia pectoralis* and *Pelobates fuscus* in the northern distribution area in Estonia and Denmark". LIFE08NAT/EE/000257.

[10] van Kleef H., van der Velde G., Leuven R.S.E.W., Esselink H. (2008). Pumpkinseed sunfish (*Lepomis gibbosus*) invasions facilitated by introductions and nature management strongly reduce macroinvertebrate abundance in isolated water bodies. Biological Invasions 10: 1481–1490.

[11] Usio N., Imada M., Nakagawa M., Akasaka M., Takamura N. (2013). Effects of pond draining on biodiversity and water quality of farm ponds. Conservation Biology 27(6): 1429-1438.

[12] Maret T.J., Snyder J.D., Collins J.P. (2006). Altered drying regime controls distribution of endangered salamanders and introduced predators. Biological Conservation 127: 129-138.

[13] Doubledee R.A., Muller E.B., Nisbet R.M. (2003). Bullfrogs, Disturbance Regimes, and the Persistence of California Red-Legged Frogs. The Journal of Wildlife Management 67(2): 424-438.

[14] Reinhardt F., Herle M., Bastiansen F., Streit B. (2003). Economic Impact of the Spread of Alien Species in Germany. Umweltbundesamt, Berlin, UBA Texte 80/03: 1-229.

[15] Nehring S., Klingenstein F. (2008). Aquatic alien species in Germany – Listing system and options for action. In: Rabitsch W., F. Essl & F. Klingenstein (Eds.): Biological Invasions – from Ecology to Conservation. NEOBIOTA 7: 19-33.

[16] Sarat E., Dupuy F. (2016). American bullfrog (*Lithobates catesbeianus*) - Managing the American bullfrog in the Périgord-Limousin regional nature park. Knowledge for action series, Onema and IUCN France. https://professionnels.ofb.fr/sites/default/files/en/doc/documentation/KFA2018_EEE-vol3_18-ABullfrog-1-Perigord.pdf

[17] Louette G. (2012). Use of a native predator for the control of an invasive amphibian. Wildlife Research 39: 271–278.

[18] Goverse E., Creemers R., Spitzen-Van der Sluijs A.M. (2012). Case study on the removal of the American bullfrog in Baarlo, the Netherlands. Stichting RAVON, Report 2010.139, Nijmegen. 31 p.

[19] Adriaens T., Devisscher S., Louette G. (2013). Risk analysis of American bullfrog, *Lithobates catesbeianus*. Risk analysis report of non-native organisms in Belgium. Rapporten van het Instituut voor Natuur- en Bosonderzoek 2013, INBO.R.2013.41, Instituut voor Natuur- en Bosonderzoek (INBO), Brussel. 56p.

[20] Kamoroff C., Daniele N., Grasso R.L., Rising R., Espinoza T., Goldberg C.S. (2020). Effective removal of the American bullfrog (*Lithobates catesbeianus*) on a landscape level: long term monitoring and removal efforts in Yosemite Valley, Yosemite National Park. Biological Invasions 22: 617–626.

[21] Reshetnikov A.N., Ficetola G.F. (2011). Potential range of the invasive fish rotan (*Perccottus glenii*) in the Holarctic. Biological Invasions 13: 2967–2980.

[22] Copp G.H., Wesley K.J., Verreycken H., Russell I.C. (2007). When an 'invasive' fish species fails to invade! Example of the topmouth gudgeon *Pseudorasbora parva*. Aquatic Invasions 2(2): 107-112.

[23] O'Keeffe S. (2009). The practicalities of eradicating red-eared slider turtles (*Trachemys scripta elegans*). Aliens: The Invasive Species Bulletin. Newsletter of the IUCN/SSC Invasive Species Specialist Group 28: 19-24.
[24] LeRoy Heman M., Campbell R.S., Redmond L.C. (1969). Manipulation of Fish Populations Through Reservoir Drawdown. Transactions of the American Fisheries Society 98(2): 293-304.
[25] Hussner A., Stiers I., Verhofstad M.J.J.M., Bakker E.S., Grutters B.M.C., Haury J., van Valkenburg J.L.C.H., Brundu G., Newman J., Clayton J.S., Anderson L.W.J., Hofstra D. (2017). Management and control methods of invasive alien freshwater aquatic plants: A review. Aquatic Botany 136: 112-137.
[26] The Humane Society of the United States (2008). The Welfare of Animals in the Aquaculture Industry. HSUS REPORTS. 5.
[27] Lim H.K., Hur J.W. (2018). Effects of Acute and Chronic Air Exposure on Growth and Stress Response of Juvenile Olive Flounder, *Paralichthys olivaceus*. Turkish Journal of Fisheries and Aquatic Sciences 18: 143-151.
[28] Cash W.B., Holberton R.L. (2005). Endocrine and Behavioral Response to a Decline in Habitat Quality: Effects of Pond Drying on the Slider Turtle, *Trachemys scripta*. Journal of Experimental Zoology 303A: 872–879.
[29] Maine Department of Inland Fisheries and Wildlife (2006). Rapid Response Plan For Invasive Aquatic Plants, Fish, and Other Fauna. Part 2: Fish and other fauna protocol. In coordination with the Maine Department of Environmental Protection, 10 p.
[30] Di Cerbo A.R., Biancardi C.M. (2013). Ecological notes on the Bullfrog *Lithobates catesbeianus* in an area of the Po plain (Emilia-Romagna, Northern Italy). In: Scillitani G., Liuzzi C., Lorusso L., Mastropasqua F., Ventrella P. (curatori), 2013. Atti IX Congresso Nazionale della Societas Herpetologica Italica (Bari - Conversano, 26-30 settembre 2012). Pineta, Conversano (BA): 328-331.
[31] Bonestroo Inc. (2009). The Shawano Lake Watershed Management Strategic Plan. Prepared for Shawano Area Waterways Management, Wisconsin, USA.
[32] Adriaens T., Brys R., Halfmaerten D., Devisscher S. (2019). Information on measures and related costs in relation to species included on the Union list – *Lithobates catesbeianus*. Technical note prepared by IUCN for the European Commission.
[33] Johansson, K-M. (2020). Solabborre i Bergadammen - Utrotning av invasiv fiskart. Länsstyrelsen i Jönköpings län, Meddelande nr 2020:21.
[40] Blaubandbärbling – Management- und Maßnahmenblatt zu VO (EU) Nr. 1143/2014 (2018). https://neobiota.bfn.de/unionsliste/art-19-management.html
[41] Buchstaben-Schmuckschildkröte – Management- und Maßnahmenblatt zu VO (EU) Nr. 1143/2014 (2018). https://neobiota.bfn.de/unionsliste/art-19-management.html
[42] Nordamerikanischer Ochsenfrosch – Management- und Maßnahmenblatt zu VO (EU) Nr. 1143/2014 (2018). https://neobiota.bfn.de/unionsliste/art-19-management.html
[43] Pfeiffenschneider, M. H., F. (2020). Plan d'action pour espèces exotiques envahissantes au Grand-Duché de Luxembourg : Perche soleil, Lepomis gibbosus (Linnaeus, 1758). In L. Administration de la nature et des forêts (Ed.), (pp. 20).

Appendix 4. Physical terrestrial barriers

1. Measure name			
1.1. English:	Physical terrestrial barriers		
1.2. Lethal or non-lethal:	Non-lethal		
1.3. Other languages (if available):			
Bulgarian	Физични прегради (бариери)	Italian	Barriere fisiche
Croatian	Fizičke pregrade	Latvian	Fiziskās barjeras
Czech	Fyzické bariéry	Lithuanian	Fizinės (mechaninės) kliūtys
Danish	Fysiske barrierer	Maltese	
Dutch	Fysische barrières	Polish	Bariery fizyczne
Estonian	Füüsilised tõkked	Portuguese	Barreiras físicas terrestres
Finnish	Fyysiset esteet	Romanian	Bariere fizice
French	Barrières physiques	Slovak	Fyzické suchozemské bariéry/prekážky
German	Physische terrestrische barrieren	Slovenian	Fizične ovire na kopnem
Greek	Φυσικοί χερσαίοι φραγμοί	Spanish	Barreras físicas
Hungarian	Fizikai akadályok	Swedish	Fysiska barriärer
Irish			

2. Technical details of measure
2.1.a. Measure description
Physical barriers may be used to prevent animals escaping from or entering into a certain area [1]. Construction of barriers (also called fences) to intentionally fragment river or land habitats may be used for invasive alien species management, to exclude them from sensitive or protected areas, to support control/eradication or to minimize the impact of invasive alien predators to threatened species. This assessment only covers physical barriers used to prevent the spreading of IAS, i.e. for the objective of 'containment' as defined by the EU IAS regulation. Physical barriers could be effective in cases where the range of the IAS is restricted to an isolated area and the eradication is not feasible or suitable [2]. In these cases, native species and/or habitats are safeguarded against the impact of IAS and its spreading outside the fenced area. The chances for a successful containment of the IAS within the fenced area are relatively good for species living in freshwater habitats [2]. Physical barriers' design differs according to the target species and also in relation to environmental conditions, e.g. in case of flooding, snow storms etc. potentially occurring in the area. Permanent amphibian fencing is made of 2 to 8 mm x 1200 mm HDPE (polypropylene) with a top return and has an average lifespan of around 15 years [3]. Temporary amphibian and reptile fencing is made of polythene sheets (1200 mm high, clear Polythene) with underground and top edge returns, affixed to wooden fence stakes and last up to two years. Gates and crossings are known to be weak points that can be exploited by vertebrates to escape or enter.

Cadi and Joly [4] used a 1 cm mesh grating (50cm high) to prevent the red-eared slider escape from invaded ponds for research purposes. Minimum mesh size, mesh skirts to prevent digging, and vertical sheets or hoods to prevent climbing or jumping all need to be designed according to target species size and behaviour; ongoing maintenance, precise construction and exceptional product quality are required for the fences to be fully effective [5]. A GIS framework may be developed to assess management options in combination with barrier network algorithms, so that the spread of invasives may be mapped and limited adequately [6].

At the moment the method has been used only to contain *Trachemys scripta* for research purposes [4], among the species included in the Union List.

Pest-exclusion fences are typically used to protect native species from the impact from alien predators. This approach, however, risk to create small expensive zoos surrounded by degraded habitat that will never be able to sustain the animal and plant species contained within the fence.

Barriers may have negative consequences on non-target species, by blocking migrations and also dividing large populations into smaller, less genetically diverse groups. Physical barriers should then be designed to preserve connectivity for non-target species. For instance, this is a legal requirement in the EU in case fences are installed in Natura 2000 areas and could affect species listed in the Habitats Directive.

Fencing has been used also to convert invasion hubs into ecological trap to control cane toads in Australia [7]. By excluding toads from dams, the researchers effectively thwarted the reinvasion of cane toads. This suggests that water exclusion devices could be potentially used to prevent bullfrog invasion and control their populations in European semi-arid habitats.

2.1.b. Integration with other measures

The use of physical barriers to manage freshwater IAS by preventing their spreading is mostly used when eradication is not feasible or necessary. Complementary measures could include active trappings and drainage of ponds, followed by euthanasia or keeping in captivity.

Fencing could also be integrated by neutering of the contained animals. Finally, physical barriers have been used to facilitate the capture and eradication of bullfrog [13] and mongooses [14].

As noted above, physical barriers used to exclude animals from areas (e.g. sensitive habitats, or potential habitats) are not covered by this assessment, but they are often used as part of broader management strategies, e.g. for *A. aegyptiacus* [15] or *P. lotor* [16,17].

2.2.a. Availability - species and objectives

Objective	Unknown objective		Rapid Eradication		Management					
					Eradication		Control		Containment	
Species	Avail.	Ref(s).	Avail.	Ref(s).	Avail.	Ref(s).	Avail.	Ref(s).	Avail.	Ref(s).
Acridotheres tristis										
Alopochen aegyptiaca										
Callosciurus erythraeus										
Corvus splendens										

Herpestes javanicus										
Lepomis gibbosus										
Lithobates catesbeianus			P						P	[7]
Muntiacus reevesi										
Myocastor coypus										
Nasua nasua										
Nyctereutes procyonoides										
Ondatra zibethicus										
Oxyura jamaicensis										
Percottus glenii										
Plotosus lineatus										
Procyon lotor										
Pseudorasbora parva										
Sciurus carolinensis										
Sciurus niger										
Tamias sibiricus										
Threskiornis aethiopicus										
Trachemys scripta			P						U	[4]

2.2.b. Application – EU Member States and objectives										
Objective	**Unknown objective**		**Rapid Eradication**		**Management**					
					Eradication		**Control**		**Containment**	
Country	Avail.	Ref(s).	Avail.	Ref(s).	Avail.	Ref(s).	Avail.	Ref(s).	Avail.	Ref(s).
Austria										
Belgium										
Bulgaria										
Croatia										
Cyprus										
Czech Republic										
Denmark										
Estonia										
Finland										
France									X	[4]
Germany										
Greece										
Hungary										
Ireland										
Italy										
Latvia										

Lithuania										
Luxembourg										
Malta										
Netherlands										
Poland										
Portugal										
Romania										
Slovakia										
Slovenia										
Spain										
Sweden										
United Kingdom*										

* Not an EU Member State

3. Humaneness of the measure			
3.1. Welfare for all measures			
Measure type (if applicable):	**Humaneness impact categories**		
Domain	**No impact**	**Mild-Moderate**	**Severe - Extreme**
1: Water deprivation, food deprivation, malnutrition	No impact in case of non-breeding populations of red-eared sliders, or in large ponds.	In case of breeding populations in small ponds, food resources could become limited in the long term, exposing the animals to starvation's risks.	
2: Environmental challenge	No impact		
3: Injury, disease, functional impairment		Common fence injuries are laceration, ischaemic and crush injury, dislocation of the hip, myopathy [9]	

4: Behavioural, interactive restriction		The ability to move freely across landscapes could be required for foraging, resting and reproduction. However, a mild-moderate impact could be considered only if fenced ponds are small or overpopulated.	
5: Anxiety, fear, pain, distress, thirst, hunger etc.	No impact in many cases.	In some cases (see above) it may lead to some mild-moderate levels of pain or distress.	

3.2. Mode of death (if relevant)

Measure type (if applicable):	**Immediate death (i.e. no suffering)**	**Not immediate death (mild - moderate suffering)**	**Not immediate death (severe - extreme suffering)**
Rationale:	N/A		

3.3. Humaneness summary	When fences are used to contain *Trachemys scripta* non-breeding populations by preventing the animals to escape from invaded ponds, the impact on the animals' welfare is limited to occasional injuries and behavioral/interactive restrictions. Under these conditions, this non-lethal method is highly humane. The additional impact of neutering the contained animals should be considered if relevant.

4. Costs and effectiveness of the measure	
General effectiveness of the measure	The measure has been shown to be effective to prevent escape of *Trachemys scripta* for research purposes. However, fences were temporary, therefore the long-term effectiveness (individuals can live for up to 40 years) is unknown and will depend upon regular upkeep and maintenance. The relatively high cost of fencing—both building and maintenance—means it is only appropriate for use in relatively small or specific areas.

4.1. Case studies	
CASE STUDY #1	
Measure type (if relevant):	Fences to contain *Trachemys scripta* in infested ponds
Species:	*Trachemys scripta*
Objective:	Containment
Combined with other measure(s):	
Country(ies) of application:	France
Geographic scale (km^2) and/or population size measure applied to:	The method has been applied for research purposes, in four 30 m X 8 m ponds. The *T. scripta* population was of 16 individuals in total.
Time period:	4 years.
Effort:	No additional food was provided to the animals during the 4-years study, then efforts only include the installation of the fences and their maintenance/monitoring. The use of fences to contain IAS is considered to be effective as long as intensive maintenance is provided [10].
Costs:	Overall costs:
	The study used 1 cm mesh grating (50cm high) to prevent the red-eared slider escape from invaded ponds. Costs are not specified. A cost-benefit analysis on exclusion fences in New Zealand concludes that the cost of fencing to control movements of IAS depends on the targeted taxon, geographical location and accompanying management measures [11], [12].
	Personnel costs:
	Not specified
	Equipment and infrastructure:
	Not specified
	Other, including overheads:
	Not specified
Effectiveness:	The measure was very effective in keeping the red-eared sliders in the studied ponds during the project duration (4 years).
Reference(s):	[4]

5. Side effects	
Non-target native species, their habitats and the broader environment:	Positive:
	Negative:
	Urban ponds including those in private gardens are known to be important for biodiversity. Building fences around ponds could negatively impact native species (particularly the European terrapin) by restricting access to water and food supplies, and preventing seasonal movement of amphibious species. Fences can have lethal consequences for non-target animals or seriously injury them when they try to pass the fence [7]. Regular inspection and maintenance should be applied to mitigate the number of injured or killed vertebrates.
Other invasive alien species:	Positive:
	Fencing the ponds might also be effective to contain other alien species (e.g. bullfrog), if present.
	Negative:
Public health and well-being:	Positive:
	This measure may have a high level of public acceptability, as animals are not removed/killed.
	Negative:
	If the purpose of the fences is not adequately explained, vandalism could be an issue.
Economic:	Positive:
	Negative:

6. Conclusion

Overall assessment of the measure (qualitative)	
So far, physical barriers have been used only for research purposes to contain the read-eared sliders in urban ponds. Therefore, its effectiveness on larger scale is unknown. However, it represents an interesting, humane non-lethal option to contain the species in small or specific areas, particularly for non-breeding populations. The relatively high cost of fencing—both building and maintenance— during the species' long lifespan (40 years) and the potential impact on non-target species should be considered. Surgical sterilisation can be undertaken to prevent captive individuals reproducing, while this would obviously increase costs.	
Assessor:	Ilaria Di Silvestre
Reviewer 1:	Riccardo Scalera
Reviewer 2:	Sandro Bertolino

7. References

1. Kotchemidova, C. (2008). The Culture of the Fence: Artifacts and Meanings. Counterblast–The Journal of Culture and Communication 2: 1–4.
2. Wittenberg, R. & Cock, M.J.W. (2001). Invasive alien species: a toolkit of best prevention and management practices. 10.1079/9780851995694.0000.
3. Thomson Group (2017). "No Title." http://www.thomsonhabitats.com/fencing-and-trapping (May 18, 2017).
4. Cadi, A., Joly, P. (2004) Impact of the introduction of the red-eared slider (*Trachemys scripta elegans*) on survival rates of the European pond turtle (*Emys orbicularis*). *Biodiversity and Conservation* **13,** 2511–2518 (2004). https://doi.org/10.1023/B:BIOC.0000048451.07820.9c
5. Day, T. and MacGibbon, R. (2007). Multiple-Species Exclusion Fencing and Technology for Mainland Sites.
6. King, S., Fraser, I., O'Hanley, J.R. (2016). Benefits transfer and the aquatic environment: An investigation into the context of fish passage improvement, Journal of Environmental Management, 10.1016/j.jenvman.2016.09.041, 183, (1079-1087), (2016).
7. Letnic, M., Webb, J., K., Jessop, T. S., Dempster , T. (2015). Restricting access to invasion hubs enables sustained control of an invasive vertebrate. Journal of Applied Ecology, Vol.52 (2) April:341-347 https://doi.org/10.1111/1365-2664.12390
8. Woodroffe, R., Hedges S., and Durant, S.M. (2014). Ecology. To Fence or Not to Fence. Science (New York, N.Y.) 344(April): 46–48.
9. Austen, R. (2008). Macropod Fence Injuries. In Paper to the National Wildlife Rehabilitation Conference, Canberra.
10. Long, K. and Robley, A. (2004). Cost Effective Feral Animal Exclusion Fencing for Areas of High Conservation Value in Australia. Department of the Environment and Heritage Canberra, Australia.
11. Cullen, R., G. A. Fairburn, and K. F. D. Hughey (2001). Measuring the Productivity of Threatened-Species Programs. (Ecological Economics) 39(1): 53–66.
12. Scofield, R., Cullen, R. and Wang, M. (2011). Are Predator-Proof Fences the Answer to New Zealand's Terrestrial Faunal Biodiversity Crisis? New Zealand Journal of Ecology: 312–17.
13. Goverse, E., Creemers, R., & Spitzen–van der Sluijs, A. (2010). Case study on the removal of the American bullfrog in Baarlo, the Netherlands. *RAVON, Report*.
14. Barun, A., Hanson, C. C., Campbell, K. J., & Simberloff, D. (2011). A review of small Indian mongoose management and eradications on islands. Island invasives: eradication and management. IUCN, Gland, Switzerland, 17-25.
15. Waschbär – Management- und Maßnahmenblatt zu VO (EU) Nr. 1143/2014 (2018). https://neobiota.bfn.de/unionsliste/art-19-management.html
16. Nilgans – Management- und Maßnahmenblatt zu VO (EU) Nr. 1143/2014 (2019). https://neobiota.bfn.de/unionsliste/art-19-management.html
17. De Sousa, T. (2019). Plan d'action pour espèces exotiques envahissantes au Grand-Duché de Luxembourg: le Raton laveur, Procyon lotor (Linnaeus, 1758). In L. Administration de la nature et des forêts (Ed.), (13/12/2019 ed.).

Appendix 5. Hand removal

1. Measure name	
1.1. English:	Hand removal
1.2. Lethal or non-lethal:	Lethal to Non-lethal

1.3. Other languages (if available):

Bulgarian	Събиране с ръце	Italian	Rimozione a mano
Croatian	Ručno uklanjanje	Latvian	Ķeršana ar rokām
Czech	Ruční sběr jedinců	Lithuanian	Pašalinimas rankomis
Danish	Fjerning ved hånd	Maltese	
Dutch	Manueel verwijderen	Polish	Usuwanie ręczne
Estonian	Käsitsi eemaldamine	Portuguese	Remoção manual
Finnish	Käsin poistaminen	Romanian	Îndepărtare manuală
French	Récolte manuelle	Slovak	Ručné odstraňovanje
German	Manuelle Entnahme	Slovenian	Ročno odstranjevanje
Greek	Χειρωνακτική απομάκρυνση	Spanish	Retirada manual
Hungarian	Kézi eltávolítás	Swedish	Handfångst
Irish			

2. Technical details of measure

2.1.a. Measure description

Hand removal includes any method where operatives make a close approach to a living animal and collect them individually by sight. The collection of individuals could be facilitated by the use of dip-netting, spearing, cannon-nets or other instruments. The method includes also the destruction of eggs, nests, and hatchlings. Once captured the animals can be dispatched with **gases**, **cranial depression**, or other techniques, or kept in captivity. A handheld net is used when sampling shallow waters or in combination with other techniques, such as **electrofishing**, where they are used to scoop stunned fish and amphibians, (e.g. bullfrog ***L. catesbeianus***) from the water. The method is known to be effective in sampling a wide range of species assemblages and it is standardized with published protocols [17]. The stone moroko ***P. parva*** was successfully eradicated in a small number of English ponds using electrofishing and hand removal [3]. In many cases, it is used in combination with other removal techniques, e.g. **trapping**, **passive netting**, or **electrofishing** [12]. Draining is effective only in small ponds, but it may have negative consequences on non-target species [2,3,12]. In some cases, drainage was anticipated by **electrofishing** [3]. Fish hand-netting could be facilitated by prolonged baiting to aggregate animals or the use of telemetry to locate natural aggregations (Bajer et al. 2019).

Marine fish could be removed with spearfishing, a technique used with lionfish [8], though it is probably more difficult to apply in smaller or less conspicuous species. Trident pole spears outfitted with rubber sling were used to capture ***L. catesbeianus*** [7].

Bird eggs can be removed or destroyed to reduce productivity as a single method or in combination with a reduction of adults, e.g. for *Alopochen aegyptiacus* [25]. In some instances, removed eggs can be replaced by dummy eggs to prevent replacement with a new clutch. Alternatively, eggs can be treated in the nest, by piercing or **oiling**, to reduce their hatching (15,18, 19, 25). In Socotra island after unsuccessful management with **live traps** and **shooting**, people were paid to bring nests first with eggs and then with fledglings [15].

Management of amphibians such as ***Lithobates catesbeianus*** is conducted walking slowly through potential breeding habitats, removing all life stages (adults, larvae, and egg masses). Crews could capture them using a variety of methods including hand grabbing, spearing, dip netting; adult and subadult bullfrogs could be located using flashlights to stupefy bullfrogs via eyeshine (7, 16). Adults have been eradicated by draining water bodies [11]. Fencing the pond in advance of animal removal avoids individual dispersal and could increase the removal rate with the use of pitfall along fences).

Trachemys scripta can be captured by hand or through various trapping devices. Eradication could be obtained by draining a water body, removing sliders by hand, and finally filling again with water [11]. When a water body is drained rapidly, up to 75% of sliders will emigrate, therefore sites should be secured with fences and pitfall traps to prevent emigration [11].

The use of cannon-nets that shoot projectiles, attached to a net, out over birds standing on the ground could be used capture *Alopochen aegyptiacus* and *Threskiornis aethiopicus* [29]. Hand nets can also be used to capture larger mammals such as ***Muntiacus reevesi*** and ***Nasua nasual*** particularly in urban settings.

2.1.b. Integration with other measures

Hand removal is considered an opportunistic, supplementary method, for population control or eradications to be combined with other methods, e.g. **live-trapping**, **electrofishing**, **shooting**. Once captured the animals must be dispatched with **gases**, **cranial depression**, or other techniques, or kept in captivity.

2.2.a. Availability - species and objectives

Objective	Unknown objective		Rapid Eradication		Management					
					Eradication		Control		Containment	
Species	Avail.	Ref(s).	Avail.	Ref(s).	Avail.	Ref(s).	Avail.	Ref(s).	Avail.	Ref(s).
Acridotheres tristis	P	6								
Alopochen aegyptiaca	P	9			P		A	25,26	A	25,26
Callosciurus erythraeus										
Corvus splendens	P		A		A	15				
Herpestes javanicus										
Lepomis gibbosus	P	1,2,3,12								
Lithobates catesbeianus	A		A	7	A	7,10	A	20,21,27	A	
Muntiacus reevesi	A									
Myocastor coypus										
Nasua nasua	A									
Nyctereutes procyonoides										

Ondatra zibethicus										
Oxyura jamaicensis	P		P		A	24	A	24	P	
Percottus glenii	P	1,2,3,12								
Plotosus lineatus	P	8								
Procyon lotor										
Pseudorasbora parva			A	2	A	3				
Sciurus carolinensis										
Sciurus niger										
Tamias sibiricus										
Threskiornis aethiopicus	P		P		A	14	A		P	
Trachemys scripta	P		A		A	11	A	13,28	A	

2.2.b. Application – EU Member States and objectives										
Objective	**Unknown objective**		**Rapid Eradication**		**Management**					
					Eradication		**Control**		**Containment**	
Country	Avail.	Ref(s).	Avail.	Ref(s).	Avail.	Ref(s).	Avail.	Ref(s).	Avail.	Ref(s).
Austria										
Belgium										
Bulgaria										
Croatia										
Cyprus										
Czech Republic										
Denmark										
Estonia										
Finland										
France					A	10				
Germany							A	25,27,28		
Greece										
Hungary										
Ireland										
Italy										
Latvia										
Lithuania										
Luxembourg							A	26		
Malta										
Netherlands										
Poland										
Portugal										

Romania										
Slovakia										
Slovenia										
Spain							A	13		
Sweden					A					
United Kingdom*					A	2,3				

* Not an EU Member State

3. Humaneness of the measure			
3.1. Welfare for all measures			
Measure type (if applicable):	**Humaneness impact categories**		
Domain	**No impact**	**Mild-Moderate**	**Severe - Extreme**
1: Water deprivation, food deprivation, malnutrition	Hand removal is usually rapid, both in adults, larvae and eggs, therefore there is no effect on food/water intake.		
2: Environmental challenge	Hand removal do not expose to environmental challenge.		
3: Injury, disease, functional impairment	Disease, injury or functional impairment should not be a consequence of hand removal.	Spearfishing could produce moderate to severe injuries, depending on the ability of the fisherman. Cannon-nets also provide a risk of injury to birds.	
4: Behavioural, interactive restriction	No intrinsic effect on the animal.		

5: Anxiety, fear, pain, distress, thirst, hunger etc.		Handling and restraint of animals almost certainly produce fear in wild species. The duration of fear will depend to the time it takes to euthanasia.	

3.2. Mode of death (if relevant)			
Measure type (if applicable):	**Immediate death (i.e. no suffering)**	**Not immediate death (mild - moderate suffering)**	**Not immediate death (severe - extreme suffering)**
Rationale:	Not applicable. After hand removal animals should be euthanized with gases, cranial depression, or other techniques (see relevant assessments).		

3.3. Humaneness summary	Hand removal is generally fast and does not involve particular severe impacts on animals. The use of spearfishing could produce moderate to severe injuries, depending to the ability of the fisherman. Flashlights to stupefy bullfrogs is used on nearby animals which are then immediately captured. Though handling and restraint of animals almost certainly will produce fear in wild species. The duration of fear will depend to the time it takes to euthanasia.

4. Costs and effectiveness of the measure	
General effectiveness of the measure	Hand removal is considered effective for eradication or control only when combined with other removal techniques, e.g. trapping, passive netting or electrofishing [12]. Spear fishing may be effective with large and easy to spot species. Hand removal of egg, larvae and adult amphibians, often combined with pond fencing, may be hampered by strong density dependence in both the larvae and post-methamorphic animals [4,5]. Likewise, the removal of adults can increase the survival of juveniles that otherwise will prey on them [4].

4.1. Case studies		
CASE STUDY #1		
Measure type (if relevant):	Hand removal, dip netting, spearing [7]	
Species:	*Lithobates catesbeianus*	
Objective:	Eradication	
Use of measure	Egg masses and larvae removed using dip nets, paint strainers, or 5-gallon buckets. Occasionally, crews used a backpack electrofisher or seine nets to remove larvae.	
Combined with other measure(s):	Crews used flashlights to locate and stupefy bullfrogs, which were removed using a variety of methods: hand grabbing, spearing (trident pole spears outfitted with rubber sling), dip netting, seine netting, or shooting with pellet rifles.	
Country(ies) of application:	United States of America	
Geographic scale (km^2) and/or population size measure applied to:	6 locations, 5 natural waterbodies and 1 manmade pond, and 15 flooded areas within a 1,500 ha area in the Yosemite Valley.	
Time period:	2005 to 2018	
Effort:	From 2005 to 2018, 1-2 full time or 1-8 part-time employees were recruited each year (see [7] for yearly details).	
Costs:	Overall costs:	
	Cost could be evaluated as man/months of work from data above.	
	Personnel costs:	
	Equipment and infrastructure:	
	Other, including overheads:	
Effectiveness:	This was the first successful eradication of bullfrogs on a landscape level. Authors effectively removed over 8000 individual bullfrogs from 6 breeding sites and 15 flooded areas throughout Yosemite Valley, an area of 1500 ha. The work highlights that the removal of bullfrogs was possible by targeting breeding populations,	

	using a variety of mechanical removal methods (incl. hand removal), and monitoring via traditional (visual surveys and audio recording devices) and eDNA survey techniques.

CASE STUDY #2	
Measure type (if relevant):	Hand removal of nests
Species:	House crow *Corvus splendens*
Objective:	Control
Use of measure	In 1999, under the guidance of the SCDP and BirdLife International, an eradication programme was started. Five attempts were made, the first three with trapping and shooting were unsuccessful, the fourth (hand removal) [15] succeeded in controlling the population, and the fifth (shooting) achieved the goal of eradication. Schoolchildren were encouraged to search for nests and rewarding them for bringing nests and young birds to be humanely dispatched by staff.
Combined with other measure(s):	Hand removal of nest was followed by adult shooting.
Country(ies) of application:	Yemen
Geographic scale (km^2) and/or population size measure applied to:	Socotra Island 3500 sqkm
Time period:	2002-2007
Effort:	242 fledgling house Crows collected
Costs:	Overall costs:
	A total of $2500 in reward payments
	Personnel costs:
	Equipment and infrastructure:
	Other, including overheads:
Effectiveness:	This control programme was successful in keeping the population under control and below 15 birds by the time it was fully operating. Eradication was achieved by shooting the last adults.

4.2. Costs effectiveness summary	The method is time consuming and requires a high degree of human effort. Its effectiveness has been demonstrated in projects that generally involved an integrated approach with the use of other techniques.

5. Side effects	
Non-target native species, their habitats and the broader environment:	Positive:
	More neutral then positive impact: hand removal is selective. The method itself is not lethal and it would allow to select and separate non-target species. No damage to the habitat or wider environment is reported.
	Negative:
	Handheld net could capture native species with limited risk of injury; however they could be easily and quickly released. Handling amphibians can help the spread of diseases, such as the chytrid fungus *Batrachochytrium dendrobatidis* which could be transmit by ***L. catesbeianus*** to native species [23].
Other invasive alien species:	Positive:
	Netting might also be effective to remove other alien species (e.g. fish, bullfrog), if present.
	Negative:
Public health and well-being:	Positive:
	Negative:
	Acceptability may depend on the technique used for euthanasia after removal.
Economic:	Positive:
	Negative:
	Hand removal of eggs, larvae and adults is time consuming. It is usually used in combination with other techniques.

6. Conclusion

Overall assessment of the measure (qualitative)

Removal by hand or facilitated by the use of dip-netting, spearing, or other instruments is time-consuming. The method includes also the removal of eggs, nests, and hatchlings. Hand removal itself can have mild to moderate humaneness impacts, however the measure is often used with methods of euthanasia which also need to be considered (see other assessments). Its effectiveness on alien vertebrate eradications and control has been demonstrated in projects that also involved the application of other techniques, e.g. **trapping**, **passive netting**, or **electrofishing**. Therefore, it should be considered a complementary technique more than a single method to manage a species. Hand removal of eggs (or their treatment in the nest to reduce hatching) and larvae may drastically reduce the replacement of adults. In the case study above, ***Lithobates catesbeianus*** was eradicated over a large area; however, adults hand removal was facilitated by electrofishing. ***Corvus splendens*** was controlled in Socotra island by nest removal; the last adults were eradicated with shooting.

Assessor:	Sandro Bertolino
Reviewer 1:	Kevin Smith
Reviewer 2:	Riccardo Scalera

7. References

[1] Bajer, P. G., Ghosal, R., Maselko, M., Smanski, M. J., Lechelt, J. D., Hansen, G., & Kornis, M. S. (2019). Biological control of invasive fish and aquatic invertebrates: a brief review with case studies. Management of Biological Invasions, 10(2), 227.

[2] Britton, J. R., Brazier, M., Davies, G. D., & Chare, S. I. (2008). Case studies on eradicating the Asiatic cyprinid Pseudorasbora parva from fishing lakes in England to prevent their riverine dispersal. Aquatic Conservation: Marine and Freshwater Ecosystems, 18(6), 867-876.

[3] Copp, G. H., Wesley, K. J., Verreycken, H., & Russell, I. C. (2007). When an 'invasive' fish species fails to invade! Example of the topmouth gudgeon Pseudorasbora parva. Aquatic Invasions, 2(2), 107-112.

[4] Doubledee, R. A., Muller, E. B., & Nisbet, R. M. (2003). Bullfrogs, disturbance regimes, and the persistence of California red-legged frogs. The Journal of wildlife management, 424-438.

[5] Govindarajulu, P., Altwegg, R., & Anholt, B. R. (2005). Matrix model investigation of invasive species control: bullfrogs on Vancouver Island. Ecological Applications, 15(6), 2161-2170.

[6] Global Invasive Species Database (GISD) 2015. Species profile Acridotheres tristis. Available from: http://www.iucngisd.org/gisd/species.php?sc=108 [Accessed 18 August 2020]

[7] Kamoroff, C., Daniele, N., Grasso, R. L., Rising, R., Espinoza, T., & Goldberg, C. S. (2020). Effective removal of the American bullfrog (*Lithobates catesbeianus*) on a landscape level: long term monitoring and removal efforts in Yosemite Valley, Yosemite National Park. Biological Invasions, 22(2), 617-626.

[8] Jiménez, C., Çiçek, B. A., Jiménez, J. U., Hadjioannou, L., & Huseyinoglu, M. F. (2018). What is the roar about? Lionfish targeted removals in Costa Rica (Central America) and Cyprus (Mediterranean Sea). In: Hüseyinoğlu, M.F., Öztürk, B. (Eds.) 2018. Lionfish Invasion and Its Management in the Mediterranean Sea. Turkish Marine Research Foundation (TUDAV) Publication no: 49, Istanbul, Turkey

[9] Marchant J. (2008). Branta canadensis (Canada goose). CABI Invasive Species Compendium.

[10] Michelin G. 2012. La Grenouille taureau en Sologne, de la lutte à l'éradication. Sciences Eaux et Territoires, 6: 50-56

[11] O'Keeffe 2009. The practicalities of eradicating red-eared slider turtles (Trachemys scripta elegans). Aliens: The Invasive Species Bulletin, 28, 19-24.

[12] Ruiz-Navarro, A., Verdiell-Cubedo, D., Torralva, M., & Oliva-Paterna, F. J. (2013). Removal control of the highly invasive fish Gambusia holbrooki and effects on its population biology: learning by doing. Wildlife Research, 40(1), 82-89.

[13] Sancho Alcayde, V., Lacomba Andueza, J.I., Bataller Gimeno, J.V. & Pradillo Carrasco, A. (2015). Manual para el Control y Erradicación de Galápagos Invasores. Colección Manuales Técnicos de Biodiversidad, 6. Conselleria d'Agricultura, Medi Ambient, Canvi Climàtic i Desenvolupament Rural. Generalitat Valenciana. Valencia.

[14] Sarat E., Mazaubert E., Dutartre A., Poulet N., Soubeyran Y., 2015. Invasive alien species in aquatic environments. Practical information and management insights. Volume 2. Management insights. Onema. Knowledge for action series. 240 pages.

[15] Suleiman, A. S., & Taleb, N. A. D. I. M. (2010). Eradication of the house crow Corvus splendens on Socotra, Yemen. Sandgrouse, 32(2), 136-140.
[16] Triece, K., Ehrenberger, J., & Davenport, N. (2018). Investigating Bullfrog Management on the Front Range. Adaptation Environmental Services.
[17] West, P., Brown, A. N., & Hall, K. (2007). Review of alien fish monitoring techniques, indicators and protocols: implications for national monitoring of Australia's inland river systems. National Land & Water Resources Audit.
[18] Pochop, P. A., Cummings, J. L., Yoder, C. A., & Steuber, J. E. (1998). Comparison of white mineral oil and corn oil to reduce hatchability in ring-billed gull eggs. In Proceedings of the Vertebrate Pest Conference (Vol. 18, No. 18).
[19] Martin, J. M., French, K., & Major, R. E. (2007). The pest status of Australian white ibis (Threskiornis molucca) in urban situations and the effectiveness of egg-oil in reproductive control. Wildlife Research, 34(4), 319-324.
[20] Schwalbe, C. R., & Rosen, P. C. (1988). Preliminary report on effect of bullfrogs in wetland herpetofaunas in southeastern Arizona. In General Technical Report-US Department of Agriculture, Forest Service (pp. 166-173).
[21] Rosen, P. C., & Schwalbe, C. R. (1995). Bullfrogs: introduced predators in southwestern wetlands. Our living resources: a report to the nation on the distribution, abundance, and health of US plants, animals, and ecosystems. US Department of the Interior, National Biological Service, Washington, DC, 452-454.
[22] Martin, J. M., French, K., & Major, R. E. (2007). The pest status of Australian white ibis (Threskiornis molucca) in urban situations and the effectiveness of egg-oil in reproductive control. Wildlife Research, 34(4), 319-324.
[23] Miaud, C., Dejean, T., Savard, K., Millery-Vigues, A., Valentini, A., Gaudin, N. C. G., & Garner, T. W. (2016). Invasive North American bullfrogs transmit lethal fungus Batrachochytrium dendrobatidis infections to native amphibian host species. Biological Invasions, 18(8), 2299-2308.
[24] MITECO. (2013). Catálogo Español de especies exóticas invasoras. *Oxyura jamaicensis*. OXYJAM/EEI/AV009 https://www.miteco.gob.es/es/biodiversidad/temas/conservacion-de-especies/oxyura_jamaicensis_2013_tcm30-69950.pdf
[25] Nilgans – Management- und Maßnahmenblatt zu VO (EU) Nr. 1143/2014 (2019). https://neobiota.bfn.de/unionsliste/art-19-management.html
[26] De Sousa, T. (2019). Plan d'action pour espèces exotiques envahissantes au Grand-Duché de Luxembourg: l'Ouette d'Égypte, Alopochen aegyptiacus (Linnaeus, 1766). In L. Administration de la nature et des forêts (Ed.), (13/12/2019 ed.).
[27] Nordamerikanischer Ochsenfrosch – Management- und Maßnahmenblatt zu VO (EU) Nr. 1143/2014 (2018). https://neobiota.bfn.de/unionsliste/art-19-management.html
[28] Buchstaben-Schmuckschildkröte – Management- und Maßnahmenblatt zu VO (EU) Nr. 1143/2014 (2018). https://neobiota.bfn.de/unionsliste/art-19-management.html
[29] Cocchi, R., Volponi, S. & Baccetti, N. (2020). Piano di Gestione Natzionale dell'Ibis Sacro *Threskiornis aethiopicus.* ISPRA Istituto Superiore per la Protezione e la Ricerca Ambientale

Appendix 6. Physical fishing methods including aquatic nets and traps

1. Measure name			
1.1. English:	Physical fishing methods including aquatic nets and traps		
1.2. Lethal or non-lethal:	Non-lethal		
1.3. Other languages (if available):			
Bulgarian	Физически риболовни методи, включително с мрежи и капани	Italian	Metodi fisici di pesca, comprese reti acquatiche e trappole
Croatian	Ribolovne metode	Latvian	Fiziskās zivju ķeršanas metodes
Czech	Fysické metody rybolovu	Lithuanian	Fiziniai (mechaniniai) žvejybos būdai
Danish	Fysiske fiskeri metoder	Maltese	
Dutch	Fysieke vismethoden, waaronder netten en vallen	Polish	Fizyczne metody połowu
Estonian	Füüsilised kalapüügimeetodid	Portuguese	Métodos de pesca físicos incluindo redes e armadilhas aquáticas
Finnish	Fyysiset kalastusmenetelmät	Romanian	Metode fizice de pescuit, inclusiv plase și capcane acvatice
French	Méthodes de pêche physiques - y compris les filets et les pièges	Slovak	Fyzické metódy odlovu – vrátane Vodných sietí
German	Befischungsmethoden, inklusive Netzbefischung und Reusen	Slovenian	Metode fizičnega ribolova - vključno z vodnimi mrežami
Greek	Μέθοδοι φυσικής αλιείας	Spanish	Métodos físicos de pesca- incluyendo redes
Hungarian	Halászati módszerek	Swedish	Fysiska fiskemetoder
Irish			

2. Technical details of measure
2.1.a. Measure description
This measure is used for the management of aquatic invasive alien species (IAS) and refers to the use of passive fishing methods, including a variety of aquatic nets and traps, through which animals are caught by actively swimming or moving into the net or trap. They are used to capture a variety of animals, both invertebrates such as crayfish, and vertebrate species such as mammals, fishes, reptiles and amphibians. **Aquatic nets** There are a number of different types of nets that can be used to manage invasive aquatic species, especially fish (but also amphibians and turtles), and their performance depends largely on the activity regime of the target species. Netting designs can have a range of sizes, colour, mesh size and materials, which should be adapted to the size and species of interest [1]. Animals are intercepted by the mesh of the net, which should preferably be

of inconspicuous colour, and remain meshed or bundled which, for fish (but not for tadpoles), generally results in death or irreversible injury. The nets can be set at the bottom of the water body, or at a certain depth without touching the ground.

Dip nets are handheld nets not very large in diameter (usually around 50 cm) that can be used to remove submerged animals in shallow waters [1]. Dipnetting can catch low numbers of highly catchable animals in small areas, but is less effective in large waterbodies [2]. Due to being labour intensive and having low effectiveness as a passive fishing method, dipnetting is often only used actively (i.e. to remove specimens detected visually) and/or as a supplementary method in combination with other techniques.

Seine nets are large, long nets that are used to haul catch from the water. Seine nets are either deployed from shore, dragged through the water by people, or by powered watercraft, actively enclosing and capturing animals as they are pulled through the water [1]. Seine nets will capture all animals within their bounds; however, this method is not efficient in cases where abundant vegetation is present or if the bottom of the water body is irregular [3]. Seine nets have been successfully used in management actions to capture *Lithobates catesbeianus* [4, 5, 6, 34] and specimens of *Pseudorasbora parva* [7, 8]. They can also be used to manage *Trachemys scripta*, but unless the seine net is hauled quietly and rapidly, the technique does not seem to be effective for this species as, when disturbed, animals usually drop to the water bottom and burrow into the mud [9, 10]. Although *Plotosus lineatus* can be captured with seine nets (also gill nets, cages and via angling), the efficacy of these fishing methods for species removal from large areas is unknown [11, 12]. Seine nets (or fine mesh fyke nets) could potentially be used to support the control of *P. glennii*, as part of an integrated strategy with the use of native predators, however high effort would be needed after each breeding season to overcome compensatory response [27].

Gill nets are another type of vertical panels of netting, but they are hung from a line usually using floats at the top, with the bottom edge being weighted down (therefore fishing passively). They are single-layer nets where the mesh size is adapted to the size of the target species, so that fish get entangled around the head or body, being highly selective for a particular size of fish [13]. Gill nets are left in the water for long periods of time (minimum three days). Gill nets have been used in the management of invasive fish species, including *Lepomis gibbosus*, but yield was shown to be quite low in terms of absolute catches and they proved ineffective in removing high quantities of fish in the pelagic zone [13, 14].

Trammel nets are a variation of gill nets, made up of three overlapping layers of netting, instead of a single layer. In trammel nets, fish can be meshed (as in gill nets) or entangled in 'bags' or pockets of netting, a fact that makes them much less selective than gill nets regarding the size and species of fish caught. Although trammel nets have been used (together with gill nets) in the management of fish species such as *L. gibbosus* [14], their use has been discarded in other management projects due to their impact on native species [13].

Trawl nets are used for trawling, a fishing method commonly used in marine environments, which consists of pulling a fishing net through the water behind a boat. Trawling can be done in the bottom of the water, by pulling the trawl along or next to the sea floor, or in midwater by pulling the trawl across the pelagic zone. It has been suggested that direct removal of *P. lineatus* via intensive trawling in shallow waters during the summer months, especially during the spawning period, could contribute to the eradication of localised, low density, newly established populations [15]. However, this is not expected to be a cost-effective, realistic or ecologically acceptable option, due to the technique's lack of catch selectivity and the physical damage that it can cause to the sea bottom [15]. Additionally, Council Regulation (EC) No 1967/2006 concerning management measures for the sustainable exploitation of fishery resources in the Mediterranean Sea, bans trawling at depths shallower than 50m throughout the year.

Longline fishing, which consists in setting lines of large hooks baited with live fish, can be used to help control populations of *T. scripta*, but this method should only be used when population abundances are very low and in water bodies devoid of recreational activities to avoid injuries [2, 16]. This technique has also been used for the management of invasive fish species, but is mostly useful for capturing large specimens of exotic predatory fish [13].

<u>Fishing with a hook and line</u> or angling, is often used as a sampling technique for different species, especially fish (e.g. *P. lineatus* [11]), and has been occasionally used as an integrated method in management of IAS (e.g. to assist eradication of *Salvelinus fontinalis* from mountain lakes [44]). For the IAS of Union concern in focus here, it has been used in eradication efforts of *L. catesbeianus* [6] and tested as a management technique for *T. scripta* [2, 16]. However, for this method to obtain positive results it has to be undertaken by an experienced fisherman and in water bodies with good visibility [16]. It should be noted that recreational fishing can play a key role in the early detection of invasive fishes.

Aquatic traps
Aquatic traps are non-destructive capture methods that involve the use of different types and models of traps. They are largely used to manage invasive aquatic vertebrates, especially (semi-aquatic) mammals, amphibians and reptiles, but also fish species, usually in combination with other methods. As traps used to manage mammals are already covered under the 'Live capture traps - cage traps' assessment, here we only refer to aquatic traps used for the management of amphibians, reptiles and fish. Trapping can be a suitable method to obtain captures in specific situations, such as when there are habitat restrictions (e.g. at the entrance to channels or streams, or in certain littoral areas) or when the management goal is to protect native fish species that should not be injured or killed in nets [13]. Aquatic traps come in a variety of shapes and formats, and the most commonly used are funnel traps and fyke nets (e.g. hoop nets, Rimov nets, Klicava traps, Cathedral traps, Gee traps), but bucket traps, plastic or steel minnow traps, cage traps, floating traps and others are also used. Fyke nets often consist of one or more 'wings' that create a barrier for the animals, guiding them into one or more funnels facing the interior of the trap, which prevents their escape [1]. Traps often contain attractants inside and, for this, different types of lures can be used depending on the target species and/or life form, such as various types of bait, light-emitting diodes, brightly coloured fishing lures, olfactory attractants, etc. [17]. Traps can be deployed from the margin of the water body or from a boat to assist set and retrieval in areas that cannot be reached from the shore. They are usually checked every day in order to collect captures and release any bycatch, but are left in place for several days.

Fyke nets and Gee traps have proven highly effective in capturing *L. catesbeianus* larvae and can also be used to remove adults, largely contributing to the control or even eradication of this species, especially in small and isolated populations [3, 5, 18]. A multiple capture trap, which has been developed to control invasive cane toads in Australia, modified to float and lured, has also shown promise in helping to control *L. catesbeianus* [19, 20]. However, funnel traps containing a mechanical lure that vibrates like an insect as an attractant were not successful in contributing to the management of this species in Yosemite National Park [6] and bucket traps have also shown to be ineffective for this species [21]. Although less used in the control of fish species, several studies have shown that fyke nets and funnel traps can be effective in catching, and be used for the management of small-bodied fishes such as *L. gibbosus* and *P. parva* [8, 22, 23, 24]. Funnel traps have also been used to capture specimens of *Perccottus glenii* [25] and, although they can potentially be used for its control when combined with other techniques, intensive trapping does not seem appropriate to eradicate this fish species [26, 27]. In Australia, fyke nets and Cathedral traps have been used to control *T. scripta* in large water bodies that cannot be drained and filled or netted with seine nets [9]. In Spain, France, Portugal and Italy, population control and eradication campaigns have successfully used various types of traps to capture specimens of *T. scripta*; fyke nets seemed to be particularly effective in canals and ditches [2, 16, 28, 29, 30, 31]. A particular type of trap that has also been effectively used in management campaigns of *T. scripta* are basking traps, which consist in floating enclosures with sloping sides and a basking surface, from which a mesh basket hangs suspended [2, 9, 10, 16, 29, 31, 32, 33]. Basking traps become increasingly effective the longer they are left in place and seem to be mostly useful for controlling small turtle populations. Their use in public or high visibility locations should be done with caution as, in case the public opposes eradication actions, they might remove or destroy the traps [9, 10].

While physical fishing can be used to support eradication of fish and bullfrog populations from localised areas, in terms of management objectives as defined by the EU IAS Regulation the measure is unlikely to be effective for 'eradication' i.e. the complete and permanent removal of a widespread population from a Member State, but rather be applied to support rapid eradication of a population at an early stage invasions (i.e. one or a few localities) or to the control or containment of a widespread population. However, the measures could be potentially applied to support the eradication of *T. scripta* in areas where it cannot breed.

2.1.b. Integration with other measures

In the case of freshwater systems, and for the vertebrate species concerned here, aquatic nets and traps are often used in integration with several other techniques, mostly electrofishing, but also hand removal, spearing/gigging, shooting, biocontrol (native predators) and pond draining [e.g. 6, 8, 14, 23, 27, 34]. For example, combining the use of aquatic nets/traps with biocontrol might be essential to achieve effective management of species which show compensatory responses in survival, growth and/or fecundity following intensive removals, as is the case of *P. parva* [8]. Aquatic nets can also be used in combination with scaring techniques, with the aim to drive fish towards them [1]. Deploying nets and traps can be especially useful in large water bodies with areas inaccessible to electrofishing, such as deep or open waters [13].

As animals are not killed in traps (and often also not in nets), these methods are usually combined with techniques that allow to euthanize the animals. For example, for *L. catesbeianus* this has been done by anaesthetising animals by submersion in a clove oil and water emulsion followed by deep freezing, by using a buffered solution of MS-222 or benzocaine (ethyl aminobenzoate), or by skull blunt force and pithing protocol [6, 34, 35]. For *T. scripta*, animals have been euthanized in Spain by injection of Eutanax, a method that is used by the veterinary staff of the Ministry responsible for these actions and in line with national regulations [28].

2.2.a. Availability - species and objectives

Objective	**Unknown objective**		**Rapid Eradication**		**Management**					
					Eradication		**Control**		**Containment**	
Species	Avail.	Ref(s).	Avail.	Ref(s).	Avail.	Ref(s).	Avail.	Ref(s).	Avail.	Ref(s).
Acridotheres tristis										
Alopochen aegyptiaca										
Callosciurus erythraeus										
Corvus splendens										
Herpestes javanicus										
Lepomis gibbosus	A	46	P				A (gill nets, fyke nets, hoop nets)	14, 22, 36	A	
Lithobates catesbeianus	P		A (seine nets, fyke nets, funnel traps, hook	4, 5, 6, 18, 35, 37			A (fyke nets, multiple capture traps)	3, 19, 36, 47	A	

			and line)							
Muntiacus reevesi										
Myocastor coypus										
Nasua nasua										
Nyctereutes procyonoides										
Ondatra zibethicus										
Oxyura jamaicensis										
Percottus glenii	P		P				P (seine nets, fyke nets)	27	P	
Plotosus lineatus							P (seine, gill and trawl nets, cages)	12, 15		
Procyon lotor										
Pseudorasbora parva	A	46	A (fyke nets)	23			A (seine nets, funnel traps)	7, 8, 48, 49	A	
Sciurus carolinensis										
Sciurus niger										
Tamias sibiricus										
Threskiornis aethiopicus										
Trachemys scripta	P		A (seine nets, variou s traps)	10, 36	P		A (seine nets, longline fishing, various traps, hook and line)	9, 16, 28, 29, 30, 31, 32, 33, 36, 38, 50, 51		

2.2.b. Application – EU Member States and objectives										
Objective	**Unknown objective**		**Rapid Eradication**		**Management**					
					Eradication		**Control**		**Containment**	
Country	Avail.	Ref(s).	Avail.	Ref(s).	Avail.	Ref(s).	Avail.	Ref(s).	Avail.	Ref(s).
Austria										

Belgium					X	37	X	3		
Bulgaria										
Croatia										
Cyprus										
Czech Republic										
Denmark										
Estonia										
Finland										
France					X	18	X	29, 30, 36		
Germany										
Greece										
Hungary										
Ireland										
Italy							X	22, 31		
Latvia										
Lithuania										
Luxembourg										
Malta										
Netherlands										
Poland										
Portugal							X	28		
Romania										
Slovakia										
Slovenia										
Spain	X	46			X	29, 38	X	14, 16, 28, 29, 32, 36, 38		
Sweden										
United Kingdom*					X	23	X	7, 8		

* Not an EU Member State

3. Humaneness of the measure			
3.1. Welfare for all measures			
Measure type (if applicable): Aquatic nets	**Humaneness impact categories**		
Domain	**No impact**	**Mild-Moderate**	**Severe - Extreme**
1: Water deprivation, food deprivation, malnutrition		For gill nets, which can be left in the water for long periods of time, animals can experience short-term food restrictions.	
2: Environmental challenge		Although most types of nets are hauled quite quickly, if kept inside the water for too long, air breathing animals can experience lack of oxygen.	
3: Injury, disease, functional impairment		If predators are caught in nets (mostly seine) with the target species and kept together for a period of time, there is a slight chance of the animals becoming injured.	Gill nets can cause severe injuries to fish, including scale loss, bruising, hemorrhaging and softening of the flesh, sometimes even causing fish death [39]. Fish caught in trawl nets often suffer scale damage and might experience compression, being unable to move their gills and suffocating, or stopping blood supply, which can result in death [40]. Both longline fishing and fishing with hook and line can cause severe injuries to animals, such as punctures to the body wall or damage to the gullet or eyes, and perforations to the digestive system [2, 41, 45].

4: Behavioural, interactive restriction		Animals caught in nets can become agitated while trying to escape, as they are not able to swim or hide from predators and, for some species, breathing air can be difficult. Fish caught in gill nets get agitated, try to swim away backwards vigorously and wrench strongly with the head and tail [39].	
5: Anxiety, fear, pain, distress, thirst, hunger etc.			Animals caught in nets, or using hooks, can experience short-term food restrictions, lack of oxygen and be subjected to severe levels of injuries and stress, which can incur mortality [2, 39, 40, 41].

Measure type (if applicable): Aquatic traps	**Humaneness impact categories**		
Domain	**No impact**	**Mild-Moderate**	**Severe - Extreme**
1: Water deprivation, food deprivation, malnutrition		If traps are left in the water without being checked for moderate periods of time, animals can experience short-term food restrictions.	
2: Environmental challenge	Traps should be set or designed to avoid potential death of air-breathing fauna, so that there is no exposure to conditions that are outside of the animals' thermoneutral range.		
3: Injury, disease, functional impairment		If handled correctly, the use of traps usually does not cause injury or impairment to the animals caught. However, it is possible that fish get gilled in the netting of the traps, becoming injured and sometimes even causing death [42]. If predators are caught in the same trap as the target species, there is also a chance of injury or even death.	

4: Behavioural, interactive restriction		Animals caught in traps can become agitated while trying to escape, as they are not able to properly swim or hide from predators, and sometimes breathing air can be difficult.	
5: Anxiety, fear, pain, distress, thirst, hunger etc.		There is a risk of the animals captured becoming injured by getting gilled in the nets or through co-presence with predators in a constrained environment. Capture and retention in the traps can also be distressing to animals [40, 42], which cannot freely swim or hide. Traps should be set or designed to avoid potential death of air-breathing fauna.	

3.2. Mode of death (if relevant)

Measure type (if applicable):	**Immediate death (i.e. no suffering)**	**Not immediate death (mild - moderate suffering)**	**Not immediate death (severe - extreme suffering)**
Rationale:	N/A		

3.3. Humaneness summary	Most types of aquatic nets used to capture animals usually involve some degree of environmental challenge (lack of oxygen), behavioural restrictions (inability to swim or hide from predators), distress and injury. The severity of injuries depends on the specific method used, the species in question and how the technique is undertaken [39]. Gill nets, which catch and retain fish by becoming gilled around their body or tangled in the net, and trawl nets, which chase fish to exhaustion, are undoubtedly the most damaging of these methods and the longer the nets are in the water the higher the levels of potential injury and death. Fishing with a hook and line is also quite damaging for the animals caught so, when possible, the use of dipnets and seine nets is preferable. Unlike nets, the use of aquatic traps usually allows for the live capture and retrieval of animals that remain inside the trap without getting injured. As such, if a proper trap design and good practices are used, injuries and casualties of animals can be largely minimised.

4. Costs and effectiveness of the measure	
General effectiveness of the measure	The use of aquatic nets and traps can be very labour and time intensive, and often does not reduce animals' abundance to the desired levels [1, 5, 19]. The effectiveness of gill nets to capture IAS depends greatly on the targeted species [43]. For traps, only when the full extent of the species invasion is known and limited to small and easily accessible water bodies, can trapping be effective in controlling, or even eradicating, invasive aquatic animals [37]. However, when species are widely distributed and present in large water bodies, both aquatic nets and traps used independently are often not effective in species eradication, and only partially effective in species management. Nevertheless, if these techniques are used together, and especially in combination with a variety of other methods (e.g. electrofishing, hand capturing, shooting, draining), as part of an Integrated Pest Management strategy, they can provide a highly valuable means of controlling aquatic invasive alien species [e.g. 5, 6].

4.1. Case studies	
CASE STUDY #1	
Measure type (if relevant):	Aquatic nets
Species:	*Lepomis gibbosus*
Objective:	Control of the population – to decimate populations of pumpkinseed by up to 80%, while eradicating common carp.
Use of measure	Use of multi-mesh gill nets (trammel-, benthic- and pelagic nets) were deployed overnight in the pelagic habitat for about 12h.
Combined with other measure(s):	The measure was combined with the use of electrofishing.
Country(ies) of application:	Spain (Lake Arreo, Basque country)
Geographic scale (km^2) and/or population size measure applied to:	Lake Arreo covers about 136 ha.
Time period:	Management actions were undertaken for a total of around 2.5 months during 2014 and 2015.
Effort:	76.3 units of effort for gill nets (45 m2 · 12 h) in 2014 and 88.9 units of effort in 2015, for a total of 76 days.
Costs:	Overall costs:
	No costs reported.
	Personnel costs:
	Equipment and infrastructure:
	Other, including overheads:

Effectiveness:	The use of different gillnet types with different mesh sizes resulted in the catch of 337 fish from the deep zone. Gillnets proved to be ineffective in removing high quantities of fish in the pelagic zone. Overall, the reduction of non-native fish populations was very high, with a 98% BPUE (biomass per unit of effort) and 81% CPUE (density per unit of effort) reduction from the beginning to the end of the management effort. A significant decrease in CPUE and BPUE of specifically *L. gibbosus* resulting from all methods was observed, but gill nets were identified as being less effective than electrofishing.
Reference	14

CASE STUDY #2	
Measure type (if relevant):	Aquatic traps
Species:	*Trachemys scripta*
Objective:	Management programme for red-eared slider turtles in Corsica - Attempt to eradicate the turtles in a former meander now cut off from the river.
Use of measure	Use of several types of aquatic traps, more specifically hoop nets, fyke nets and sundeck (basking) traps.
Combined with other measure(s):	No integration with other measures.
Country(ies) of application:	France (Corsica)
Geographic scale (km^2) and/or population size measure applied to:	No mention of the scale of the actions, but it is mentioned that the meander was selected for eradication due to being isolated and because of the large number of *T. scripta* found to be present there.
Time period:	A total of around 3.5 months of trapping in 2010 and then again in 2011.
Effort:	In 2010, traps were set along the banks, one trap every 15 metres, including 38 hoop nets from 8 June to 31 August, 5 fyke nets and 15 sundeck traps from 1 to 16 September. Traps were checked once daily, toward the end of the day. In 2011, approximately 40 hoop nets were set in three sectors in the northern part of the site using the same protocol.
Costs:	Overall costs:
	The overall costs of the management actions were 25.000 EUR in 2010 and 20.000 EUR in 2011.
	Personnel costs:
	Equipment and infrastructure:
	Other, including overheads:

Effectiveness:	In 2010, a total of 37 turtles (out of the estimated 46 turtles present) were captured over the 101 days of trapping and in 2011 a total of 34 turtles were captured. The use of the trapping technique proved relatively effective in this confined and isolated area, but did not eradicate the species. During the project, only 72 of the 84 identified turtles were captured.
Reference	36

4.2. Costs effectiveness summary	Aquatic nets and traps are usually not very expensive equipment to acquire, with the bulk of the resources needed referring to the required labour. However, given that management actions using these methods usually have to be sustained for long periods of time in order to achieve meaningful results, their use ends up requiring large and constant amounts of resources. As such, these methods are most practical and cost-effective for the management of isolated IAS populations in small and shallow water bodies, especially when combined with methods to prevent species reproduction, or for management actions integrating several other methods.

5. Side effects	
Non-target native species, their habitats and the broader environment:	Positive:
	Negative:
	Bycatch is a negative consequence of using these passive fishing methods. Non-target aquatic animals that use the same habitat as the target species, such as crayfish, amphibians, turtles, fish and even small aquatic mammals or birds, are often captured in aquatic nets and traps, sometimes attracted by the presence of prey animals inside. Aquatic nets are the most damaging, with mortality of non-target species increasing with longer net set durations [42], making them unsuitable for use in sensitive areas or where native species are protected or endangered. Trammel and trawl nets have particularly strong negative effects on non-target fauna and the environment, and their use often accidentally kills bycatch [40]. Aquatic traps are usually less invasive and, if used correctly, allow returning non-target animals uninjured to the environment [13, 34]. Traps can be set or designed with a view to avoid the accidental capture and potential death of non-target air-breathing fauna, through only partially submerging them or by creating an opening at the top, so that fauna can get out easily or remain on the surface and not drown [13, 43]. If traps are completely submerged, an appropriate size of breathing air space should be provided for non-target air-breathing animals [35, 42]. There is, however, a risk of non-target animals captured becoming injured, or being killed, by predators caught in the same trap, or reaching them from the outside. In any case, traps should be checked regularly to avoid these potential negative impacts on non-target animals.

	In case traps or nets are lost in the water body, ghost fishing may occur, whereby non-target animals become trapped in the nets or inside the traps and end up succumbing.
Other invasive alien species:	Positive:
	Aquatic nets and traps can also capture other invasive alien species, such as crayfish, amphibians, turtles, fish and even small aquatic mammals (e.g. muskrats and coypu) [42].
	Negative:
Public health and well-being:	Positive:
	Negative:
Economic:	Positive:
	If traps or nets are set to remove IAS that are predators of commercially important fish species, this can have positive side effects on the economic fishing activities of that area.
	Negative:
	If traps or nets have to be set for management purposes in a commercially important fish pond, this can have negative side effects on the economic fishing activities of that area.

6. Conclusion	
Overall assessment of the measure (qualitative)	
In conclusion, both aquatic nets and traps can be very useful in management actions of aquatic IAS, due to their practicality and low costs. However, their use is only cost-effective and worth considering when used either in small and confined water bodies and/or in integration with a variety of other measures, such as electrofishing, hand capturing, shooting, draining or biocontrol. Because of the lack of humaneness and strong negative effects on non-target fauna, the use of any type of gill net, trawl net and hook and line should be avoided, with a preference for the use of seine nets. Aquatic traps are usually less invasive to animals caught and, if designed correctly and checked regularly, only have mild effects on non-target animals.	
Assessor:	Ana Nunes
Reviewer 1:	Riccardo Scalera
Reviewer 2:	Sandro Bertolino

7. References

[1] West P., Brown A., Hall K. (2007). Review of alien fish monitoring techniques, indicators and protocols: Implications for national monitoring of Australia's inland river systems. Invasive Animals Cooperative Research Centre, Canberra.

[2] Sancho Alcayde V., Lacomba Andueza J.I., Bataller Gimeno J.V., Pradillo Carrasco A. (2015). Manual para el Control y Erradicación de Galápagos Invasores. Colección Manuales Técnicos de Biodiversidad, 6. Conselleria d'Agricultura, MediAmbient, Canvi Climàtic i Desenvolupament Rural. Generalitat Valenciana. Valencia.

[3] Louette G., Devisscher S., Adriaens T. (2013). Control of invasive American bullfrog *Lithobates catesbeianus* in small shallow water bodies. European Journal of Wildlife Research 59: 105–114.

[4] D'Amore A., Kirby E., McNicholas M. (2009). Invasive species shifts ontogenetic resource partitioning and microhabitat use of a threatened native amphibian. Aquatic Conservation: Marine and Freshwater Ecosystems 19: 534–541.

[5] Lukey N. (2017). Management of introduced American bullfrogs (*Lithobates catesbeiana* Shaw 1802) in the South Okanagan, British Columbia. MSc Thesis, University of Waterloo. Waterloo, Ontario, Canada.

[6] Kamoroff C., Daniele N., Grasso R.L., Rising R., Espinoza T., Goldberg C.S. (2020). Effective removal of the American bullfrog (*Lithobates catesbeianus*) on a landscape level: long term monitoring and removal efforts in Yosemite Valley, Yosemite National Park. Biological Invasions 22: 617–626.

[7] Britton J.R., Davies G.D., Brazier M. (2010). Towards the successful control of the invasive *Pseudorasbora parva* in the UK. Biological Invasions 12: 125–131.

[8] Davies G.D., Britton J.R. (2015). Assessing the efficacy and ecology of biocontrol and biomanipulation for managing invasive pest fish. Journal of Applied Ecology 52: 1264–1273.

[9] O'Keeffe S. (2005). Investing in conjecture: eradicating the red-eared slider in Queensland. Proceedings of the 13th Australasian Vertebrate Pest Conference, Wellington, New Zealand, 169-176.

[10] O'Keeffe S. (2009). The practicalities of eradicating red-eared slider turtles (*Trachemys scripta elegans*). Aliens: The Invasive Species Bulletin. Newsletter of the IUCN/SSC Invasive Species Specialist Group 28: 19-24.

[11] Davies T.E., Beanjara N., Tregenza T. (2009). A socio-economic perspective on gear-based management in an artisanal fishery in south-west Madagascar. Fisheries Management and Ecology 16(4): 279-289.

[12] Galanidi M., Turan C., Öztürk B., Zenetos A. (2019). Europen Union (EU) Risk Assessment of *Plotosus lineatus* (Thunberg, 1787); a summary and information update. Journal of the Black Sea/Mediterranean Environment 25(2): 210-231.

[13] Consorci de l'Estany (2014). Protocolo de control de peces exóticos. Protocolo de control de peces exóticos en el lago de Banyoles y otras masas de agua menores de su entorno. LIFE12 NAT/ES/001091 - "Conservación de fauna fluvial de interés europeo en red Natura 2000 de las cuencas de los ríos Ter, Fluvià y Muga".

[14] Haubrock P.J., Criado A., Monteoliva A.P., Monteoliva J.A., Santiago T., Inghilesi A.F., Tricarico E. (2018). Control and eradication efforts of aquatic alien fish species in Lake Caicedo Yuso-Arreo. Management of Biological Invasions 9(3): 267–278.

[15] Galanidi M., Zenetos A., Sewell J. (2017). Study on Invasive Alien Species – Development of risk assessments to tackle priority species and enhance prevention. Final Report, *Plotosus lineatus* (Thunberg, 1787). Annex 12. Evidence on measures and their implementation cost and cost-effectiveness. Contract No 07.0202/2016/740982/ETU/ENV.D2.

[16] LIFE INVASEP (2016). Manual de captura y plan de control de galápago de Florida. Acción C.2 "Ensayos para el control y erradicación de la Tortuga de Florida (*Trachemys scripta elegans*)" del Proyecto LIFE10 NAT/ES/000582 (INVASEP). https://vdocuments.mx/document/manual-de-captura-y-plan-de-control-de-galpago-de-2019-04-15-3-1-introduccin.html

[17] Groffen J., Kong S., Jang Y., Borzée A. (2019). The invasive American bullfrog (*Lithobates catesbeianus*) in the Republic of Korea: history and recommendations for population control. Management of Biological Invasions 10(3): 517–535.

[18] Sarat E., Dupuy F. (2016). American bullfrog (*Lithobates catesbeianus*) - Managing the American bullfrog in the Périgord-Limousin regional nature park. Knowledge for action series, Onema and IUCN France. https://professionnels.ofb.fr/sites/default/files/en/doc/documentation/KFA2018_EEE-vol3_18-ABullfrog-1-Perigord.pdf

[19] Snow N.P., Witmer, G. (2010). American Bullfrogs as Invasive Species: A Review of the Introduction, Subsequent Problems, Management Options, and Future Directions. Proc. 24th Vertebr. Pest Conf. (R. M. Timm and K. A. Fagerstone, Eds.). University of California, Davis, 86-89.

[20] Snow N.P., Witmer G.W. (2011). A field evaluation of a trap for invasive American bullfrogs. Pacific Conservation Biology 17: 285–291.

[21] Triece K., Ehrenberger J., Davenport N. (2018). Investigating bullfrog management on the front range. Jefferson County Colorado Open Space.

[22] Pietro V., Claudio F., Silvia G., Paolo S., Silvia Z. (n.d.). Reducing the spread of invasive fish species through a canal connecting two lakes: assessment of efficacy and practicality of different gears. Project IdroLIFE LIFE15 NAT/IT/000823. https://idrolife.eu/wp-content/uploads/2020/02/Comunicazione-scientifica_poster-2.pdf

[23] Davison P.I., Copp G.H., Créach V., Vilizzi L., Britton J.R. (2017). Application of environmental DNA analysis to inform invasive fish eradication operations.

The Science of Nature 104: 35.
[24] Czeglédi I., Preiszner B., Vitál Z., Kern B., Boross N., Specziár A., Takács P., Erős T. (2019). Habitat use of invasive monkey goby (*Neogobius fluviatilis*) and pumpkinseed (*Lepomis gibbosus*) in Lake Balaton (Hungary): a comparison of electrofishing and fyke netting. Hydrobiologia 846: 147–158.
[25] Pupina A., Pupins M., Skute A., Pupina A., Karklins A. (2015). The distribution of the invasive fish Amur sleeper, rotan *Perccottus glenii* Dybowski, 1877 (Osteichthyes, Odontobutidae), in Latvia. Acta Biologica Universitatis Daugavpiliensis 15(2): 329-341.
[26] De Vries W., Rannap R., Briggs L. (2012). Guidelines for eradication of invasive alien aquatic species. Project Report "Securing *Leucorrhinia pectoralis* and *Pelobates fuscus* in the northern distribution area in Estonia and Denmark". LIFE08NAT/EE/000257.
[27] Verreycken, H. 2019. Information on measures and related costs in relation to the species included on the Union list: *Perccottus glenii*. Technical note prepared by IUCN for the European Commission.
[28] LIFE-Trachemys (2012). Memoria intermedia de actuaciones. Años 2011-2012. Informes LIFETrachemys n° 14. Conselleria d'Infraestructures, Territori i Medi Ambient, 26 pp.
[29] García-Díaz P., Ramsey D.S.L., Woolnough A.P., Franch M., Llorente G.A. et al. (2017). Challenges in confirming eradication success of invasive red-eared sliders. Biological Invasions 19: 2739–2750.
[30] Blottière D., Lascève M. (2018). Red-eared slider turtle (*Trachemys scripta elegans*) - Managing a population of red-eared slider turtles on the Vieux Salins site in Hyères (Var department). Knowledge for action series, Onema and IUCN France. http://www.especes-exotiques-envahissantes.fr/wp-content/uploads/2018/09/rex-red-eared-slider-turtle-hyre-uk-v4.pdf
[31] Zanetti M. (2018). River functionality index as planning instrument for a good governance of Sile's ecosystem. Azione C7. Controllo e eradicazione della specie *Trachemys scripta* spp. e altre testuggini palustri esotiche. Relazione annuale e conclusiva. LIFE14 NAT/IT/000809, Anno 2018. 31p.
[32] Valdeón A., Crespo-Diaz A., Egaña-Callejo A., Gosá A. (2010). Update of the pond slider *Trachemys scripta* (Schoepff, 1792) records in Navarre (Northern Spain), and presentation of the Aranzadi Turtle Trap for its population control. Aquatic Invasions 5: 297–302.
[33] Drost C.A., Lovich J.E., Madrak S.V., Monatesti A.J. (2011). Removal of nonnative slider turtles (*Trachemys scripta*) and effects on native Sonora mud turtles (*Kinosternon sonoriense*) at Montezuma Well, Yavapai County, Arizona: U.S. Geological Survey Open-File Report 2010–1177, 48 pp.
[34] Adriaens T., Devisscher S., Louette G. (2013). Risk analysis of American bullfrog, *Lithobates catesbeianus*. Risk analysis report of non-native organisms in Belgium. Rapporten van het Instituut voor Natuur- en Bosonderzoek 2013, INBO.R.2013.41, Instituut voor Natuur- en Bosonderzoek (INBO), Brussel. 56p.
[35] Vogel J., Sternberg M., Fraser K., Bates E. (2017). American Bullfrog Surveillance and Eradication Program. Prepared with financial support of the Fish and Wildlife Compensation Program on behalf of its program partners BC Hydro, the Province of BC, Fisheries and Oceans Canada, First Nations and public stakeholders.
[36] Sarat E., Mazaubert E., Dutartre A., Poulet N., Soubeyran Y. (2015a). Invasive alien species in aquatic environments. Practical information and management insights. Volume 2. Management insights. Onema. Knowledge for action series. 252 pages.
[37] Adriaens T., Brys R., Halfmaerten D., Devisscher S. (2019). Information on measures and related costs in relation to species included on the Union list – *Lithobates catesbeianus*. Technical note prepared by IUCN for the European Commission.
[38] Sancho V., Lacomba J.I. (2013). Expansion of *Trachemys scripta* in the Valencian Community (Eastern Spain). Proceedings of the International Symposium on Freshwater Turtles Conservation, Vila Nova de Gaia, Portugal, 41–49.
[39] Potter E.C.E, Pawson M.G. (1991). Gill netting. Laboratory leaflet Number 69. Ministry of Agriculture, Fisheries and Food Directorate of Fisheries Research. Lowestoft, UK, 34 pp.
[40] Fishcount (2019). Trawling. http://fishcount.org.uk/fish-welfare-in-commercial-fishing/capture/trawl.
[41] Cooke S.J., Suski C.D., Barthel B.L., Ostrand K.G., Tufts B.L., Philipp D.P. (2003). Injury and Mortality Induced by Four Hook Types on Bluegill and Pumpkinseed. North American Journal of Fisheries Management 23:3: 883-893.
[42] Larocque S.M., Colotelo A.H., Cooke S.J., Blouin-Demers G., Haxton T., Smokorowski K.E. (2012). Seasonal patterns in bycatch composition and mortality associated with a freshwater hoop net fishery. Animal Conservation 15: 53–60.
[43] Sarat E., Mazaubert E., Dutartre A., Poulet N., Soubeyran Y. (2015b). Invasive alien species in aquatic environments. Practical information and management insights. Volume 1. Practical information. Onema. Knowledge for action series. 252 pages.
[44] Tiberti R., Bogliani G., Brighenti S., Iacobuzio R., Liautaud K., Rolla M., von Hardenberg A., Bassano B. (2019). Recovery of high mountain Alpine lakes after the eradication of introduced brook trout *Salvelinus fontinalis* using non-chemical methods. Biological Invasions 21: 875–894.
[45] Borkowski R. (1997). Lead poisoning and intestinal perforations in a snapping turtle (*Chelydra serpentina*) due to fishing gear ingestion. J Zoo Wildlife Med 28: 109–113.
[46] Real Decreto 630/2013, de 2 de agosto, por el que se regula el Catálogo español de especies exóticas invasoras. (BOE n° 185 de 3 de agosto de 2013).

[47] Pfeiffenschneider, M. H., F. (2020). Plan d'action pour espèces exotiques envahissantes au Grand-Duché de Luxembourg : Perche soleil, *Lepomis gibbosus* (Linnaeus, 1758). In L. Administration de la nature et des forêts (Ed.), (pp. 20).
[48] Blaubandbärbling – Management- und Maßnahmenblatt zu VO (EU) Nr. 1143/2014 (2018). https://neobiota.bfn.de/unionsliste/art-19-management.html
[49] De Sousa, T. (2020). Plan d'action pour espèces exotiques envahissantes au Grand-Duché de Luxembourg: le Goujon asiatique, *Pseudorasbora parva* (Temminck & Schlegel, 1846). In L. Administration de la nature et des forêts (Ed.), (pp. 21).
[50] Buchstaben-Schmuckschildkröte – Management- und Maßnahmenblatt zu VO (EU) Nr. 1143/2014 (2018). https://neobiota.bfn.de/unionsliste/art-19-management.html
[51] De Sousa, T. (2019). Plan d'action pour espèces exotiques envahissantes au Grand-Duché de Luxembourg: la Tortue de Floride, Trachemys scripta ssp. (Schoepff, 1792). In L. Administration de la nature et des forêts (Ed.), (13/12/2019 ed.).

Appendix 7. Egg oiling

1. Measure name			
1.1. English:	Egg oiling		
1.2. Lethal or non-lethal:	Lethal		
1.3. Other languages (if available):			
Bulgarian	Омасляване на яйцата	Italian	Oliare le uova
Croatian	Nauljivanje jaja	Latvian	Putnu olu eļļošana ar parafīnu
Czech	Olejování vajec	Lithuanian	Kiaušinių vaškavimas (parafinizavimas)
Danish	Oliering af æg	Maltese	
Dutch	Oliën van eieren	Polish	Olejowanie jaj
Estonian	Munade õlitamine - munad määritakse õliga kokku ja asetatakse pesadesse tagasi	Portuguese	Cobertura de ovos com parafina
Finnish	Munien öljyäminen	Romanian	Acoperirea ouălor cu ulei
French	Huiler les oeufs	Slovak	Olejovanie vajec
German	Einölen von Eiern, Eianstechen, Einsatz von Gipseiern	Slovenian	Oljenje jajc
Greek	Λίπανση αυγών	Spanish	Control de puestas con parafina
Hungarian	Tojáslakkozás	Swedish	Oljering av ägg
Irish			

2. Technical details of measure
2.1.a. Measure description
This measure includes egg oiling, pricking, shaking and replacement. Egg oiling is a method of population management whereby bird eggs are coated with a substance such as mineral or corn oil. The coating prevents the necessary gas exchange through the shell which results in the death of the embryo. For this method the eggs are removed from the nest, the coating is applied and then the eggs are returned [1]. Egg addling is the broader term used to define the several different processes that result in a fertilised egg losing viability (including egg oiling, but also egg prickling, shaking, freezing, removal and destruction) [2]. The advantage of the egg oiling technique is that the female will then continue to incubate the eggs, generally for a longer period in comparison to the other techniques where the eggs are still returned but more often result in shorter incubation times or early nest abandonment, e.g. with egg prickling. By returning the eggs and prolonging the incubation, this will reduce the chance of the female from re-laying during the breeding season [3]. In the EU, egg oiling substances, e.g. paraffin oil, when used for coating eggs in order to control the population size of nesting birds, is not considered to be a restricted biocidal product for the purposes of Article 3(1)(a) of Regulation (EU) No. 528/2012 concerning making available on the market and use of biocidal products.

Pricking a pin-hole in the egg, or shaking the egg also renders them nonviable [2]. In such cases, the female will continue to incubate for longer than the usual incubation period. The drawback to this method is the same as if nesting material was removed: the birds will eventually lay another clutch, often in a new nesting site that may contain an active nest. Many experienced nest monitors concede that nest removal and prolonged incubation are temporary and not long-term solutions. Provided bird nests can be located and are accessible, then eggs can be removed or destroyed to reduce productivity (see Hand removal measure). In some instances, removed eggs can be replaced by dummy eggs to prevent replacement with a new clutch [3].

2.1.b. Integration with other measures

As egg oiling on Egyptian geese is not deemed to be very effective (see 4. Costs and effectiveness of the measure below), this method is best implemented in conjunction with other eradication measures such as **trapping and direct shooting.** In certain areas where other eradication measures are not applicable/permitted - such as when hunting is restricted in protected areas - egg oiling can be implemented as an individual measure, but it will likely contribute more to population control compared to rapid eradication [1].

It is suggested that shooting/hunting are the most effective for rapid eradication, alongside evidence that Larsen traps (live traps with decoys) can also be employed effectively [4].

2.2.a. Availability - species and objectives

Objective	**Unknown objective**		**Rapid Eradication**		**Management**					
					Eradication		**Control**		**Containment**	
Species	Avail.	Ref(s).	Avail.	Ref(s).	Avail.	Ref(s).	Avail.	Ref(s).	Avail.	Ref(s).
Acridotheres tristis			P		P		P		P	
Alopochen aegyptiaca					P		A	1, 17	A	
Callosciurus erythraeus										
Corvus splendens			P				A	8		
Herpestes javanicus										
Lepomis gibbosus										
Lithobates catesbeianus										
Muntiacus reevesi										
Myocastor coypus										
Nasua nasua										
Nyctereutes procyonoides										
Ondatra zibethicus										
Oxyura jamaicensis					A	12	A	12	A	
Perccottus glenii										
Plotosus lineatus										
Procyon lotor										
Pseudorasbora parva										
Sciurus carolinensis										
Sciurus niger										
Tamias sibiricus										

Threskiornis aethiopicus			P		P		A	7	P	
Trachemys scripta										

2.2.b. Application – EU Member States and objectives

Objective	**Unknown objective**		**Rapid Eradication**		**Management**					
					Eradication		**Control**		**Containment**	
Country	Avail.	Ref(s).	Avail.	Ref(s).	Avail.	Ref(s).	Avail.	Ref(s).	Avail.	Ref(s).
Austria							X	Expert opinion		
Belgium										
Bulgaria										
Croatia										
Cyprus										
Czech Republic										
Denmark										
Estonia										
Finland										
France										
Germany										
Greece										
Hungary										
Ireland										
Italy										
Latvia										
Lithuania										
Luxembourg							X	17		
Malta										
Netherlands										
Poland										
Portugal										
Romania										
Slovakia							X	Expert opinion		
Slovenia										
Spain										
Sweden										
United Kingdom*							X	1, 12		

* Not an EU Member State

3. Humaneness of the measure			
3.1. Welfare for all measures			
Measure type (if applicable):	**Humaneness impact categories**		
Domain	**No impact**	**Mild-Moderate**	**Severe - Extreme**
1: Water deprivation, food deprivation, malnutrition	The embryos are not restricted from food or water as these provisions are accessible via the yolk within the egg [5].		
2: Environmental challenge	No impact before the mode of death is applied.		
3: Injury, disease, functional impairment	There are no obvious signs of chick abnormalities in the embryos that were treated with paraffin [3].		
4: Behavioural, interactive restriction	The embryo is not impacted behaviourally as it is in a sleep like unconscious state [6].	Nesting birds actively defend their eggs and it could be necessary to drive them away or hold the bird off the nest for the shortest time needed to complete oiling. This may cause some stress or injuries. Potentially increase incubation times beyond what is normally experienced in the species could affect the brooding female as there will be prolonged time where she is unable to prioritise her only physiological requirements.	
5: Anxiety, fear, pain, distress, thirst, hunger etc.	With the evidence that unconscious states are likely predominant in unhatched eggs before 80% of the incubation has been attained [6, 15], the ability of the foetus to be consciously aware and suffering is limited.	In some cases there may be mild levels of distress caused to parent birds (see above).	

	Little is known about the effect of this method on the subsequent behaviour of nesting birds and their potential effects on population dynamics. However, research has shown that the stage of incubation when egg oiling is performed can delay when nest abandonment occurs [16].		

3.2. Mode of death (if relevant)			
Measure type (if applicable):	**Immediate death (i.e. no suffering)**	**Not immediate death (mild - moderate suffering)**	**Not immediate death (severe - extreme suffering)**
Rationale:	The embryos die from asphyxia, as they are deprived of their environmental oxygen [3]. However conscious perception is not thought to be present in chicks until 80% of incubation has been attained, although species specific differences may occur [15]. Prior to this point, the embryo is experiencing sleep-like unconsciousness [6] it can be assumed that no impact is perceived by the embryo if the asphyxiation is operated during this early stage.	Bird embryos that have attained > 80% incubation demonstrate a level of consciousness which is sufficient for pain and breathlessness perception. Consequently, egg oiling should be used only with eggs that have attained less than 80% of incubation. Because research is still evolving and there are species-specific differences in development, euthanasia of embryos should be performed based on the best available data and with attention to assuring, as best as possible, that conscious suffering does not occur [15].	

3.3. Humaneness summary	An embryo in development is unable to perceive pain and suffering until more than 80% of incubation has been attained. Therefore, this measure can be considered a humane control measure if applied before more than 80% of the incubation has been attained. However, research on Canadian geese indicates that egg oiling should be applied as late as possible in incubation to result in geese remaining at the nest site well past the expected hatch date [16]. This suggests that the more humane implementation of the method could reduce its effectiveness.

4. Costs and effectiveness of the measure	
General effectiveness of the measure	The application of egg oiling is extremely effective once it is applied to an egg at any stage of egg development. Various studies record very high success rates with many documenting 100% success rate with egg oiling treatment [7,8]. The effectiveness of the measure in terms of population control is more dependent on the ease of locating nests and the ability to treat a vast majority of nests as it has been estimated that 88% of nests need to be detected in order to revert population growth [9, 10]. In the case studies that focused on smaller populations where nest detection was high, this measure could be effectively employed. However, with larger population, the effort required to locate all nests increases as well as the likelihood that nests will go undetected, especially in species that do not breed colonially, like the Egyptian goose [1]. Shooting and culling are generally identified as the more effective measure of population control and eradication in these avian species [1, 11, 9]. If the egg oiling was completely successful in preventing all reproduction in a population, the numbers would look to reduce at the rate of normal adult mortality – which is species dependent, but in the Canadian goose is estimated at 10-15% annually [3].

4.1. Case studies	
CASE STUDY #1	
Measure type (if relevant):	Egg control (specific destruction method not listed) [12]
Species:	Ruddy Duck (*Oxyura jamaicensis*)
Objective:	Assessment on suitability for use in population control
Use of measure	This was a comparison study comparing egg control measures with shooting and nest-trapping to identify effectiveness of each on the population management. The study identified factors such as the hours of control effort and then used population modelling techniques to identify which measure is most efficient overall.
Combined with other measure(s):	Egg control was utilised and compared to the other methods of shooting and nest-trapping.
Country(ies) of application:	England and Wales
Geographic scale (km^2) and/or population size measure applied to:	Three methods were analysed on an initial population of 3,300 and were compared in order of the effectiveness of reducing the population to less than 50 birds. Population modelling incorporated information such as number of animals, distribution, recruitment, mortality, immigration and emigration.
Time period:	Egg control methods were employed over a two-year period from 1993-1994.
Effort:	Egg-control was conducted at a rate of one nest per 5.3 hours in 1993 and one nest per 10.1 hours in 1994. The marked difference between the two years was attributed to a higher such rate by field workers in 1993 as well as a higher number of nests attempted to be obtained in 1994 – meaning that more hours would be required for the more difficult nests to be located, obtained and treated.

Costs:	Overall costs:	
	No specific costs for the method were listed.	
	Personnel costs:	
	The time commitment comparatively per measure would be an indication of the costs associated with personnel needed to apply the measure. Shooting required significantly less hours to control one bird 0.6-2.9 hours, compared to the egg-control technique which required 5.3-10.1 hours per nest, which was also comparative more time efficient than nest-trapping which required 14.7-25.6 hours per bird captured.	
	Equipment and infrastructure:	
	Other, including overheads:	
Effectiveness:	When the three methods were compared with the effort needed (measures in man-hours) compared to the direct effect on the population (how effective each measure is at reducing population numbers), shooting was deemed the most effective overall, followed by nest-trapping and lastly egg control despite nest-trapping requiring significantly more hours to capture a single bird. Egg oiling was also used on a small number of sites during the campaign but was not considered a cost-effective method. It raised significant health and safety issues for operatives as many ruddy duck nests are on floating vegetation mats	

CASE STUDY #2	
Measure type (if relevant):	Egg oiling [8]
Species:	Indian House Crow (*Corvus splendens*)
Objective:	Population control
Use of measure	In this case study the measure of egg oiling was applied using equipment that allowed for access of highly situated nests (up to 18m high). The device (https://patentscope.wipo.int/search/en/detail.jsf?docId=WO2019021265&tab=PCTBIBLIO) was controlled by humans on the ground and therefore allowed for the administration of the oil without direct human contact. This reduces the difficulty experienced with other species in which egg oiling has been applied where access to the nest sites has been a restricting factor to the methods success.
Combined with other measure(s):	Used as a stand-alone method
Country(ies) of application:	Eilat region in southern Israel
Geographic scale (km^2) and/or population size measure applied to:	300-400 nests, 91 were subjected to oiling and others were subjected to nest removal.
Time period:	The measure was applied over a two-year period from 2016 – 2017.
Effort:	
Costs:	Overall costs:

	Personnel costs:
	Unknown, however the implementation of the device which allows for delivery of oil without direct human access would reduce the personnel costs and decrease safety risks when. Dealing with nests in difficult to reach areas.
	Equipment and infrastructure:
	See device https://patentscope.wipo.int/search/en/detail.jsf?docId=WO2019021265&tab=PCTBIBLIO
	Other, including overheads:
Effectiveness:	When revisited, none of the oiled nests (91) had hatched. 45% of the nests that were oiled were abandoned, with the other 55% of treated nests being incubated for three weeks or more. This approach resulted in a 19% decrease to the population.

CASE STUDY #3	
Measure type (if relevant):	Egg oiling [7]
Species:	The Australian White Ibis (*Threskiornis molucca*) [incl. as similar to *T. aethiopicus*]
Objective:	Population control
Use of measure	Canola oil was used to coat the Ibis eggs through a handheld spray bottle. There were several treatments which involved multiple application of the oil and at different stages of egg development in order to test the success of the treatment in different application and situations. There were also control groups which did not receive the oil treatment. The nests were monitored for hatching success.
Combined with other measure(s):	Used as a stand-alone method
Country(ies) of application:	Australia
Geographic scale (km^2) and/or population size measure applied to:	Field trials were conducted at two ibis nesting colonies: Cabramatta Creek and Lake Gillawarna. The number of eggs that were treated throughout the study was approximately 584.
Time period:	Treatments occurred from August to October 2005. The population was monitored from August 2005 to February 2006.
Effort:	This study recommends that the most effective interval for checking nests for the application of this measure on the ibis is every 19 – 21 days. At this interval there will be limited number of nests that could have been laid and reached hatching without detection, as the average incubation period is 23 days for this species. The study recommends that the measure be applied to a population for the entire laying duration or until the group disbands, this can be up to 6 months.
Costs:	Overall costs:

	The cost effectiveness in this study is compared to other nest and egg destruction techniques. In these measures the costs are mainly associated with personnel requirements as the equipment necessary for the measure is not particularly expensive. It is suggested in the study that this is considered a cost-effective method with low labour requirements and costs.
	Personnel costs:
	For the suggested application of this measure on this species, personnel would be required for 1-2 days every 19-21 days over a 6-month period depending on the size of the population and the ease of nest detection.
	Equipment and infrastructure:
	Ladder was required to access nests approximately 3-5m high. Plastic tags for monitoring nests. Canola oil delivered by a 500ml hand-held spray bottle
	Other, including overheads:
Effectiveness:	The field study found that the application of canola oil was 98% effective in preventing the treated eggs from hatching. This was irrespective of how often the treatment was applied, indicating that a one-off application of the canola oil was sufficient in achieving the desired outcome. This study also found the treatment was effective irrespective of the stage of development of the egg when it received the treatment, which would be beneficial when applying the measure over multiple nests that were laid at different times of the breeding season. As the ibis is a multi-brooding species, the extended incubation period associated with the treatment will also aid in preventing further clutches being laid throughout the breeding season. Egg oiling is potentially more efficient than nest and egg destruction measures, as these could be responsible for the fragmentation of larger breeding populations and create smaller populations dispersed over a larger area, making further management more difficult and require more effort, time and higher costs. This study showed a significant decrease in the number of eggs and nests over a 6-month period, indicating the possibility of implementation of the measure for population control. It is also highlighted that this measure has been shown to be more socially accepted compared to culling measures.

4.2. Costs effectiveness summary	It is estimated that destruction of eggs could cost up $40 USD per egg [13]. Most of the costs associated with the measure of egg oiling relate to the labour costs, which will be directly impacted by the size of the populations managed, the distribution and the ability to locate and treat nests. Whilst some studies indicate that this measure is more cost effective that other egg/nest destruction methods [7], most studies indicate that the effectiveness, time required and therefore costs are not reasonable to make this method feasible for larger population control in comparison to culling methods.

5. Side effects	
Non-target native species, their habitats and the broader environment:	Positive:
	The quick drying nature of the liquid paraffin and no evidence of plumage affected in the nesting female would suggest that there are little residual effects from the oil on the natural habitat [3].
	Negative:
Other invasive alien species:	Positive:
	No obvious signs of distress in the brooding Canadian geese whose nests were treated with liquid paraffin. Furthermore, there were no significant impacts for those that experienced longer than normal incubation periods, no obvious signs of plumage affected by incubation on treated eggs [3].
	Negative:
	When humans are accessing the nest site, this may cause disturbance to nesting birds as well as other native animal living in the habitat that are not used to the presence of humans.
Public health and well-being:	Positive:
	It has been shown that egg oiling is a more socially accepted method of population control [7].
	Negative:
Economic:	Positive:
	The measure could be more economically viable when compared to other nest destruction techniques. Which may result in fragmentation of populations making the populations harder to manage and therefore more expensive [7].
	Negative:

6. Conclusion
Overall assessment of the measure (qualitative)
Overall, the measure of egg oiling is deemed to be humane on the target animals (particularly if applied in an early stage of the incubation period, see above) and cause little adverse effects on non-target animals. Once applied, the method is extremely effective on preventing the hatching of treated eggs. However, this method shows to require significant time commitment when dealing with larger populations which creates a significant cost due to personnel requirements as location of nests in order to achieve the appropriate number to effectively reduce population requires more effort. The

method has been shown to reduce population size [8], but other evidence indicates that it is the lesser of effective measures behind shooting and nest-trapping. This measure would be useful to employ as a combination with other measures, or where more effective measures are inapplicable due to the location of the population e.g. national protected areas, or if shooting or other culling methods are not socially accepted. Egg oiling can be effective on small populations where nests are easily accessible and detectable.	
Assessor:	EAZA
Reviewer 1:	Riccardo Scalera
Reviewer 2:	Ilaria Di Silvestre

7. References

[1] Strubbe, D. (2017). Information on measures and related costs in relation to species included on the Union list: Alopochen aegyptiaca. *Technical note prepared by IUCN for the European Commission.*

[2] Pochop, P. A, Cummings, J. L, Yoder, C. A, & Steuber, J. E. (1998). Comparison of white mineral oil and corn oil to reduce hatchability in ring-billed gull eggs. *Proceedings of the Vertebrate Pest Conference*, 18. http://dx.doi.org/10.5070/V418110097 Retrieved from https://escholarship.org/uc/item/6xc8r0gp

[3] S. J. Baker, C. J. Feare, C. J. Wilson, D. S. Malam & G. R. Sellars (1993). Prevention of breeding of Canada Geese by coating eggs with liquid paraffin, *International Journal of Pest Management*, 39:2, 246-249, DOI: 10.1080/09670879309371798

[4] Adriaens, T., Huysentruyt, F., De Bus, K., Van Moer, K., Standaert, S., & Casaer, J. (2013). P.TL.02 Testing the efficacy of a floating multicapture trap for invasive Egyptian geese. *In Programme & Abstract Book 31st IUGB Congress 27-29.08.2013*. Brussels, Belgium. (pp. 227). International Union of Game Biologists.

[5] van der Wagt, I., de Jong, I. C., Mitchell, M. A., Molenaar, R., & van den Brand, H. (2020). A review on yolk sac utilization in poultry. Poultry science, 99(4), 2162–2175. https://doi.org/10.1016/j.psj.2019.11.041

[6] Mellor, D.J. & Diesch, T.J, (2007). Birth and hatching: Key events in the onset of awareness in the lamb and chick. *New Zealand Veterinary Journal*. 55:2, 51-60, DOI: 10.1080/00480169.2007.36742

[7] Martin, J., French, K., & Major, R., (2007). The pest status of Australian white ibis (Threskiornis molucca) in urban situations and the effectiveness of egg-oil in reproductive control. *Wildlife Research*. 34. 319-324. 10.1071/WR07005.

[8] Motro, Yoav. (2017). In- situ sterilization of eggs in high nests – Indian House Crows. Borowski, Z., Olech, W. & Suchecka, A. (eds.). *11th European Vertebrate Pest Management Conference*. Warsaw, Poland. 25-29/09/2017.

[9] Klok, C., van Turnhout, C., Willems, F., Voslamber, B., Ebbinge, B. & Schekkerman, H., (2010). Analysis of population development and effectiveness of management in resident greylag geese Anser anser in the Netherlands. *Animal Biology*. 60. 373-393. 10.1163/157075610X523260.

[10] Van Daele, P., Adriaens, T., Devisscher, S., Huysentruyt, F., Voslamber, B., De Boer, V., ... Casaer, J. (2012). *Management of summer geese in Flanders and Zeelandic Flanders: report prepared in the context of the INVEXO - INTERREG project* . (Reports from the Institute of Nature and Forest Research; No. INBO.R.2012.58). Brussels: Institute for Nature and Forest Research.

[11] Henderson, I. & Robertson, P., (2007). Control and eradication of the North American Ruddy Duck in Europe. *Managing Vertebrate Invasive Species: Proceedings of an International Symposium.* paper 16, http://digitalcommons.unl.edu/nwrcinvasive/16.

[12] Hughes, B., Kirby, J.S. & Rowcliffe, J.M. (1999). Waterbird conflicts in Britain and Ireland: Ruddy Ducks Oxyura jamaicensis, Canada Geese Branta canadensis, and Cormorants Phalacrocorax carbo. *Wildfowl* 50: 77-99.

[13] Keefe, T. (1996). Feasibility study on processing nuisance Canada geese for human consumption. Minnesota Department of Natural Resources, Section of Wildlife, St. Paul, USA

[14] Lensink, R., (1999). Aspects of the biology of Egyptian Goose Alopochenaegyptiacus colonizing The Netherlands. *Bird Study*. 46:2, 195-204, DOI:10.1080/00063659909461131

[15] American Veterinary Medical Association (2020) AVMA Guidelines for the Euthanasia of Animals: Version 2020.0.1, https://www.avma.org/sites/default/files/2020-02/Guidelines-on-Euthanasia-2020.pdf

[16] Beaumont, Matthieu & Rodrigue, Jean & Pilotte, Catherine & Chalifour, Émilie & Giroux, Jean-François. (2018). Behavioral response of canada geese to egg-oiling and nest removal: Controlling Temperate-Nesting Canada Geese. The Journal of Wildlife Management. 82. 10.1002/jwmg.21486
[17] Waschbär – Management- und Maßnahmenblatt zu VO (EU) Nr. 1143/2014 (2018). https://neobiota.bfn.de/unionsliste/art-19-management.html

Appendix 8. Electrofishing

1. Measure name			
1.1. English:	Electrofishing		
1.2. Lethal or non-lethal:	Lethal to Non-lethal		
1.3. Other languages (if available):			
Bulgarian	Електроулов	Italian	Pesca elettrica, Elettropesca
Croatian	Elektroribolov	Latvian	Elektrozveja
Czech	Odlovy elektrickým agregátem	Lithuanian	Žūklė elektra
Danish	Elektrofiskeri	Maltese	
Dutch	Elektrisch vissen	Polish	Elektropołowy ryb
Estonian	Kalade elektripüük	Portuguese	Pesca elétrica
Finnish	Sähkökalastus	Romanian	Pescuit electric
French	Électropêche	Slovak	Odlov elektrickým agregátom
German	Elektrofischerei	Slovenian	Elektroribolov
Greek	Ηλεκτραλιεία	Spanish	Pesca eléctrica
Hungarian	Halászat elektromos halászgéppel	Swedish	Elfiske
Irish			

2. Technical details of measure
2.1.a. Measure description
Electrofishing is a method based on the use of a field of electric currents in water to capture fish and amphibians (but also insects and other invertebrates, both in freshwater and marine environments) [5, 33]. The use of electrical devices capable of killing and stunning mammals and birds is prohibited under the Bern Convention. Under the effect of a weakly polarized electric field, a fish will align with such field, and swim toward an electrode (a phenomenon known as electrotaxis), while they will cease to swim in case of a stronger field rolling on their sides and appearing unconscious, a neuromuscular response that seems to be best understood in terms of epilepsy [22, 23]. In the case of its use on frogs, the method is also known as electro-frogging [38]. Frogs (both adult and subadult) will stretch out their hind limbs and remain immobilized for a short period, while tadpoles will show a passive and lethargic behavior [20]. The most common electrofishing systems include backpack-mounted and boat-mounted 'electrofisher' units [6, 27, 31, 40, 45], as well as electric seines [28, 46], though the latter seem less popular. The units used for frogs are also named "electro-froggers" [38, 39], see also review by Allan and Riley [20]. As a side note this technique is also adopted in marine environments, e.g. to increase the catch efficiency and/or reduce fuel costs of bottom trawls, in which case is known as "electrotrawling" [50].

The method is known to have some harmful and even fatal effects on fish, particularly on salmonids (it seems not having major side effects on other fish and amphibians, particularly if properly used), but results on the issue are questionable and not definitive, as the level of reaction depends in general upon the strength of the electric field, and the size of the fish (bigger the fish the greater the affects), see for example the review by Snyder [22]. Damages to fish can be reduced to a minimum by the strict regulations of the fisheries authorities and by the training of the fishermen, therefore only persons with a special license should be allowed to fish with electricity [24]. Some dedicated guidance on the issue is available [32].

In general, information on specific legal issues in the EU was rarely found, with some exceptions. In Scotland, for example, fishing with electrofishing is prohibited, but the method can be used for research purpose, provided a specific permission is obtained by the relevant authorities [35]. A specific license for conducting electrofishing survey was required also in Poland [16] and Greece [48].

Dedicated protocols, manuals and guidelines for a sound implementation of the method are available, although mostly focusing on its application to surveys aimed at sampling fish population [34, 35, 36, 37, 48, 49, 54, 55], mostly dedicated to salmonids [32]. Guidance specifically aimed at the management of alien species is also available [41], although sometime only indirectly: i.e. when it was aimed at assessing the effect of the eradication/management carried out with other means (as in some eradication attempts on ***Pseudorasbora parva*** with rotenone, de-watering and disinfection) [56, 58, 59].

In relation to the common objectives of its use, electrofishing was used for the eradication of ***Lithobates catesbeianus*** [38, 57], and is considered the principal method for **species detection and assessing fish assemblage** and abundance in streams [5] for purposes which may be not necessarily linked to the management/eradication of the species. For example, in relation to the target species of this study, ***P. parva***, it was used as a monitoring method to assess the success of eradication achieved in UK by using Rotenone [4].

Also in a study aimed at assessing the result of biological control measures in Lithuania (by introducing the native piscivorous fishes *Esox lucius* and *Perca fluviatilis*), all target fish, including ***Perccottus glenii***, were captured by electrofishing through a boat [7]. In Poland the use of electrofishing was aimed at the study of the distribution of ***P. glenii*** (some individual of ***P. parva*** was also recorded) [6] and other life-history traits of the species [14]. In one of the studies, after identification the target fish all individuals of ***P. glenii*** were killed with an overdose of 2-phenoxyethanol [6]. Electrofishing was used also in Hungary, where specimens of ***P. glenii*** were collected to study the feeding ecology of the species [11]. In Romania, the first record (**detection**) of ***P. glenii*** was obtained following captures made through electrofishing [12]. Also the feeding behavior of the species in Romania was studied by capturing fish by electrofishing (it is not clear how fish were killed, but according to the authors "fish were deposited in situ on an ice bed, in a portable cooler box to avoid digestion of the stomach contents") [13]. Also in Slovakia electrofishing was used as a method to collect ***P. glenii*** with the objective to study their feeding ecology [8]. Outside the EU, experience with the use of electrofishing to capture ***P. glenii*** is available for Serbia, along the Danube, but the aim was to check the species status [10] and study the local population [9].

In relation to ***Lepomis gibbosus*** in Poland, some life-history traits of the species were studied collecting fish by electrofishing (this was conducted with a backpack electroshocker, with power output set to immobilise small fish with minimal stress and injury) [16].

Electrofishing is unlikely to be used to support the eradication of species as defined in the EU IAS regulation, i.e. in the total permanent removal of a widespread population from a Member State. However, the measure can be used to support rapid eradication and control, through supporting eradication of populations from isolated water bodies.

2.1.b. Integration with other measures

Once the animals are stunned through electrofishing, they need to be captured with conventional nets, trawls etc. to gather up the animals [25, 29, 31, 32, 35, 45], therefore the method requires by its very nature to be used in association with other ones.

For example in the case of ***P. glennii*** electrofishing was used for detection and management, combined with **drawdown (**the water level of infested ponds can be lowered, or the pond can be completely emptied**)** and with physical removal (e.g. **netting** through **fykes, traps, seine nets**) [42, 1], Similarly, ***P. parva*** was sampled in Poland and UK, using a combination of electro-fishing and **baited fish traps** [47]. Also in Germany, a minor control survey was carried using electrofishing equipment along with **landing nets** [15].

According to a study focusing on the control of the brown trout (*Salmo trutta*) in Sardinia [29], the electrofishing method was shown to be generally applicable to headstreams in Mediterranean climatic zones prone to summer droughts, in combination with **electric barriers** (e.g. to prevent upstream reinvasion of the target species). The use of **blocking nets** to prevent fish immigration is also discussed [32].

Also in the case of frogs captured though electrofishing, alternate capture methods were occasionally utilized including **hand netting** and **pellet rifle** [39]. Within the EU, the only specific experience of eradication of ***Lithobates catesbeianus*** with electrofishing was in Germany and the Netherland, in association with **fencing, seine netting, fyke netting, and drainage pond** [57, 63]

As a conclusive remark, electrofishing may require captured fish to be killed through other methods, e.g. by the use of an overdose of **anaesthetics (2-phenoxyethanol)** [6, 7] or **clove oil** [11, 16, 17]. Similarly, frogs captured though electrofishing may need to be anesthetized by submersion in a clove oil (eugenol) and water emulsion, and then euthanized by deep freezing and frozen to kill [39].

2.2.a. Availability - species and objectives

Objective	Unknown objective		Rapid Eradication		Management					
					Eradication		Control		Containment	
Species	Avail.	Ref(s).	Avail.	Ref(s).	Avail.	Ref(s).	Avail.	Ref(s).	Avail.	Ref(s).
Acridotheres tristis										
Alopochen aegyptiaca										
Callosciurus erythraeus										
Corvus splendens										
Herpestes javanicus										
Lepomis gibbosus	A	17*, 60*	P				P	65	P	
Lithobates catesbeianus	P		A	38, 57			P		P	
Muntiacus reevesi										
Myocastor coypus										
Nasua nasua										
Nyctereutes procyonoides										
Ondatra zibethicus										
Oxyura jamaicensis										

Perccottus glenii	A	6*, 8*,11*, 12*, 13*	A	1, 7**			A	15	P	
Plotosus lineatus										
Procyon lotor										
Pseudorasbora parva	A	6*, 17*, 47*, 56*, 58*, 59*	A	1, 4**			A		P	
Sciurus carolinensis										
Sciurus niger										
Tamias sibiricus										
Threskiornis aethiopicus										
Trachemys scripta										

2.2.b. Application – EU Member States and objectives										
Objective	**Unknown objective**		**Rapid Eradication**		**Management**					
					Eradication		**Control**		**Containment**	
Country	Avail.	Ref(s).	Avail.	Ref(s).	Avail.	Ref(s).	Avail.	Ref(s).	Avail.	Ref(s).
Austria										
Belgium										
Bulgaria										
Croatia										
Cyprus										
Czech Republic										
Denmark										
Estonia										
Finland										
France	X	47*, 60*					X	31		
Germany					X	38, 57	X	15		
Greece										
Hungary	X	11*, 17*								
Ireland										
Italy					X	29				
Latvia										
Lithuania					X	7**				
Luxembourg										
Malta										

Netherlands										
Poland	X	6*, 14*, 47*								
Portugal										
Romania	X	12*, 13*								
Slovakia	X	8*								
Slovenia										
Spain					X	44				
Sweden										
United Kingdom***	X	47*, 56*, 58*, 59*			X	1, 4**				

* surveys not directly aimed at the species management
** assessment of eradication through other methods
*** Not an EU Member State

3. Humaneness of the measure			
3.1. Welfare for all measures			
Measure type (if applicable):	**Humaneness impact categories**		
Domain	**No impact**	**Mild-Moderate**	**Severe - Extreme**
1: Water deprivation, food deprivation, malnutrition	The measure will be completed (i.e. the fish/amphibians will be stunned) before water or food restrictions are an issue.		
2: Environmental challenge		The measure will expose the animal to environmental conditions, e.g. electric fields, which are outside the normal range encountered by the animal. However, the impact will depend on the strength of the electric field used and the fish size/physiology. The aim of the measure's application will be to solicit a moderate impact, i.e. marked short-term environmental challenges that elicit body responses beyond the physiological	As the severity of the impacts depend on the strength of the electric field used and the fish size/physiology, the measure could lead to severe impacts, i.e. environmental challenges that lead to serious physiological compromise or permanent dysfunction, or injury.

		adaptive capacity of the animal, but where the untoward effects are readily reversed by restoration of normal conditions.	
3: Injury, disease, functional impairment		Regarding the use of electrofishing on amphibians some authors stressed that the effects on their health and behaviour is sparse and largely limited to agency reports [18]. Electrofishing equipment was successfully used (outside the EU) to capture and remove *L. catesbeianus* from streams and lakes; in particular, over 20,000 individuals were captured with zero direct mortalities [20], in fact according to the authors all captured individuals were euthanized using a separate protocol (freezing). Some basic experiments on eggs of *L. catesbeianus* showed no effects of electroshocking on them [20].	In general, to stun fishes, either AC or DC current and its pulsing frequency are controlled at a level that alters their behaviour, normally to cause paralysis but no or limited injury and possibly no death [45]. However, the harmful effects of electrofishing on fish are often not externally obvious or fatal [21, 22]. A published review and synthesis of literature on electrofishing and its harmful effects on fish stressed that in some cases spinal injuries and associated haemorrhages have been documented in over 50% of examined fish, particularly in salmonid, and less on other fish [22]. It is clear that injury rates to fishes from electrofishing may differ among ecosystems and/or habitats, and may be the result of a combination of multiple environmental variables, fish morphology, the conductivity of the fish themselves, and electrofishing settings [55].
4: Behavioural, interactive restriction		For both fish and amphibians, there is a full range of impacts reported, from no impact to fatal injuries, depending on sizes etc, which are described in detail below, under section 3.3.	For both fish and amphibians, there is a full range of impacts reported, from no impact to fatal injuries, depending on sizes etc, which are described in detail below, under section 3.3.

5: Anxiety, fear, pain, distress, thirst, hunger etc.		For both fish and amphibians, there is a full range of impacts reported, from no impact to fatal injuries, depending on sizes etc, which are described in detail below, under section 3.3.	For both fish and amphibians, there is a full range of impacts reported, from no impact to fatal injuries, depending on sizes etc, which are described in detail below, under section 3.3.

3.2. Mode of death (if relevant)			
Measure type (if applicable): Electrofishing	**Immediate death (i.e. no suffering)**	**Not immediate death (mild - moderate suffering)**	**Not immediate death (severe - extreme suffering)**
Rationale:	In general, if properly used, the method itself should not lead to the death of the animal. However, depending on the environmental conditions of the target areas, on the equipment used and the modalities of usage, and on the peculiarities of the target species, the application of this method can have harmful effects on the animals targeted (as well as to by catch) ranging across the full range of humanness impact categories in relation to the various domain of the measure type (including death, which in fact could be even not immediate).		

3.3. Humaneness summary	In general, if properly used, the method itself should not lead to the death of the animal. However, depending on the environmental conditions of the target areas, on the equipment used and the modalities of usage, and on the peculiarities of the target species, the application of this method can have harmful effects on the animals targeted (as well as to by catch) ranging across the full range of humanness impact categories in relation to the various domain of the measure type (including death, which in fact could be even not immediate).

	Overall, the information available on this regard is far from leading to any definitive conclusion, the results from studies being usually very different. The effects of electrofishing on fish, in accordance to the cumulative body of studies and research carried out across the years lead to many questions which remained unanswered and whose results are either difficult to interpret/understand or questionable [22]. In general, to stun fishes, either AC or DC current and its pulsing frequency are controlled at a level that alters their behaviour, normally to cause paralysis but no or limited injury and possibly no death [45]. However, this is not always straightforward. In fact the harmful effects of electrofishing on fish, especially endangered species, are receiving some major concern because they are often not externally obvious or fatal [21, 22]. A published review and synthesis of literature on electrofishing and its harmful effects on fish stressed that in some cases spinal injuries and associated haemorrhages have been documented in over 50% of examined fish [22]. Although electrofishing has been considered as a valuable sampling technique for over half a century, the very dynamic and complex mix of physics, physiology, and behaviour, which characterise this method remains poorly understood. New hypotheses have been advanced regarding ''power transfer'' to fish and the epileptic nature of their responses to electric fields, but these too need to be more fully explored and validated [22]. It is evident there is still much to learn about electrofishing and its potential effects on fishes [55] as there is documented evidence of high injury rates which is not consistent with the results of other studies, although there is some difference between AC and DC electrofishing. Moreover it is clear that injury rates to fishes from electrofishing may differ among ecosystems and/or habitats, and may be the result of a combination of multiple environmental variables, fish morphology, the conductivity of the fish themselves, and electrofishing settings [55]. Early studies on fish mortality and injuries (e.g. including tissue haemorrhaging, vertebral compressions, and vertebral fractures) as a consequence of electrofishing showed that their frequency varied with the type of electric current used, with spinal injury rates being about 12%, and mortality rates 11% (but figures were even higher in laboratory tests) [27]. The same authors [27] did not detect any injuries in some species but high injury rates in others, apparently depending on factors such as conductivity, temperature, and pulse frequency. Other reports with data on fish injuries caused by electrofishing are available [18, 21] and results are not always consistent. For example, results of a study on *Cottus bairdi* suggest that fish were not acutely injured during collection as has been reported in other cases [26]. A study carried out in a tropical stream in the French Guiana found that electrofishing had a low mortality rate (<2%) which is more acceptable than the 100% rate using rotenone: approximately 5,800 fish of 93 species were captured, with an electrofishing mortality rate of 1.83% [5]. This result suggests that the method can be more selective compared to other tools, and allow for a significant reduction of bycatch through identification of the target species before dispatch. Regarding the use of electrofishing on amphibians some authors stressed that the effects on their health and behaviour is sparse and largely limited to agency reports [18].

	Electrofishing equipment was successfully used (outside the EU) to capture and remove *Lithobates catesbeianus* from streams and lakes; in particular, over 20,000 individuals were captured with zero direct mortalities [20], in fact according to the authors all captured individuals were euthanized using a separate protocol (freezing). Some basic experiments on eggs of *Lithobates catesbeianus* showed no effects of electroshocking on them [20]. Another report [20], stressed that "Extensive literature has demonstrated the potential for direct injury, death, or stress-related mortality to fish as a result of electrofishing. Modern advances in electrofishing equipment and greater awareness of proper field protocols has reduced the potential for electrofishing to negatively impact fish populations. Unlike for fish, very little focused research has been directed towards assessing electrofishing impacts on amphibians. However, limited studies, including a field study conducted as part of this review, has verified a large body of qualitative observations demonstrating that amphibians, particularly pre- and post-metamorphosed frogs, are more robust against electrofishing injuries than are fish. An expert panel assembled to discuss the relationship between electrofishing injuries to fish and potential injuries to amphibians concluded that metamorphosed frogs are unlikely to suffer significant injuries, but acknowledged that greater potential exists for electrofishing-related injuries to tadpoles due to greater similarity between fish and tadpoles in tail muscle and spinal structure. However, several factors associated with tadpole biology were identified that are expected to minimize electrofishing-related injuries to tadpoles, such as differences between tadpole and steelhead habitat selection, the tadpoles naturally sedentary feeding behaviour, and the bodily transformation into a whole new creature. The robustness of frogs to electrofishing was supported by email correspondence from over 20 field biologists, none of whom reported any direct mortality or other acute effects. Three of the individuals reportedly have electrofished over 30,000 amphibians in sum, but they observed little or no short-term impacts to stunned animals and did not report any direct mortalities. Those results are in direct contrast to results from fish studies, where direct mortalities may exceed 2% of stunned individuals. At that mortality rate, electrofishing 30,000 fish would be expected to result in 600 fish mortalities. A field study with 30 adult treefrogs and two northern red-legged frogs described in this report resulted in no mortalities over a 6-day-period. Short-term results did show that electrofishing at high frequencies (60 Hz) produced short-term effects on frog jumping ability and feeding, but those effects were no longer evident after 6 days. No significant differences were evident between frogs shocked at a lower frequency of 30 Hz and control frogs that were not shocked." Also the conclusions of a recent review of electrofishing and its harmful effects on fish call for a more cautious use of this method: "Electrofishing is a valuable tool for fishery management and research, but when resultant injuries to fish, or other adverse effects, are a significant problem and cannot be adequately reduced by changes in procedure, gear, and technique, we must abandon or severely limit its use and seek less harmful alternatives. This is our ethical responsibility to the fish, the populace we serve, and ourselves" [22]. As a side note, electric stunning (although done with tools different than those used for electrofishing) is considered as sound alternative to trout asphyxiation in ice slurry as a commercial slaughter method, and according to the conclusion of a dedicated study "high quality, humanely slaughtered trout can be met by the use of an electric stunning procedure" [52], although other authors claim opposite effects [53].

4. Costs and effectiveness of the measure	
General effectiveness of the measure	Electrofishing is usually considered very efficient to catch fish [25]. A recent systematic review on fish removal techniques [40] identified 20 interventions whose stated goal eradication, 47 at population control, and 9 at either eradication or population control. According to the result of the analysis made to assess the effectiveness of different types of removal methods targeting alien fish [40], successful eradication with electrofishing was possible (58% success rate) but required intensive effort and multiple treatments over a number of years (compared to methods more effective such as those with chemical treatments), while electrofishing had the highest success for population size control (56% of data sets, of these studies with sufficient information). However, as a side note most of the studies were conducted in the US and were targeting trout. Electrofishing equipment was successfully used to also capture and remove *L. catesbeianus* from streams and lakes, i.e. outside the EU [20]. A study aimed at assessing the frog response (behavior and injury) to electrofishing on *L. catesbeianus* was carried out, including (e.g. voltage gradient and power density thresholds that resulted in immobilization of early life stages) [18]. As a result, caution should always be used when electrofishing is employed to capture an organism, as it may depend on several factors such as voltage gradients, power densities, and exposure time [18]. In the EU, a study focusing on the control of the brown trout (*Salmo trutta*) in Sardinia confirmed the efficiency of the electrofishing method to eradicate the trout populations [29], but results on the same species were not as definitive in France, where despite multiple-electrofishing campaigns, the target population was not entirely eradicated, and some natural recruitment persisted [31]. Information on measures and related costs in relation to species included on the Union list are available for *Lepomis* spp. [62], *L. catesbeianus* [63], *P. parva* [64], *P. glenii* [42]. In the UK the eradication of *P. parva* by electrofishing was deemed successful after 3 years of monitoring [1]. However, in relation to *P. parva,* because of the species small size (12-70 mm) electrofishing is considered not feasible, alongside other conventional measures, such as netting [56]. As a side note, most studies on the effectiveness of electrofishing refer to its use as a sampling method for surveys. Some guidance stressed that electrofishing may not be the most appropriate method of sampling "coarse fish" [34], but this mostly refers to fish surveys and not to fish management or eradication. In fact, when concerning detection only, other methods are receiving greater attention for their cost-effectiveness, for example the use of eDNA [3]. On the other hand, in shallow water environments, it can replace net fishing, which is less efficient and more traumatic to fish [5]. For example, electrofishing resulted effective enough to collect 30 times more fish per unit of fishing time than gill netting [30]. The effectiveness of the method, similarly to what discussed in relation to its harmful effects on fish, depends on several factors. For example, it is known that water conductivity is an important factor in determining effectiveness of the technique: while low-conductivity waters are poor conductors of electricity and decrease the effectiveness of the method, very high-conductivity waters allow the charge to dissipate too rapidly [45]. For example, as a sampling technique, it is considered most effective in clear shallow waters (e.g. less than 4

	meters depth, as in small rivers and lake margins), where turbidity is low and water conductivity is mid-range [46]. It is interesting to note that a number of studies exists to compare the effectiveness of electrofishing versus other methods, and electrofishing often resulted as a best option. For example, in Hungary electrofishing was compared with fyke netting as a sampling method to study the habitat preference of *Lepomis gibbosus* (along with *Neogobius fluviatilis*), but other species were captured as well (including e.g. *P. parva*) [17]. In this study electrofishing resulted in the capture of larger individuals in greater proportion than fyke nets, showing that by using the two methods in parallel it is possible to complement each other and achieve better result (for example it may compensate the fact that electrofishing is not effective in deep waters) [17]. The results of this study were considered a prerequisite for the sound management and eradication of the species [17]. In relation to *L. gibbosus*, the mechanical removal of centrarchid sunfishes can be done by gill netting, seine netting and electrofishing. Protocols for removal are well developed [46] but electrofishing is preferred because it has the least amount of bycatch and damage to native fish populations [62]. In conclusion, it is worth pointing out that the results of a study carried out in small tropical streams of low water conductivity but high biodiversity value in French Guiana show that with the right equipment and settings, electrofishing can be an efficient alternative to poison fishing surveys, so it can substitute rotenone treatments [5].

4.1. Case studies	
CASE STUDY #1	
Measure type (if relevant):	Electrofishing [38, 43]
Species:	*Lithobates catesbeianus*
Objective:	Eradication
Use of measure	According to the relevant paper [38] from which the following text is excerpted “In 2006, a prototype electrode-fitted pole (electro-frogger) was developed and field tested, and more refined, patent-pending versions have been employed since 2007. During the summers of 2007 to 2009, a two-person team applied this manual capture technique for four-hour sessions on every evening that weather permitted. A four-hour session included loading and unloading equipment, so the time locating and capturing bullfrogs was approximately three hours. Teams worked at night from an inflatable boat, with one person to manoeuvre and position the boat while the second person located and caught juveniles (< 80 mm body length) and adults (> 80 mm) frogs. Pond and lake margins were scanned by spotlight to detect bullfrogs by their eye reflections. Vocalisations from adult male bullfrogs also independently identified their whereabouts. Bullfrogs were dazzled and transfixed by the spotlight’s beam as we approached. Then the electrode-fitted pole was used to generate a subsurface concentrated electrical field of < 50 cm diameter near the target

	bullfrog. The electrical field stunned and temporarily paralysed juvenile and adult bullfrogs for 30 seconds to one minute, which was enough time to get them into a container.
Combined with other measure(s):	According to the relevant paper [38] from which the following text is excerpted "For euthanasia, bullfrogs were placed into a chest freezer modified to lower their core body temperature to just below 2° C. After at least 12 hours they are transferred to a conventional deepfreeze that quick-freezes the now cold-stupified bullfrogs. They remain in the second freezer for at least 48 hours. Cold is a natural anaesthetic for amphibians".
Country(ies) of application:	Canada (Vancouver Island, British Columbia)
Geographic scale (km^2) and/or population size measure applied to:	Amy's Pond (0.4 km perimeter distance) Glen Lake (2 km perimeter distance)
Time period:	Between 2007 and 2009.
Effort:	In total: 93 nights in 2007 (19 sites/4,479 bullfrogs), 114 nights in (2009). (20 sites/3,430 bullfrogs), and 125 nights in 2009 (28 sites/3872 bullfrogs). NB: the case study is part of a larger regional programme that encompassed many more sites, hence figures may not reflect the effort in the single target sites below. At Amy's Pond, 1587 adult and juvenile bullfrogs were collected after 23 nights of effort spread over 3 years. At Glen Lake, 1774 bullfrogs were collected after 41 nights of effort spread over 3 years.
Costs:	Overall costs:
	The cost of running this programme is currently $400* (257.58 EUR)/night/2-person team, or CAN$37,200 (23,955 EUR) in 2007, CAN$45,600 (29,364.20 EUR) in 2008, and CAN$50,000 (32,197.60 EUR) in 2009. At Amy's Pond the total cost over 3 years was CAN$9200 (5,924.35 EUR). At Glen Lake the total cost over 3 years was CAN$16,000 (10,303.20 EUR). *NB: the paper does not specify whether it is CAN$ or US$, but in line with the other reported costs it is assumed to be CAN$
	Personnel costs:
	Equipment and infrastructure:
	Other, including overheads:
Effectiveness:	According to the authors [38] "By the end of the 2009 field season, all age-classes of bullfrogs had been successfully removed from both sites. Excluding repopulation through natural immigration or human translocation, both Amy's Pond and Glen Lake were then free of bullfrogs." Also "The technique is humane, species-specific and only targets one bullfrog or small groups of bullfrogs in very close proximity to one another". In conclusion the authors stated that the method applied to bullfrog "is time-efficient, cost-effective, humane, and safe for personnel and the environment".

CASE STUDY #2

Measure type (if relevant):	Electrofishing [1]
Species:	*Pseudorasbora parva*
Objective:	Eradication
Use of measure	In the summer of 2001, the pond was drained down, with concurrent fish removal, and then de-silted. In November 2001, the pond was refilled. Electrofishing was carried out, with all captured fishes removed, in March 2003, June 2005 and February 2006.
Combined with other measure(s):	Target pond was drained down
Country(ies) of application:	UK
Geographic scale (km^2) and/or population size measure applied to:	0.38 ha area (maximum depth ≈ 1.5 m)
Time period:	2001-2006
Effort:	N/A
Costs:	Overall costs:
	N/A
	Personnel costs:
	Equipment and infrastructure:
	Other, including overheads:
Effectiveness:	The species was successfully removed from the pond (along with other alien fish). However, it is worth pointing out that according to the authors "It is not clear whether the species actually succeeded in reproducing in the pond, nor is it known exactly when the fish were introduced (i.e. only just prior to their capture, and therefore not permitted the opportunity to breed)". In fact, "it is possible that all of the topmouth gudgeon captured in the pond were simply abandoned pet fish".

4.2. Costs effectiveness summary	Based on the evidence provided it is a cost-effective measure to eradicate populations of fish and amphibians (i.e. bullfrogs) from small isolated ponds/lakes and streams/rivers, though repeated applications are needed, sometime in combination with other methods. Costs increase and effectiveness decreases as the size of the treated area gets larger. However, the measure requires specific equipment for its application as well as duly trained skilled staff which can ensure its proper use depending on the target species.

5. Side effects	
Non-target native species, their habitats and the broader environment:	Positive:
	Native fish are not normally harmed or fatally injured (but see negative aspects below). As this method is expected to be not lethal, it would allow to select and separate non target species. No damage to the habitat or wider environment is reported.
	Negative:
	May affect other species present in the same targeted area. Some research dedicated to endangered native species has shown some of the harmful effects that can occur while electrofishing, e.g. the susceptibility of all life stages of native fish and amphibians to stress, injuries and mortality from electrofishing [18, 19, 22]. “Most of the studies on injuries to fish focus on the spinal injuries and related haemorrhaging. The morphological structure of fish is likely the reason they are susceptible to spinal injury from electrical shock. Compressed, broken or misaligned vertebrae and related injuries are believed to be caused by the simultaneous muscular convulsions on both sides of the spinal column”. For this reason it would be advisable to collect as many eggs, tadpoles and frogs of endangered native species from any target site for off-site holding (until the end of the activities with electrofishing), but also avoid the contamination of sites with petroleum products related to the use of equipment, as well as the transfer of disease and pathogens [19], However, other studies seem to confirm a greater robustness of frogs than expected [20]. A study for the capture of a marine clam through electrofishing (hence in the marine environment) showed no major side-effects on invertebrates, fish, birds and marine mammals [33].
Other invasive alien species:	Positive:
	May affect other species present in the same targeted area, therefore could be used to capture of multiple species. It was the case of some individual of ***P. parva*** caught while searching for ***P. glenii*** [6]
	Negative:
Public health and well-being:	Positive:
	The method is well known and operators should be trained appropriately to avoid injuries due to electric shock [34, 35]. See discussion on protocols and manuals above.
	Negative:
	There is some risk of electric shock, which however can be overcome by an appropriate training of the equipment. Specific guidance on health and safety issues is available on this regard [34, 35]. See discussion on protocols and manuals above.
Economic:	Positive:
	Negative:

6. Conclusion	
Overall assessment of the measure (qualitative)	
Most of the information on the impact of this method derives from its use as a sampling technique rather than for the management/control of the fish/frogs populations. However, such information is discussed here because the relevant studies provided useful data for the assessment of the humanness and effectiveness of the method. Moreover it provided indications about its use in different EU countries. In general, electrofishing is considered very efficient [25]. The method seems to have some harmful effects, particularly on salmonids, but seems not to have side effects on other fish and amphibians, particularly if used properly. Moreover it seems to be more effective and less harmful than other chemical and mechanical methods, although it requires to be used in conjunction with other tools (various kinds of nets etc.). As the method does not foresee the direct dispatch of the target animals, the removal needs to be accompanied by measures to dispatch the animals (e.g. through an overdose of anaesthetics, such as 2-phenoxyethanol, or clove oil). As shown by the case studies above on ***Pseudorasbora parva*** [1] and ***Lithobates catesbeianus*** [38, 43] the method has been employed effectively for some of the species of Union concern. Also in Spain, the results of a recent study [44] demonstrate how electrofishing can be a costly but effective method for the eradication of introduced fish and the conservation of stream-dwelling amphibians. Moreover, electrofishing has been considered the one of the least harmful techniques for collecting fish, and as such as been used as a valuable sampling technique for decades [21]. According to a study focusing on the control of the brown trout (*Salmo trutta*) in Sardinia [29] mechanical removal methods, such as repeated electrofishing was used as (along with gill netting) a viable alternative to chemical methods to allow selective species removal. Similar evidence, of electrofishing being more appropriate for removing introduced fish from streams than the use of chemicals, was obtained in Spain [44]. Some harmful effects of electrofishing on fish and amphibians (e.g. stress, injuries and mortality) have been noted [22], but they are common to most other collection techniques [20]. However it is clear that "Electrofishing is often considered the most effective and benign technique for capturing moderate to large-size fish, but when adverse effects are problematic and cannot be sufficiently reduced, its use should be severely restricted" [22]. As summarized by IUCN [61] and references therein, this measure is manpower intensive; additionally, when the equipment is not properly used the electrical current can cause serious injuries to the fish, e.g. internal bleeding or broken vertebrae, or be fatal. The IUCN note [61] also points out that poor netting practices and the overheating of the water holding buckets, in which the captured fish is placed, can harm the fish. However, when personnel are properly trained the risks to the fishes welfare are significantly reduced. On this regard, several protocols, manuals and guidance documents for good practices with electrofishing are available, including recommendations on equipment, safety and training, sampling design and precision requirements (including to minimize harmful effects on fish) [21, 32 34, 35, 36, 37, 48, 49, 54, 55], most with a focus on salmonids (guidelines for electric fishing best practice were also available in the UK, but the document was considered out of date and was withdrawn in 2016 [51]). In some protocols, ethical recommendations are available too [37].	
Assessor:	Riccardo Scalera
Reviewer 1:	Kevin Smith
Reviewer 2:	Sandro Bertolino

7. References

[1] Copp, G.H., Wesley, K.J., Verreycken, H. and Russell, I.C. (2007). When an 'invasive' fish species fails to invade! Example of the topmouth gudgeon Pseudorasbora parva. Aquatic Invasions 2, 107–112.

[2] Nathan T. Evans, Patrick D. Shirey, Jamin G. Wieringa, Andrew R. Mahon & Gary A. Lamberti (2017). Comparative Cost and Effort of Fish Distribution Detection via Environmental DNA Analysis and Electrofishing, Fisheries, 42:2, 90-99

[3] Stebbing PD, (2016). The management of invasive crayfish. In: Longshaw M, Stebbing PD (2016). Biology and ecology of crayfish. CRC Press, pp 337-357.

[4] Robinson, C., Garcia De Leaniz, C., Rolla, M., Consuegra, S., Consuegra del Olmo, S. (2019). Monitoring the eradication of the highly invasive topmouth gudgeon (Pseudorasbora parva) using a novel eDNA assay. Environmental DNA, 1:74–85.

[5] Pottier G, Beaumont WR, Marchand F, et al. (2019). Electrofishing in streams of low water conductivity but high biodiversity value: Challenges, limits and perspectives. Fish Manag Ecol.00:1–12

[6] Rechulicz, J., Plaska, W. & Nawrot, D. (2015). Occurrence, dispersion and habitat preferences of Amur sleeper (Perccottus glenii) in oxbow lakes of a large river and its tributary. Aquatic Ecology, 49:389–399

[7] Rakauskas V, Virbickas T, Stakėnas S, Steponėnas A. (2019). The use of native piscivorous fishes for the eradication of the invasive Chinese Sleeper, Perccottus glenii. Knowl. Manag. Aquat. Ecosyst., 420, 21.

[8] Koščo O., P. Manko, D. Miklisova, L. Košuthova. (2008). Feeding ecology of invasive Perccottus glenii (Perciformes, Odontobutidae) in Slovakia. Czech J. Anim. Sci., 53 (11): 479–486

[9] Skorić S., B. Mićković, D. Nikolić, A. Hegediš & G. Cvijanović (2017) A Weight-length Relationship of the Amur Sleeper (Perccottus glenii Dybowski, 1877) (Odontobutidae) in the Danube River Drainage Canal, Serbia. Acta zool. bulg., Suppl. 9: 155-159

[10] Simonovic P, Maric S, Nikolic V, (2006). Records of Amur sleeper Perccottus glenii (Odontobutidae) in Serbia and its recent status. Arch. Biol. Sci., Belgrade, 58 (1), 7P-8P.

[11] Kati, S., Mozsár, A., Árva, D., Cozma, N. J., Czeglédi, I., Antal, L., ... Erős, T. (2015). Feeding ecology of the invasive Amur sleeper (Perccottus gleniiDybowski, 1877) in Central Europe. International Review of Hydrobiology, 100(3-4), 116–128.

[12] Nalbant, T., Battes, K.W., Pricope, F. & Ureche, D. (2004). First record of the Amur sleeper Perccottus glenii (Pisces: Perciformes, Odontobutidae) in Romania. Travaux du Museum National d'Histoire Naturelle Grigore Antipa 47:279-284.

[13] Rau, M. A., Plavan, G., Strungaru, S. A., Nicoara, M., Rodriguez-Lozano, P., Mihu-Pintilie, A., ... Klimaszyk, P. (2017). The impact of amur sleeper (Perccottus glenii Dybowsky, 1877) on the riverine ecosystem: food selectivity of amur sleeper in a recently colonized river. Oceanological and Hydrobiological Studies, 46(1):96-107

[14] Grabowska, J., Pietraszewski, D., Przybylski, M., Tarkan, A. S., Marszał, L. and Lampart-Kałużniacka, M., (2011). Life-history traits of Amur sleeper, Perccottus glenii ,in the invaded Vistula River:early investment in reproduction but reduced growth rate. Hydrobiologia, 661,197-210.

[15] Nehring S., J. Steinhof (2015). First records of the invasive Amur sleeper, Perccottus glenii Dybowski, 1877 in German freshwaters: a need for realization of effective management measures to stop the invasion. BioInvasions Records, 4(3): 223–232

[16] Zięba, G., Dukowska, M., Przybylski, M., Fox, M.G. Smith, C. (2018). Parental care compromises feeding in the pumpkinseed fish (Lepomis gibbosus). The Science of Nature 105, 26.

[17] Czeglédi, I., Preiszner, B., Vitál, Z., Kern, B., Boross, N., Specziár, A., Takács, P., Erős (2019). Habitat use of invasive monkey goby (Neogobius fluviatilis) and pumpkinseed (Lepomis gibbosus) in Lake Balaton (Hungary): a comparison of electrofishing and fyke netting. Hydrobiologia 846:147–158

[18] Gilbert EI, Dean JC, Maglothin MR (2017). Responses of American Bullfrog, Lithobates catesbeianus, and Southern Leopard Frog, Lithobates sphenocephalus, to low voltages in uniform aquatic electrical fields. The Southwestern Naturalist 62: 148–154

[19] Southwest Endangered Species Act Team (2008). Chiricahua leopard frog (Lithobates [Rana] chiricahuensis): considerations for making effects determinations and recommendations for reducing and avoiding adverse effects. U.S. Fish and Wildlife Service, New Mexico Ecological Services Field Office, Albuquerque, New Mexico. 75 pp.

[20] Allen M, Riley S (2012). Effects of Electrofishing on Adult Frogs. Normandeau Associates, Inc. Casitas Municipal Water District, Oak View, Canada, 57 pp.

[21] Snyder, D.E. (2003a). Electrofishing and its harmful effects on fish, Information and Technology Report USGS/BRD/ITR-2003-0002: U.S. Government Printing Office, Denver, CO, 149 p.

[22] Snyder, D.E. (2003b). Invited overview: conclusions from a review of electrofishing and its harmful effects on fish. Reviews in Fish Biology and Fisheries 13:445-453.

[23] Norman G. Sharber & Jane Sharber Black (1999). Epilepsy as a Unifying Principle in Electrofishing Theory: A Proposal, Transactions of the American Fisheries Society, 128:4, 666-671

[24] Schulz U (1995). Electrofishing From Animal Welfare Aspects. Dtsch Tierarztl Wochenschr. 102(3):125-7.
[25] Northrop, R.B. (1967). Electrofishing. IEEE Trans. Biomed. Eng. 14: 191–200.
[26] Barrett, J.C. & Grossman, G.D. (1988). Effects of direct current electrofishing on the mottled sculpin. North American Journal of Fisheries Management 8:112-116
[27] Culver E, Chick JH (2015). Shocking Results: Assessing the Rates of Fish Injury from Pulsed-DC Electrofishing. North American Journal of Fisheries Management 35:1055–1063
[28] Bayley PB, Larimore RW, Dowling DC. (1989) Electric Seine as a Fish-Sampling Gear in Streams. Transactions of the American Fisheries Society 118(4):447-453
[29] Sabatini A, C. Podda, G. Frau, M. V. Cani, A. Musu, M. Serra, & F. Palmas (2018). Restoration of native Mediterranean brown trout Salmo cettii Rafinesque, 1810 (Actinopterygii: Salmonidae) populations using an electric barrier as a mitigation tool. The European Zoological Journal, 85(1):137–149
[30] Growns IO, Pollard DA, Harris JH. (1996). A comparison of electric fishing and gillnetting to examine the effects of anthropogenic disturbance on riverine fish communities. Fisheries Management and Ecology 3:13–24.
[31] Caudron A, Champigneulle A. (2011). Multiple electrofishing as a mitigate tool for removing nonnative Atlantic brown trout (Salmo trutta L.) threatening a native Mediterranean brown trout population. Eur. J. Wildl. Res. 57(3): 575-583
[32] Bohlin, T., S. Hamrin, T. G. Heggeberget, G. Rasmussen, and S. J. Saltveit. (1989). Electrofishing–theory and practice with special emphasis on salmonids. Hydrobiologia 173:9–43.
[33] Woolmer, A., Maxwell, E. and Lart, W. (2011). Effects of Electrofishing for Ensis spp. on Benthic Macrofauna, Epifauna and Fish Species, Seafish Report SR652
[34]. FCC - Scottish Fisheries Co-ordination Centre, (2007). Manage Electrofishing Operations. Training Manual. Fisheries Management SVQ Level 3, Inverness/Barony College, pp.78.
[35] FCC - Scottish Fisheries Co-ordination Centre, (2007). Catch Fish Using Electrofishing Techniques. Introductory Electrofishing Training Manual. Fisheries Management SVQ Level 2, Inverness/Barony College, pp.44.
[36] ISPRA (2014). Protocollo di campionamento e analisi della fauna ittica dei sistemi lotici guadabili. Manuali e linee guida, ISPRA, Roma,111: 20 pp.
[37] Kennard, M. J., Pusey, B. J., Perna, C., Burrows, D. & Douglas, M. (2011). Field manual-Including protocols for quantitative sampling of fish assemblages, habitat, water quality and sample preservation. Australian Rivers Institute, Griffith University, Australia.
[38] Orchard S.A. (2011) Removal of the American bullfrog Rana (Lithobates) catesbeiana from a pond and a lake on Vancouver Island, British Columbia, Canada. Pages 217–221 in: C. R. Veitch, M. N. Clout & D. R. Towns (eds) Island invasives: eradication and management. , IUCN, Gland, Switzerland.
[39] Vogel J, M. Sternberg, K. Fraser, E. Bates (2017). American Bullfrog Surveillance and Eradication Program FWCP COL-F15-W-1222
[40] Rytwinski T, Taylor JJ, Donaldson LA, Britton JR, Browne D, Gresswell RE, Lintermans M, Prior K, Pellatt MG, Vis C, Cooke SJ. (2019). The effectiveness of non-native fish removal techniques in freshwater ecosystems: a systematic review. Environmental Reviews. 27: 71-94.
[41] Orueta, J. (2003). Manual práctico para el manejo de vertebrados invasores en islas de España y Portugal, Proyecto LIFE2002NAT/CP/E/000014, Gobierno de las Islas Baleares y Gobierno de Canarias, 254 pp
[42] Verreycken, H. (2019). Information on measures and related costs in relation to the species included on the Union list: *Perccottus glenii*. Technical note prepared by IUCN for the European Commission.
[43] Sarat E., Mazaubert E., Dutartre A., Poulet N., Soubeyran Y., (2015). Invasive alien species in aquatic environments. Practical information and management insights. Volume 2. Management insight. Animal species.
[44] Bosch J, Bielby J, Martin-Beyer B, Rinco ń P, Correa-Araneda F, Boyero L (2019). Eradication of introduced fish allows successful recovery of a stream-dwelling amphibian. PLoS ONE 14(4): e0216204. https://doi.org/10.1371/journal. pone.0216204
[45] Dodds W. Whiles M (2020). Fish Ecology, Fisheries, and Aquaculture. Freshwater Ecology, 699–722.
[46] West, P., Brown, A. and Hall, K. (2007). Review of alien fish monitoring techniques, indicators and protocols: Implications for national monitoring of Australia's inland river systems. Report to the National Land & Water Resources Audit. Invasive Animals Cooperative Research Centre, Canberra.
[47] Fletcher D (2018). Biological invasion risk assessment, considering adaptation at multiple scales : the case of topmouth gudgeon Pseudorasbora parva. Biodiversity and Ecology. Université Montpellier, 258 pp
[48] IMBRIW (2013). Inland waters fish monitoring operations manual: electrofishing health and safety / HCMR rapid fish sampling protocol. Hellenic Centre for Marine Research - HCMR Special Publication, Draft Version 1. 79 pp.
[49] Goodchild, G.A. (1991). Code of practice and guidelines for safety with electric fishing. EIFAC Occasional Paper. No. 24. Rome, FAO. (1991). 16p.
[50] Soetaert, M., Boute, P. G., and Beaumont, W. R. C. (2019). Guidelines for defining the use of electricity in marine electrotrawling. – ICES Journal of Marine Science 76(7):1994-2007

[51] Beaumont W R C , A A L Taylor, M J Lee and J S Welton (2002) Guidelines for Electric Fishing Best Practice. R&D Technical Report W2-054/TR. CEH Report Ref. No: C01614. Environment Agency, UK
[52] Lines, J. A., Robb, D. H., Kestin, S. C., Crook, S. C. & Benson, T. (2003). Electric stunning: a humane slaughter method for trout. Aquaculture Engineering 28, 141–154.
[53] Huntingford, F. A.; Adams, C.; Braithwaite, V. A.; Kadri, S.; Pottinger, T. G.; Sandoe, P.; Turnbull, J. F. (2006). Review paper: current issues in fish welfare. Journal of Fish Biology, 68 (2). 332-372.
[54] NSW Fisheries (1997). The Australian Code of Electrofishing Practice. NSW Fisheries Management Publication No. 1.
[55] Temple, G.M.; Pearsons, T.N. (2007). Electrofishing: backpack and drift boat. In Johnson, D.H.; Shrier, B.M.; O'Neal, J.S.; Knutzen, J.A.; Augerot, X.; O'Neil, T.A.; Pearsons, T.N. (Eds): Salmonid field protocols handbook: techniques for assessing status and trends in salmon and trout populations. American Fisheries Society in association with State of the Salmon. 478 p.
[56] Britton, J.R., and Brazier, M. (2006). Eradicating the invasive topmouth gudgeon, Pseudorasbora parva, from a recreational fishery in northern England. Fisheries Manag. Ecol. 13(5):329-335.
[57] Reinhardt, F., M. Herle, F. Bastiansen, and B. Streit. (2003). Economic impact of the spread of alien species in Germany. Research Report 201 86 211, Federal Ministry of the Environment, Nature Conservation and Nuclear Safety.
[58] Britton, J.R., Brazier, M., Davies, G.D., and Chare, S.I. (2008). Case studies on eradicating the Asiatic cyprinid Pseudorasbora parva from fishing lakes in England to prevent their riverine dispersal. Aquat. Conserv. 18(6):867-876.
[59] Britton, J.R., Davies, G.D., and Brazier, M. (2009). Eradication of the invasive Pseudorasbora parva results in increased growth and production of native fishes. Ecol. Freshwat. Fish. 18(1):8-14.
[60] Evangelista, C., Britton, R.J., and Cucherousset, J. (2015). Impacts of invasive fish removal through angling on population characteristics and juvenile growth rate. Ecol. Evol. 5(11):2193-2202.
[61] IUCN (2017). Information on non-lethal measures to eradicate or manage vertebrates included on the Union list. Technical note prepared by IUCN for the European Commission.
[62] Zogaris, S. (2017). Information on measures and related costs in relation to species considered for inclusion on the Union list: Lepomis spp. Technical note prepared by IUCN for the European Commission.
[63] Adriaens, T., Brys, R., Halfmaerten, D., & Devisscher, S. (2019). Information on measures and related costs in relation to species included on the Union list – Lithobates catesbeianus. Technical note prepared by IUCN for the European Commission.
[64] Britton, R. (2019). Information on measures and related costs in relation to species included on the Union list: Pseudorasbora parva. Technical note prepared by IUCN for the European Commission.
[65] Pfeiffenschneider, M. H., F. (2020). Plan d'action pour espèces exotiques envahissantes au Grand-Duché de Luxembourg : Perche soleil, *Lepomis gibbosus* (Linnaeus, 1758). In L. Administration de la nature et des forêts (Ed.), (pp. 20).

Appendix 9. Chemical fertility control

1. Measure name	
1.1. English:	Chemical fertility control Hormonal sterilisation Chemical sterilisation Sterilisation via Injection Immunocontraception Fertility chemical control in bait Triploidy
1.2. Lethal or non-lethal:	Non-lethal

1.3. Other languages (if available):

Bulgarian	Химическо регулиране на плодовитостта	Italian	Controllo chimico della fertilità
Croatian	Kontola plodnosti – kemijska (u mamcima) i injekcijska	Latvian	Ķimiskā auglības kontrole (ēsmā & injekcijas)
Czech	Chemická kontrola plodnosti	Lithuanian	Cheminė vaisingumo kontrolė (masalas & injekcija)
Danish	Kemisk fertilitets kontrol	Maltese	
Dutch	Chemische sterilisatie (via aas of injectie)	Polish	Chemiczna kontrola płodności/sterylizacja chemiczna
Estonian	Viljakuse keemiline kontroll (sööt & süstimine)	Portuguese	Controlo de fertilidade - químico (com isco) e injeção
Finnish	Hedelmällisyyden kontrollointi – kemiallinen (syötti) & injektio	Romanian	Control chimic al fertilității
French	Contrôle chimique de la fertilité	Slovak	Chemická kontrola plodnosti
German	Chemische Fertilitätskontrolle	Slovenian	Kemični nadzor plodnosti
Greek	Χημικός έλεγχος γονιμότητας	Spanish	Control químico de fertilidad
Hungarian	Sterilizálás – kémiai (csalétek) és injekció	Swedish	Kemisk sterilisering
Irish			

2. Technical details of measure

2.1.a. Measure description

This measure identifies the use of chemical control mechanisms as a wildlife management tool to regulate populations (e.g. capture, treat, release, or self-administered drugs). Chemical fertility control describes several processes involving a broad range of chemical agents that work to prevent reproduction by blocking ovulation, oogenesis, sperm production and other reproductive mechanisms [1]. In captivity, use of chemical contraception has been reported in mammals, aves, reptiles, and fish [27, 28]. In mammals, chemical contraception is administered as implants, injections, or orally, while in aves, reptiles, and fish, injections and implants are predominantly used [27]. While there are no reports of chemical contraception being used in amphibians, there is evidence that reproduction in amphibians can be disrupted by synthetic oestrogens and progestogens [29, 30]. In the field, preliminary studies have looked into innovative ways to administer chemical fertility control, (for example, through the use of infectious vectors [2]) however, the primary mode of delivery has been through injection, with some early research that is exploring the possibility of oral consumption with baits for mammals, and oral contraceptives being used in avian species [26]. However, much work still needs to be done in this area before it can be suggested for use as a management tool [3].

Immunocontraceptives specifically relate to the use of chemical agents, such as vaccines, used to prevent conception by stimulating the production of antibodies against chemicals or hormones that are essential for reproduction [4]. There are several commercial products that are available for use in wild populations with mammalian gonadotropin-releasing hormone (GnRH) and mammalian zona pellucida (ZP) proteins being two highly studied immunocontraceptives, however there is very little research into the use of avian contraceptives or sterilants [1, 3]. GnRH vaccines (immuno-contraceptive) have been most commonly used for ungulate population control with a variety of injectable products available (Improvac, Improvest, GonaCon). GonaCon was developed for use in wild populations, and along with ZP are single dose injections while the other products are developed for use on captive animals and require multiple or repeated doses making them of limited use for wild populations.

A different form of immunocontraception is the use of vaccines targeting the Zona pellucida (ZP), a layer of glycoproteins that surround the ovulated mammalian egg which facilitates the binding of sperm [3]. This vaccine is only effective in females as it is acts on the female egg and its effectiveness across different species varies due to the variation in glycoproteins present. The Porcine ZP vaccines have been shown to be effective in ungulate species [6], with fertility control being evident in the white tailed deer for one year following a vaccination with one booster [4], but is likely to be less efficient for implementation on other IAS species [3]. There have been further developments of rodent specific ZP vaccines, however the feasibility of these in wild population control is limited due to the need for booster shots. In comparison, GnRH vaccines are less species-specific and have also been shown to be effective on ungulate species such as the White-tailed deer [7].

GnRH agonists are another form of contraception which can be administered via injection, nasal spray or as an implant. The agonist suppresses the reproductive endocrine system by binding to the gonadotrophs in the pituitary, which effectively blocks GnRH receptors and thus prevents production of pituitary and gonadal hormones. It is important to note that a GnRH agonist initially stimulates the reproductive system,which can result in a potential increase in hormonal production resulting in an acute fertility enhancemen,and thereftore, this should be mitigated by additional contraception during this time [3,5]. This method of fertility control is likely too expensive to feasibly employ for population management at this stage.

Other fertility control agents to consider include Progestin injections and implants which act as estogen antagonists/blockers and cholesterol inhibitors, such as DiazaCon, which reduces the cholesterol available for the production of reproductive steroids [5,3].

Some contraceptives that have been shown to be effective on individual animals have not been tested at the population level. Chemical contraception methods used in captive non-domestic species, are maintained within the Contraception Database, a database maintained by the AZA Reproductive Management Center and the EAZA Reproductive Management Group, with the aim of centralising information about contraceptive use in exotic wildlife. These records, of which there are over 48000, are used to analyse trends in efficacy, safety, and future fertility of contraceptive products, with the aim of ensuring evidence-based use. Within the database are the following methods:

Nyctereutes procyonoides: The Contraception Database has 10 records of chemical contraception use in this species (all female). This includes the use of GnRH agonist implants (Suprelorin), progestin-implants (MGA), and oral progestins (Megace). All records were effective at preventing reproduction

with no side effects. The Contraception Database is a database maintained by the AZA Reproductive Management Center and the EAZA Reproductive Management Group, with the aim of centralising information about contraceptive use in exotic wildlife. These records, of which there are over 48000, are used to analyse trends in efficacy, safety, and future fertility of contraceptive products, with the aim of ensuring evidence-based use. However, it is important to note that these are all methods developed for use on captive animals and of limited use for wild populations given the need for repeated treatments. *Procyon lotor*: The Contraception Database has 27 records of chemical contraception use in this species (1 in males, 26 in females). In males, progestin injections were used (Delvosteron). Please note that this is likely to prevent reproductive behaviour and would not stop spermatogenesis. In females, progestin-implants (MGA and Implanon), GnRH agonist implants (Suprelorin), progestin-injections (Delvosteron, Depo-Provera) have been used successfully and with no side effects. Additionally, although not technically 'chemical sterilisation' but rather 'genetic' the creation of triploid individuals in lower vertebrate species, which are rendered sterile through cold, heat or pressure treatments in early embroyonic development e,g, for *L. catesbeianus* [31,32,34], is considered within the scope of this assessment. The release of sterile males is also believed to have future potential for fish species. For population control, effectiveness and duration of contraception are only two of the many factors that should be considered when evaluating fertility control to control populations. Other factors include feasibility, species-specificity, effects on non-target species (including humans through meat consumption), social acceptance, effects on behaviour,animal welfare, costs, and sustainability.
2.1.b. Integration with other measures
For all methods of contraception that require direct contact with the animal (e.g. injection) this measure would also need to be coupled with an appropriate live-capture measure. The humaneness and practicality of a measure should consider the frequency of application that the animal be subjected to. Depending on the chemical fertility control agent that is applied, there will be different requirements for capture or delivery of the agent. If re-administration of the contraceptive agent is required to maintain fertility control, then the animals will be subject to systematic recapture. To limit welfare impacts and costs involved in live-capture measures, remote administration techniques (syringe darts, bio-bullets, biodegradable projectiles etc.) might also be considered. The downsides of this method of administration is difficulty to identify individual animals, regulation of dose and incomplete intra-muscular injection [1]. This measure can also be integrated with 'kept in captivity'. There is evidence that integrating culling with fertility control can achieve eradication or a significant population reduction. Culling will be more effective on reducing the initial population size, whereas fertility control can prevent the rebounding of the population to its previous level. Therefore, using a combination of culling and fertility control can help achieve a maintained population at a reduced level [1].

2.2.a. Availability - species and objectives										
Objective	Unknown objective		Rapid Eradication		Management					
					Eradication		Control		Containment	
Species	Avail.	Ref(s).	Avail.	Ref(s).	Avail.	Ref(s).	Avail.	Ref(s).	Avail.	Ref(s).
Acridotheres tristis										
Alopochen aegyptiaca										
Callosciurus erythraeus							P	8,15		
Corvus splendens										
Herpestes javanicus										
Lepomis gibbosus										
Lithobates catesbeianus					U (Triploidy)	31	U (Triploidy)	31, 32	U (Triploidy)	32
Muntiacus reevesi							P	1, 5, 6, 7, 18, 19, 20		
Myocastor coypus										
Nasua nasua							P	21		
Nyctereutes procyonoides							P		P	27
Ondatra zibethicus							P	3		
Oxyura jamaicensis										
Percottus glenii										
Plotosus lineatus										
Procyon lotor							P		P	27
Pseudorasbora parva										
Sciurus carolinensis							A	8, 15		
Sciurus niger							P	8, 15		
Tamias sibiricus							P	8, 15		
Threskiornis aethiopicus										
Trachemys scripta										

2.2.b. Application – EU Member States and objectives										
Objective	Unknown objective		Rapid Eradication		Management					
					Eradication		Control		Containment	
Country	Avail.	Ref(s).	Avail.	Ref(s).	Avail.	Ref(s).	Avail.	Ref(s).	Avail.	Ref(s).
Austria										
Belgium										
Bulgaria										
Croatia										
Cyprus										
Czech Republic										

Denmark										
Estonia										
Finland										
France										
Germany										
Greece										
Hungary										
Ireland										
Italy										
Latvia										
Lithuania										
Luxembourg										
Malta										
Netherlands										
Poland										
Portugal										
Romania										
Slovakia										
Slovenia										
Spain										
Sweden										
United Kingdom*										

* Not an EU Member State

3. Humaneness of the measure			
3.1. Welfare for all measures			
Measure type (if applicable): Chemical fertility control (analysed separately to the capture method)	**Humaneness impact categories**		
Domain	**No impact**	**Mild-Moderate**	**Severe - Extreme**
1: Water deprivation, food deprivation, malnutrition	The injection of the animal with a vaccination or other chemical substance is generally a quick procedure. The injection is often coupled with other necessary procedures that are performed to support the overall measure application (such as		

	tagging, microchipping and blood sampling). With the addition of the coupled procedures the whole process is still unlikely to be a period long enough to create an issue of water or food deprivation.		
2: Environmental challenge	With the short-term restraint that is required for an injection to take place within that animal's environment, there is minimal to no environmental impact as the animal is released after the procedure has taken place.		
3: Injury, disease, functional impairment		There is the possibility of injury as a result of the animal resisting restraint. In some species abscesses can form at the injection site, [3]. Pai et al. [8] noted a 7.62% incidence of injection site reactions. GnRH-based immuno-contraceptives should not be administered to antlered animals as the vaccine will interfere with the antler cycle. For both GnRH and PZP-based vaccines, no effect was found on ongoing pregnancies [1] GnRH agonists can cause abortion, with a consequent impact on the welfare of females if administred during the breeding season [3].	
4: Behavioural, interactive restriction	Immunocontraception does not appear to have ongoing significant effects on social behaviour, but instead keeps the animal's behaviour in non-breeding season [3]. Animal subjected to immunocontraceptions and GnRH agonists may however lose secondary sexual characteristics [8].		

5: Anxiety, fear, pain, distress, thirst, hunger etc.	No impact for immunocontraception administered through bait.	Short terms effects of handling, restraint, prevention of resource access, inability to hide, proximity to humans and pain associated with the procedure would all contribute to varying degrees of anxiety, fear and distress in the captured animals [11]. The species and individual animal's ecology (i.e. if they are prey or predator animals and how close they regularly come to human contact) will affect the level of stress experienced throughout the procedure. The length of time that handling is required for the procedure and potential duration of stress experienced by the animal will vary according to the species and the methods required (i.e. if additional tagging, and blood draws are required). However, the stress induced may be limited if the procedure is made to be quick and efficient as possible with minimal handling by trained personnel, i.e. the method of darting removes all direct human contact, but the trade-off is that it reduces effectiveness of delivery of vaccines [1].	

3.2. Mode of death (if relevant)

Measure type (if applicable):	**Immediate death (i.e. no suffering)**	**Not immediate death (mild - moderate suffering)**	**Not immediate death (severe - extreme suffering)**
Rationale:	N/A		

3.3. Humaneness summary	This measure is considered to have mild welfare impact. Measures should be applied in a way to reduce the welfare impact as much as possible. With measures that require recapture and booster injections the humanness impact should be increased accordingly for the number of times the same animals need to be subjected to the procedure. The use of triploidy in American bullfrogs, can be considered to have no impact on welfare. Although little information is available on the sensory capacity of amphibian eggs at the stage of development [33], since the procedure takes place 10 mins after fertilisation [31], negligible impacts are to be expected.

4. Costs and effectiveness of the measure	
General effectiveness of the measure	Non-oral contraceptives are best utilised on isolated and small wild populations due to immigration and emigration that might affect the proportion of contracepted animals. Lethal methods are more effective in achieving a rapid reduction in population size [1, 13]. In addition, where hunting is otherwise not permitted, fertility control can be applied to control populations. In some instances, both ethical and public perception should be considered when choosing control methods; in some species in some places, thesocial acceptance of lethal methods could make fertility control a option to consider. Its success at population level may however vary in relation to the feasibility of targeting different proportions of a population [14]. The population biology and trappability of the IAS of Union concern may allow the technique to contribute to their management, at least in some local scale contexts, but each would require a species-specific feasibility study to explore this potential. The availability of chemical fertility control that could be delivered orally via species-specific baits would potentially greatly increase the scope for the application of these agents as a IAS management tool, particularly where injectable vaccine is not a viable option [15]. However, the delivery of fertility through baits is still in its research phase for the IAS of Union concern, so before there can be conclusions made about its effectiveness in controlling wild populations there would need to be the application of this method in field trials. In relation to the release of sterile triploid *L. catesbeianus*, research has found that sterile triploid males can be produced at sufficient numbers to eradicate a small target population [32,34]. This approach, combined with traditional management (e.g. fish traps) is now being applied through the LIFE 3n-Bullfrog project which began in late 2019 with the aim of control and containment in Belgium [33].

4.1. Case studies	
CASE STUDY #1	
Measure type (if relevant):	GonaCon Immunocontraceptive Vaccine [14]
Species:	White-tailed deer (Odocoileus virginianus)
Objective:	Population control
Use of measure	Single injection of GonaCon, an immunocontraceptive vaccine was administered upon capture and immobilization of the female deer. The two subsequent years of the study, the deer were non-invasively monitored for evidence of reproduction through visual observation of udders for signs of lactation.
Combined with other measure(s):	The deer were captured and immobilised using tranquiliser darts. After the vaccine or control was administered as well as the appropriate tagging complete the deer were given Tolazine to reverse the anaesthesia to allow for quick recovers and release.
Country(ies) of application:	Maryland, USA
Geographic scale (km^2) and/or population size measure applied to:	The study looked at two adjoining sites: • The White Oak site, was a mostly forested, fully fenced, 268-ha (662-acre) and contained an estimated 50–80 deer. In the study 28 does were captured for treatment. • The Adelphi site consisted of suburban residential development and was fully fenced, and its vegetation was similar to the White Oak site. The site was 82 hectares and contained an estimated 50 deer. In the study 15 does were captured for the control treatment.
Time period:	The 2-year study commenced with the injections in July 2004. Monitoring of fertility was assessed during the summers of 2005 and 2006.
Effort:	Unable to calculate
Costs:	Overall costs:
	Not explored in the study
	Personnel costs:
	Not explored in the study, however experienced personnel would be required for capture of the deer and administration of the vaccine. The captures took place over a two-month period and resulted in 43 captures.
	Equipment and infrastructure:
	GonaCon immunocontraceptive vaccine, syringes, refrigeration storage facilities, tranquiliser guns, tranquiliser darts, ear tags, radio-telemetry collars, Tolazine injections.
	Other, including overheads:
Effectiveness:	In the first year after the vaccine was administered the control group showed signs of lactation in 85% of the does, whereas in the vaccinated group only 12% showed signs of lactation. In the second subsequent year, 100% of the control group showed signs of pregnancy compared to 53% of the group that were given the single vaccination dose. This shows the effects of the single dose fertility control decreased over the second year and suggests boosters may be required for longer term fertility control management. Due to similar species' characteristics , similar outcomes could be expected on *Muntiacus reevesi*.

CASE STUDY #2	
Measure type (if relevant):	Immunocontraceptive Gonacon [8]
Species:	Eastern Grey Squirrel (*Sciurus carolinensis*)
Objective:	Population control
Use of measure	The immunocontraceptive was gives as an injection after the live capture of the grey squirrels. They were handled with a restraint cone throughout the procedure. The mean handling time was 10 minutes before the squirrels were released at the capture site.
Combined with other measure(s):	This measure was used in conjunction with live capture box traps which were checked every hour they were set. The squirrels were handled with a restraint cone. They were sexed, weighed, ear-tagged, microchipped as well as being vaccinated with GonaCon™ or a control. Blood samples were also taken for analysis.
Country(ies) of application:	Northwestern South Carolina, USA
Geographic scale (km^2) and/or population size measure applied to:	Clemson University's (CU) main campus of approximately 3.25 Km^2. This area included a number of buildings interspersed with about 6600 trees in addition to shrubs and bushes. This study estimated 9 grey squirrels per hectare, approximately 5,103 squirrels.
Time period:	March 2008 to June 2009 and included four trapping sessions. • The first trapping session was conducted during March – April 2008 with 55 captured. • The second trapping session was conducted in July 2008. • The third trapping session was conducted in November 2008. • The last session corresponded with the May – June 2009 breeding season.
Effort:	Traps were set from dawn to dusk and were checked on the hour over 6 months of trapping. Handling of the squirrels took approximately 10 minutes, and in this study there were 317 squirrels captured and treated.
Costs:	Overall costs:
	The study looked at a previous study [12] which showed that over 90% of the population of grey squirrels needed to have effective fertility control before the desired population control due to high birth rates. From this model the study concluded that it would take 1000 days at a cost of $15USD per grey squirrel to catch the 4593 squirrels necessary to achieve the 90% treatment rate. Therefore, the labour costs alone (assuming minimum wage of $7.25/hr) would amount to around $70,000.
	Personnel costs:
	Qualified personnel would be required in order to administer the vaccination, and in this case, the additional procedures carried out on the grey squirrels e.g. blood draw.
	Equipment and infrastructure:
	GonaCon™ (vaccination), storage facility (vaccines require refrigeration), syringe, appropriate waste disposal system, gloves, box traps, restraint collar.
	Other, including overheads:

Effectiveness:	The study concluded that the single dose of the GonaCon™ (GnRH immuno-contraceptive vaccine) produced sufficient immunological response in the treated squirrels to indicate immunocastration in males and likely to have inhibited reproduction in females.

CASE STUDY #3	
Measure type (if relevant):	Oral chemical contraceptive Agent DiazaCon [15]
Species:	Grey squirrels (*Sciurus carolinensis*)
Objective:	Effectiveness and potential side effects on individual animals
Use of measure	Squirrels were fed peanuts coated with DiazaConTM. This agent acts to reduce the ability of the animal to produce cholesterol, which is needed to produce steroid reproductive hormones and therefore reduces the animal's reproductive abilities. The squirrels were offered treated peanuts for 8 consecutive days.
Combined with other measure(s):	If set up with appropriate feeding stations that only allow the targeted species to consume (e.g. through the warfarin-poisoned bait feeder) then this method would not need to be coupled with any other measure. However, this has not been proven at a population level.
Country(ies) of application:	USA
Geographic scale (km^2) and/or population size measure applied to:	This study was conducted in a laboratory where 48 squirrels were kept in individual cages.
Time period:	The measure was applied over 8 days of feeding, which resulted in 2 months of cholesterol at the necessary level to reduce reproduction.
Effort:	Preparation of bait.
Costs:	Overall costs:
	The oral delivery of chemical fertility agents reduces the costs of capture, treatment and release.
	Personnel costs:
	Equipment and infrastructure:
	Other, including overheads:
Effectiveness:	Although the study looked at feeding peanuts on consecutive days, DiazaCon does not need to be fed consecutively to be effective as it accumulates in the liver. To be effective in reproductive control the plasma cholesterol concentrations must be reduced by 40%. This was achieved in this study for 2 months after treatment. No side effects were noted in this study.

4.2. Costs effectiveness summary	Case study 2 highlights the potential costs associated with a method that utilises a capture, treat, release fertility vaccine measure. Cost will very much depend on whether volunteers can join trained staff , for instance to recapture squirrels and monitor reproductive status. If so, cost will decrease significantly. However, with the high costs associated as well as trained and experienced personnel required to perform the handling and administration of the product for the fertility control, it is likely that the costs of such a measure would be much higher than a lethal eradication measure which also are more effective in terms of population control [15]. Oral delivery of a contraceptive, formulated in bait or feed, is likely to be substantially less costly than methods requiring capture, treatment and release of individual animals. This could potentially be a useful tool to contribute to the population management of IAS. Recent and ongoing research has provided promising results (see case study 3), but further species-specific research and field testing are needed. It is important to note that species specific methods of bait delivery pose an obstacle to the application of this approach.

5. Side effects	
Non-target native species, their habitats and the broader environment:	Positive:
	An advantage of the use of immune-contraceptive vaccines is that the antibodies that are produced by the animal in response to the vaccine which target their individual reproductive proteins or hormones are unlikely to affect another animal or species in the food chain, as the antibodies are destroyed in the gastro-intestinal tract [3].
	Negative:
	Any oral contraceptives that are administered need to be done with extreme care as they are indiscriminate in terms of the species they contracept unless a unique delivery system is created. However, species-specific delivery systems are available to reduce or eliminate risks for non-target species. These include the warfarin hoppers for squirrels suggested above, rafts for mink (and coypu) and Boar-Operated-System for wild boar [1]. The release of large numbers of sterile triploid males (e.g. for *L. catesbeianus*) could cause environmental harm through competition and predation [34]. However, this impact could be reduced if the measure is combined through a manual removal programme and also if the sterile individuals are released at an early stage of development (i.e. they are smaller) [34].
Other invasive alien species:	Positive:
	Negative:
Public health and well-being:	Positive:

	Fertility control has shown to be a more accepted measure than lethal controls in the eye of the public. Public views concerning population control of invasive grey squirrels showed preferred method chosen by the majority of >3000 respondents is fertility control [16].
	Negative:
	For species like muntjac, which may end up in the human food chain, the effects on consumers must be considered, especially if the contraceptive is a hormone.
Economic:	Positive:
	Negative:

6. Conclusion

Overall assessment of the measure (qualitative)

The use of different fertility control methods will depend on the availability of the product in Member States. GnRH vaccines are available in Europe but the legislative requirements of the use of Improvac on free-living wildlife will need to be explored, and it is worth noting that Improvac effects do not last as long as GonaCon as Improvac was developed for the pig industry to suppress boar taint. Vacsincel is only available in Spain and GonaCon is not commercially available yet. PZP - can be ordered from the Science and Conservation Center, Zoo Montana but cannot be imported to the UK.

GnRH agonists implants such as deslorelin acetate (Suprelorin) are available throughout Europe, as well as the GnRH agonist injection product leuprolide acetate (Lupron depot) but these are generally more expensive than the immuno-contraceptives. Progestins implants - etonogestrel (Nexplanon/Implanon) are also available throughout Europe as well as Levonorgestrel (Jadelle), however this is not frequently used. Progestin Injection - medroxyprogesterone acetate (Depo-Provera) is available throughout Europe. Oral birth control pills, altrenogest (Regumate) should be readily available. However, all of these methods require repeated doses, limiting their effectiveness for use on wild populations. In addition, the use of triploidy is a promising measure under development, with low welfare impacts though efforts requires construction of a dedicated facility to rear sterile triploid bullfrogs in sufficient numbers for release [32,34].

It is recognised that for a rapid reduction in population numbers, culling is the most efficient method at the moment. However, a combination of culling and fertility control can be employed to first reduce and then maintain a population. Fertility control can also be effective as a stand alone measure where the population is small and contained as this will reduce the likelihood of immigration of fertile individuals [1, 14, 13]. The measure of fertility control and management appear to be quite costly, but this could be mitigated by reduced reliance on capture and handling (through the use of remote administration methods) and increasing volunteers who can accompany trained staff [6]. The use of remote administration can also reduce the welfare impacts on the animal, however this may be coupled with reduced ability to identify individuals as increase the potential of an incomplete administration [1]. Humanness should also be considered in a manner that reflects if the product requires re-administration and therefore the animals are subject to re-capture and/or re-treatment.

Fertility control methods could be useful in areas where hunting or culling methods are not feasible. It is also important to note that public attitudes are generally more accepting of this method in comparison to other lethal methods [16]. Preliminary results on the development of oral

contraceptive/vaccination are encouraging, but more research needs to be conducted in field trials on the use of fertility control in baits before they can be considered as an effective method for population control [15].

Assessor:	EAZA
Reviewer 1:	Riccardo Scalera
Reviewer 2:	Ilaria Di Silvestre

7. References

[1] Massei, G. & Cowan, D. (2014). Fertility control to mitigate human-wildlife conflicts: A review. CSIRO Wildlife Research. 41. 1-21. 10.1071/WR13141.

[2] McLeod, S., Saunders, G., Twigg, L., Arthur, A. D., Ramsey, D. and Hinds, L. (2008). Prospects for the future: is there a role for virally vectored immunocontraception in vertebrate pest management? *Wildlife Research*, 34, 555-566.

[3] IUCN. 2017. Information on non-lethal measures to eradicate or manage vertebrates included on the Union list. Technical note prepared by IUCN for the European Commission.

[4] Miller, L. A. and Killian, G. J. (2002). In search of the active PZP epitope in white-tailed deer immunocontraception. Vaccine, 20, 2735-2742.

[5] EAZA Group on Zoo Animal Contraception. (2017). Cervidae & Tragulidae [fact sheet]. Retrieve from https://www.egzac.org/home/viewdocument?filename=Cervidae%20and%20tragulidae%20EGZAC%20taxon%20sheet%202017.pdf

[6] Kirkpatrick, J.F., Lyda, R.O. & Frank, K.M. (2011), Contraceptive Vaccines for Wildlife: A Review. American Journal of Reproductive Immunology, 66: 40-50. doi:10.1111/j.1600-0897.2011.01003.x

[7] Miller, L. A., Gionfriddo, J. P., Fagerstone, K. A., Rhyan, J. C., & Killian, G. J. (2008). The single-shot GnRH immunocontraceptive vaccine (GonaCon) in white-tailed deer: comparison of several GnRH preparations. *American journal of reproductive immunology* (New York, N.Y. : 1989), 60(3), 214–223. https://doi.org/10.1111/j.1600-0897.2008.00616.x

[8] Pai, M., Bruner, R., Schlafer, D. H., Yarrow, G. K., Yoder, C. A. and Miller, L. A. (2011). Immunocontraception in eastern gray squirrels (Sciurus carolinensis): morphologic changes in reproductive organs. Journal of Zoo and Wildlife Medicine, 42, 718-722.

[9] West, G., Heard, D. J., & Caulkett, N. (Eds.). (2014). *Zoo animal and wildlife immobilization and anesthesia*. John Wiley & Sons.

[10] Bednarski, R., Grimm, K., Harvey, R., Lukasik, V. M., Penn, W. S., Sargent, B., & Spelts, K. (2011). AAHA anesthesia guidelines for dogs and cats. *Journal of the American Animal Hospital Association*, *47*(6), 377-385.

[11] Lindsjö, J., Cvek, K., Spangenberg, E., Olsson, J., & Stéen, M. (2019). The Dividing Line Between Wildlife Research and Management-Implications for Animal Welfare. *Frontiers in veterinary science*, 6, 13. https://doi.org/10.3389/fvets.2019.00013

[12] Hosey, G., & Melfi, V. (Eds.) (2018). Anthrozoology: human-animal interactions in domesticated and wild animals. Oxford University Press.

[13] Mcleod, S.R. & Saunders, Glen. (2014). Fertility control is much less effective than lethal baiting for controlling foxes. Ecological Modelling. 273. 1–10. 10.1016/j.ecolmodel.2013.10.016.

[14] Gionfriddo, J., Eisemann, J., Sullivan, K., Healey, R., Miller, L., Fagerstone, K., Engeman, R., & Yoder, C., (2009). Field test of a gonadotrophin-releasing hormone immunocontraceptive vaccine in female white-tailed deer. *CSIRO Wildlife Research*. 36. 10.1071/WR08061.

[15] Yoder, C. A., Mayle, B. A., Furcolow, C. A., Cowan, D. P., & Fagerstone, K. A. (2011). Feeding of grey squirrels (Sciurus carolinensis) with the contraceptive agent DiazaCon™: effect on cholesterol, hematology, and blood chemistry. *Integrative zoology*, 6(4), 409–419. https://doi.org/10.1111/j.1749-4877.2011.00247.x

[16] Dunn, M., Marzano, M., Forster, J. & Gill, R., (2018). Public attitudes towards "pest" management: Perceptions on squirrel management strategies in the UK. *Biological Conservation*. 222C. 10.1016/j.biocon.2018.03.020.

[17] Moore, H.D.M., N.M. Jenkins and C. Wong. 1997. Immunocontraception in rodents: A review of the development of a sperm-based immunocontraceptive vaccine for the gray squirrel (Sciurus carolinensis). Reprod. Fertil. Dev., 9, 125-129.

[18] Gionfriddo, J.P., Denicola, A.J., Miller, L.A. and Fagerstone, K.A. (2011), Health effects of GnRH immunocontraception of wild white-tailed deer in New Jersey. Wildlife Society Bulletin, 35: 149-160. doi:10.1002/wsb.17

[19] Curtis, P., Richmond, M., Miller, L. & Quimby, F., (2008). Physiological Effects of Gonadotropin-Releasing Hormone Immunocontraception on White-Tailed Deer. Human-Wildlife Conflicts. 2.
[20] Miller, L.; Rhyan, J., & Killian, G., (2004). GonaConTM, a Versatile GnRH Contraceptive for a Large Variety of Pest Animal Problems (2004). *USDA National Wildlife Research Center,* Staff Publications. 371.
[21] EAZA Group on Zoo Animal Contraception. (2017). Small carnivores (Procyonidae, Herpestidae/Eupleridae, Mustelidae, Viverridae, Mephitidae, Ailuridae) [fact sheet]. Retrieve from https://www.egzac.org/home/viewdocument?filename=Small%20carnivores%20taxon%20sheet%202017.pdf
[22] Jewgenow, K., Dehnhard, M., Hildebrandt, T. B., & Göritz, F. (2006). Contraception for population control in exotic carnivores. *Theriogenology*, 66(6-7), 1525–1529. https://doi.org/10.1016/j.theriogenology.2006.01.027
[23] Cowan, D. P. and Massei, G. (2008). Wildlife contraception, individuals and populations: how much fertility control is enough? Proceedings Vertebrate Pest Conference, 23, 220–228.
[24] Cowan, D. P., Massei, G. and Mellows, R. J. B. (2006). A modelling approach to evaluating potential applications of emerging fertility control technologies in the UK. Proceedings Vertebrate Pest Conference, 22, 55–62.
[25] Krause, S. K., Kelt, D. A., Van Vuren, D. H., & Gionfriddo, J. P. (2014). Regulation of tree squirrel populations with immunocontraception: a fox squirrel example. *Human–Wildlife Interactions*, *8*(2), 3.
[26] Avery, L. A. , Keacher, K. L., and Tillman, E. A. (2008). Nicarbazin bait reduces reduction by pigeons (Columba livia). *Wildlife Research* 35(1), 80-85
[27] AZA RMC, EAZA RMG (2020) Contraception Database. Viewed 15th September 2020.
[28] Asa, C.S., & Porton, I. J. (2005). Wildlife Contraception: Issues, Methods, and Applications. (C.S. Asa & I. J. Porton, Eds.). Baltimore, Maryland: The Johns Hopkins University Press.
[29] Hoffmann, Frauke, and Werner Kloas. "Estrogens can disrupt amphibian mating behavior." PloS one 7.2 (2012): e32097.
[30] Ziková, Andrea, et al. "Endocrine disruption by environmental gestagens in amphibians–A short review supported by new in vitro data using gonads of Xenopus laevis." Chemosphere 181 (2017): 74-82.
[31] Descamps, S. and De Vocht, A., 2017. The sterile male release approach as a method to control invasive amphibian populations: a preliminary study on Lithobates catesbeianus. Management of Biological Invasions, 8(3), p.361.
[32] LIFE 3n-Bull Frog. LIFE18 NAT/BE/001016
https://ec.europa.eu/environment/life/project/Projects/index.cfm?fuseaction=search.dspPage&n_proj_id=7240
[33] Underwood, W. and Anthony, R., 2013. AVMA Guidelines for the Euthanasia of Animals: 2020 Edition. Retrieved on March, 30(2020), pp.2020-01.
[34] Teem, J.L., et al. (2020). Genetic control of invasive species. Frontiers in Bioengineering and Biotechnology. 8:452. doi: 10.3389/fbioe.2020.00452

Appendix 10. Hunting dogs (tracking/ baying)

1. Measure name	
1.1. English:	Hunting dogs (tracking/ baying)
1.2. Lethal or non-lethal:	Non-lethal

1.3. Other languages (if available):

Bulgarian	Ловни кучета (проследяване/ преследване)	Italian	Cani da caccia (inseguimento / punta)
Croatian	Lovački psi (praćenje/gonjenje)	Latvian	Medību suņi (izsekošana/izspiešana)
Czech	Lovečtí psi (stopování/stavění)	Lithuanian	Medžiokliniai šunys (sekimas/baidymas)
Danish	Jagthunde	Maltese	
Dutch	Jachthonden	Polish	Psy myśliwskie (tropienie/płoszenie)
Estonian	Jahikoerad (jälitamine/haukumine)	Portuguese	Cães de caça (rastreamento / latidos)
Finnish	Metsästyskoirat (jäljestys/haukkuminen)	Romanian	Câini de vânătoare
French	Chiens de chasse	Slovak	Poľovné upotrebiteľné psy (stopovanie/durenie)
German	Jagdhunde	Slovenian	Lovski psi (sledenje / lov)
Greek	Κυνηγετικοί σκύλοι (ιχνηλάτιση/γαύγισμα)	Spanish	Peros de caza (Rastreo/ladrido)
Hungarian	Vadászkutyák (nyomkövetés/ugatás)	Swedish	Jakthundar
Irish			

2. Technical details of measure

2.1.a. Measure description

Hunting dogs are an intergrated part of many hunting cultures worldwide. Many types of dogs have been bred to track, find and bay or point at a multitude of different quarrys for hunting [1], but their skills are also used in conservation [2], and wildlife research projects, e.g.moose (Alces alces) [3], brown bear (Ursus arctos) [4], and grouse (Tetraonidae) [5]. Dogs are also used for IAS work. Detection dogs detect, but never hunt or come in direct contact with the IAS, i.e. similar to customs dogs detecting and alerting for narcotics, and are used to find egg laying places for ***Trachemys*** species in Spain [6], **Raccoon Dog** tracks in Norway [7], and **Nutria** in USA [8]. Tracking/baying dogs track, find and put the IAS at bay when released and are used to find and bay **Nutria** in USA [9], **Raccoon** in USA [10,11], and **Raccoon *Dog*** in Sweden/Finland [7]. Appropriately trained tracking/baying dogs, i.e. that are selective for the target species and do not interact with the IAS in a manner that could harm the target animal, should be used. Some dogs do not want to, or are so well trained that they will not, interact physically with the IAS (or other animals). In other cases the target species have a behavior that makes it impossible to interact physically, e.g. tree-climbing species. If untrained dogs are used, e.g. young dogs in training, and there is risk for physical confrontation they should wear a muzzle to prevent harming the IAS or other species. Once found and brought to bay, the animals are captured or culled with other measures. The hunter will only capture or cull the target species, leaving any non-target species, possibly stressed, but physically unharmed.

Dogs can be an important tool for IAS management in both areas of low densities, such as areas of new introductions/releases of an IAS, as well as a way to support the cull of large numbers of individuals in an established population. Swedish and Finnish professional IAS hunters have assessed that by using dogs they capture/cull over 90 % of all raccoon dogs reported by MMS cameras in low density populations. Without hunting dogs less than 10 % would be captured or culled, with traps or shot on a bait, according to their assessment [7]. About 50% of the total annual cull of 150,000 – 200,000 **raccoon dogs** in Finland is culled by local hunters with the help of their private hunting dogs [M Alhainen 2020, Pers. comm.]. The dogs are trained and used according to the Finnish hunting law. In the Nordic countries **raccoon dogs** are typically hunted with only one dog which minimizes the risk for invasive confrontations for the IAS. The legislation for using, and if so how and when to use, private hunting dogs may differ between species and countries in Europe.

2.1.b. Integration with other measures

Tracking/baying hunting dogs can be used together with shooting, to cull the animal humanely once put at bay, in medium to high density populations where the dog is released on free search in an area suitable for the target species. In low density populations there is little use in releasing a tracking/baying dog on free search. The tracking/baying hunting dog in low density areas is put to use after the target animal first have been detected by other measures, such as a game camera, a citizen science system, a Judas animal, or a detection dog. None of these other measures can handle putting the target animal at bay so that it can be captured or culled with yet another measure such as an animal control pole or by shooting. Often several measures are combined, for example a person spots an IAS and reports it to the citizen science system whereafter a game camera is set up to confirm the observation, and when the IAS shows itself a professional hunter releases a tracking/baying hunting dog to put the IAS at bay, which can then be captured or culled. Traps can and are also commonly put out on the same spot and at the same time as the camera, but usually it is difficult to get the IAS to go into the trap and a hunting dog is still often needed.

2.2.a. Availability - species and objectives

Objective	Unknown objective		Rapid Eradication		Management					
					Eradication		Control		Containment	
Species	Avail.	Ref(s).	Avail.	Ref(s).	Avail.	Ref(s).	Avail.	Ref(s).	Avail.	Ref(s).
Acridotheres tristis										
Alopochen aegyptiaca										
Callosciurus erythraeus	P		P	Expert opinion	P	Expert opinion	P	Expert opinion	P	Expert opinion
Corvus splendens										
Herpestes javanicus	A	12					P		P	
Lepomis gibbosus										
Lithobates catesbeianus										
Muntiacus reevesi	P	Expert opinion			P		P		P	
Myocastor coypus	A	8,9	P		P		P		P	
Nasua nasua	P	Expert opinion								
Nyctereutes procyonoides	P		A	7	A	7	A	7	A	7

Ondatra zibethicus	P	Expert opinion	P		P		P		P	
Oxyura jamaicensis										
Perccottus glenii										
Plotosus lineatus										
Procyon lotor	P		A	10, 11	A	10, 11	A	10, 11	A	10, 11
Pseudorasbora parva										
Sciurus carolinensis	P		P	Expert opinion	P	Expert opinion	P	Expert opinion	P	Expert opinion
Sciurus niger	P		P	Expert opinion	P	Expert opinion	P	Expert opinion	P	Expert opinion
Tamias sibiricus	P		P	Expert opinion	P	Expert opinion	P	Expert opinion	P	Expert opinion
Threskiornis aethiopicus										
Trachemys scripta			P	Expert opinion	P	Expert opinion	A	6		

2.2.b. Application – EU Member States and objectives										
Objective	**Unknown objective**		**Rapid Eradication**		**Management**					
					Eradication		**Control**		**Containment**	
Country	Avail.	Ref(s).	Avail.	Ref(s).	Avail.	Ref(s).	Avail.	Ref(s).	Avail.	Ref(s).
Austria										
Belgium										
Bulgaria										
Croatia										
Cyprus										
Czech Republic										
Denmark			X	13	X	13	X	13	X	13
Estonia										
Finland			X	13	X	13	X	13	X	13
France										
Germany										
Greece										
Hungary										
Ireland										
Italy										
Latvia										
Lithuania										

Luxembourg										
Malta										
Netherlands										
Poland										
Portugal							X	6		
Romania										
Slovakia										
Slovenia										
Spain							X	6		
Sweden			X	13	X	13	X	13	X	13
United Kingdom*										

* Not an EU Member State

3. Humaneness of the measure			
3.1. Welfare for all measures			
Measure type (if applicable): Tracking/ baying hunting dogs	**Humaneness impact categories**		
Domain	**No impact**	**Mild-Moderate**	**Severe - Extreme**
1: Water deprivation, food deprivation, malnutrition	No impact for either the hunted animals or the hunting dogs.		
2: Environmental challenge	No impact for either the hunted animals or the hunting dogs. Game animals hunted with dogs are naturally also hunted by predators.		
3: Injury, disease, functional impairment		Mild/moderate impact for the hunted animals or the hunting dogs, given the use of appropriately trained dogs or dogs that are wearing a Muzzle. Hunted animals may experience	

		mild to moderate damage to muscles and some destruction of red blood cells when trying to escape [14].	
4: Behavioural, interactive restriction	No, only natural antipredator behavior.		
5: Anxiety, fear, pain, distress, thirst, hunger etc.		Mild/moderate impact for the hunted animals or the hunting dogs, given the use of appropriately trained dogs or dogs that are wearing a Muzzle. Target animals will experience stress similar to their natural level of stress when being chased by a predator.	

Measure type (if applicable): Detection dogs	**Humaneness impact categories**		
Domain	**No impact**	**Mild-Moderate**	**Severe - Extreme**
1: Water deprivation, food deprivation, malnutrition	No impact for either the detected animals or the hunting dogs.		
2: Environmental challenge	No impact for either the detected animals or the hunting dogs.		
3: Injury, disease, functional impairment	No impact for either the detected animals or the hunting dogs.		

4: Behavioural, interactive restriction	No impact for either the detected animals or the hunting dogs.		
5: Anxiety, fear, pain, distress, thirst, hunger etc.	No impact for either the detected animals or the hunting dogs.		

3.2. Mode of death (if relevant)			
Measure type (if applicable):	**Immediate death (i.e. no suffering)**	**Not immediate death (mild - moderate suffering)**	**Not immediate death (severe - extreme suffering)**
Rationale:	N/A		

3.3. Humaneness summary	No impact for or caused by the detection dogs. They detect, but never hunt or come in direct contact with the IAS. Mild/moderate impact for the hunted animals and the tracking/baying hunting dogs, given the use of appropriately trained dogs, or dogs that are wearing a Muzzle. Time between finding the animal and death/handling of the animal is short with dogs (minutes), for example compared with non-lethal trapping, where animals can be restrained for hours or more. After the capture, animals are usually shot (if not captured), which causes the immediate death of the animals if made correctly.

4. Costs and effectiveness of the measure	
General effectiveness of the measure	Hunting with tracking/baying dogs can be very efficient to reduce high density populations of IAS in combination with shooting. In low density populations tracking/baying dogs are very efficient combined with other measures, such as game cameras or Judas animals, to put the IAS at bay for capture or culling once detected by the other measures. Detection dogs can be efficient to confirm presence of IAS when there is nothing there to find for the human eye as well as to prove zero presence after an eradication. Most reports from citizen science systems are for example difficult to validate if there is no picture or DNA included in the observation. There is little use of putting up a camera or a trap since the observed animal is usually far away within days. A detection dog can however confirm if it actually was a target species that was observed, or that it was not, several days after the observation was made.

4.1. Case studies	
CASE STUDY #1	
Measure type (if relevant):	Tracking/baying Hunting dogs
Species:	Raccoon dog
Objective:	Containment
Use of measure	Used to keep the population at a low level and contain it to the present distribution area (which today is much smaller than ten years ago). It is not eradication since individuals are immigrating from Finland, so eradication is not possible as long as there are raccoon dogs on the other side of the country border.
Combined with other measure(s):	Citizen science systems, game cameras, Judas animals, shooting The tracking/baying hunting dog in low density areas is put to use after the target animal first have been detected by other measures, such as a game camera, a citizen science system or a Judas animal. None of these other measures can handle putting the target animal at bay so that it can be captured or culled with yet another measure such as an animal control pole or by shooting. Often several measures are combined, for example a person spots an IAS and reports it to the citizen science system whereafter a game camera is set up to confirm the observation, and when the IAS shows itself a professional hunter releases a tracking/baying hunting dog to put the IAS at bay, which can then be captured or culled. Traps can and are also commonly put out on the same spot and at the same time as the camera, but usually it is difficult to get the IAS to go into the trap and a hunting dog is still often needed.
Country(ies) of application:	Sweden
Geographic scale (km^2) and/or population size measure applied to:	150 000 Km2
Time period:	10 years.
Effort:	5 appropriately trained dogs at all time for ten years. Dogs are not used all the time but need to be cared for continuously.
Costs:	Overall costs:

	The whole project has costed approx. 800 000 Euros/year 2010-2020, but then incorporates all combined measures (citizen science system, game cameras, Judas' animals, professional hunters and hunting dogs). Breaking out only hunting dogs is difficult since all measures are used simultaneously. Approximately 20-25% of the budget is used for the work with hunting dogs, to capture partners to Judas animals, to capture raccoon dogs disclosed by game cameras, etc.
	Personnel costs:
	Equipment and infrastructure:
	Other, including overheads:
Effectiveness:	Very effective.
Reference(s):	13

CASE STUDY #2	
Measure type (if relevant):	Detection dogs
Species:	*Trachemys scripta*
Objective:	Control
Use of measure	Specifically trained dogs are used to identify nests, and eggs, and to verify terrestrial sightings when exact locations is unknown.
Combined with other measure(s):	Trapping, ground penetration radar, gravid female radio tracking.
Country(ies) of application:	Spain and Portugal
Geographic scale (km^2) and/or population size measure applied to:	23.000 exotic invasive specimens of tortoises have been caught in the wild during the project implementation, in 33 Spanish and 5 Portuguese wetlands.
Time period:	2011-2013
Effort:	No information provided.
Costs:	Overall costs:
	No information provided. However, steps need to be taken to train the dogs (familiarization, searching for dummy nests and eggs, searching for real nests), which shows that the resources required can be high depending on the number of dogs trained.
	Personnel costs:

	Equipment and infrastructure:
	Other, including overheads:
Effectiveness:	LIFE Trachemys mentioned that the measure works for new laid nests or for hatchlings, but that older nests are missed by sniffer dogs which reduces the effectiveness of the measure. The method seems particularly effective for early detection/rapid eradication projects.
Reference(s):	6

4.2. Costs effectiveness summary	Difficult to summarize. Ten years ago the professional hunters and their dogs worked only with raccoon dogs in Northern Sweden. Today they are responsible for the eradication/mitigation of all EU-listed mammals, birds and reptiles in Sweden and the staff works all over the country with all these species for the same cost as for one species 10 years ago. Today the hunting dogs are very cost-effective, and ten years ago, while building up the integrated system, including the hunting dogs, it was still effective, but the cost was higher. When dogs are used to detect nests of Trachemys scripta, the method seems particularly effective for early detection/rapid eradication projects.

5. Side effects	
Non-target native species, their habitats and the broader environment:	Positive:
	Well trained hunting dogs are selective for the target species and do not interact physically with the IAS. If untrained dogs are used, e.g. young dogs in training, and there is risk for physical confrontation they should wear a muzzle to prevent harming the IAS or other species. In any of these cases the hunter will only capture or cull the target species, leaving any non-target species, possibly stressed, but physically unharmed.
	Negative:
	Untrained dogs may find and take on non-target species, especially in areas with high density of game. If tracking/baying hunting dogs are not appropriately trained or adequately muzzled both target and non target animals can be seriously injured/ killed by dogs, or escape wounded. In both cases, severe suffering can be involved [15].
Other invasive alien species:	Positive:
	Dogs trained for one IAS can often also be used on similar IAS.
	Negative:

Public health and well-being:	Positive:
	Hunting dogs in general are no threat for humans.
	Negative:
Economic:	Positive:
	Dogs trained for one IAS can also be used on similar IAS which lead to savings on the available budget.
	Negative:
	The training of the hunting dogs is time-consuming and may require high resources, depending on the number of the dogs.

6. Conclusion

Overall assessment of the measure (qualitative)

The measure is, if correctly used, very effective and also relatively humane, Mild-Moderate. Someone, however, has to train and care for the dogs. Costs depend on the setup of the management. If fully professional it will be quite expensive and labour intense. If like in the Nordic countries there are many hunters that already have hunting dogs the cost of hiring them with their dogs are much lower, such a system is for example practiced in the Island of Åland. However, this may imply higher risks of impact on animal welfare and non-target species, if the best practice recommendations are not followed, i.e. the hired dogs are not well trained or are not wearing a muzzle. During this assessment we notice a large unused potential for hunting dogs to detect, track and bay IAS in Europe. Several of the listed IAS in Europe has a strong hunting dog culture in their native range that could be imported and adopted for use here. Some of these hunting dog traditions (Raccoons) are so effective that game management governments has put bag limits for hunting with dogs to avoid population declines of the target animal [11]. For several other IAS in Europe, that are not commonly hunted with dogs in their native range, potentials based on hunting dogs use on similar species can be seen. Squirrels, for example, can certainty be hunted with dogs traditionally used for squirrel hunting in Sweden and Finland, but which are today mainly used for capercaillie and black grouse [1]. Land access and hunting laws in other parts of Europe, outside the Nordic countries, may be a major obstacle to adapt hunting dogs as an IAS-management tool.

Assessor:	Fredrik Dahl, Per-Arne Åhlen
Reviewer 1:	Ilaria Di Silvestre
Reviewer 2:	Riccardo Scalera

7. References

1. Bergström R, Huldt H, Nilsson T (1992) Swedish game—biology and management. Almqvist & Wiksell, Uppsala
2. Gompper, M. (2014). Free-ranging dogs and wildlife conservation. Oxford University Press, Oxford.
3. Ruusila, V., & Pesonen, M. (2004). Interspecific cooperation in human (Homo sapiens) hunting: The benefits of a barking dog (Canis familiaris). Annales Zoologici Fennici, 41(4), 545-549. Retrieved July 9, 2020, from www.jstor.org/stable/23735938
4. Bischof, R., Fujita, R., Zedrosser, A., Söderberg, A. & Swenson, J.E. (2008) Hunting patterns, ban on baiting, and harvest demographics of brown bears in Sweden. Journal of Wildlife Management, 72, 79– 88.
5. Smith, A. & Willebrand, T. (1999) Mortality causes and survival rates of hunted and unhunted willow grouse. Journal of Wildlife Management, 63, 722– 730.
6. Project LIFE+Trachemys (LIFE09 NAT/ES/000529). https://ec.europa.eu/environment/life/project/Projects/index.cfm?fuseaction=home.showFile&rep=file&fil=LIFE09_NAT_ES_000529_LAYMAN.pdf
7. Dahl, F., & Åhlén, P. A. (2017). Information on measures and related costs in relation to species included on the Union list: Nyctereutes procyonoides. Technical note prepared by IUCN for the European Commission.
8. Pepper, M., Sullivan, K., Colona, R., & McKnight, J. (2018). Chesapeake Bay Nutria Eradication Project: 2017 Update. Proceedings of the Vertebrate Pest Conference, 28.
9. Kendrot, S. R. (2014). Chesapeake Bay Nutria Eradication Project: Update 2009-2014. Proceedings of the Vertebrate Pest Conference, 26.
10. Rakow, T & Rakow, B. (2006) Raccoon Hunting Basics and Beyond. Rock Dove Publications.
11. Raccoon hunting. https://en.wikipedia.org/wiki/Coon_hunting.
12. Mongoose hunting. https://www.hawaiinewsnow.com/story/19487659/dog-joins-hunt-for-mongoose-on-kauai/
13. MIRDINEC. (2014). Management of the invasive Raccoon Dog (Nyctereutes procyonoides) in the north-European countries. MIRDINEC LIFE09 NAT/SE/000344 MIRDINEC. https://ec.europa.eu/environment/life/project/Projects/index.cfm?fuseaction=search.dspPage&n_proj_id=3784
14. Bateson, P., and Harris, R. (2000). 'The Effects of Hunting with Dogs in England and Wales on the Welfare of Deer, Foxes, Mink and Hare. Contract 7 Report to the Burns Inquiry.' Home Office, London.
15. Committee of Inquiry into Hunting with Dogs in England and Wales (2000). The Final Report of the Committee of Inquiry into Hunting with Dogs in England and Wales, Norwich: TSO.

Appendix 11. Judas animals

1. Measure name	
1.1. English:	Judas animals
1.2. Lethal or non-lethal:	Non-lethal

1.3. Other languages (if available):

Bulgarian	Животни Юда	Italian	Animali Giuda
Croatian	Trenirane životinje	Latvian	Jūdas dzīvnieki (viens dzīvnieks aizved aiz sevis baru un to atved noteiktajā vietā)
Czech	Jidášští jedinci	Lithuanian	Apgaulūs (Judo) gyvūnai
Danish	Judas dyr	Maltese	
Dutch	Judasdieren	Polish	Wykorzystanie osobników obcego gatunku inwazyjnego do wykrywania innych osobników (ang. metoda Judasza)
Estonian	Juudase loomad - üks isend püütakse kinni, varustatakse raadiosaatjaga ja lastakse tagasi loodusesse eesmärgiga naasta liigikaaslaste juurde; jahimehed jälitavad saatjaga looma ja surmavad kõik tema leitud liigikaaslased	Portuguese	Animais Judas
Finnish	Juudas-eläin	Romanian	Animalele lui Iuda, Capra lui Iuda
French	Animaux Judas	Slovak	Uporaba živali vodnice (iste vrste)
German	Judastiere	Slovenian	Animalele lui Iuda, Capra lui Iuda
Greek	Ζώα προδότες	Spanish	Animales Judas
Hungarian	Júdás állatok	Swedish	Judasdjur, Sändardjur
Irish			

2. Technical details of measure

2.1.a. Measure description

The technique of using tagged individuals to find conspecifics, in species that are known to aggregate, has been termed the Judas technique [1]. The Judas technique and its name originates from its use in sheep (*Ovis aries*) and cattle (*Bos taurus*) herding in the 1800s. The Judas animals were trained to associate with sheep or cattle, leading them to a specific destination. The term refers to Judas Iscariot, a disciple of Jesus Christ who betrayed him [2, 39]. The use of the technique for livestock management has diminished over the years, but has since the 1980s been picked up as a useful tool in nature conservation projects [1]. One of the more influential conservation projects using Judas animals are the feral goat (*Capra hircus*) eradication projects on the Galapagos Islands [3, 4, 5]. The Judas technique has since then spread to a multitude of invasive taxa and species, e.g. other feral livestock [6, 7, 8], birds [9], insects [10, 11], predators [12], fish [13], rodents [14], primates [33], and reptiles [15].

Some species are social by nature, searching for and pairing up with conspecifics if they become alone. This natural behavior can be used to find new animals by letting one animal (the Judas animal) disclose others [4]. The Judas animal has to have some kind of tracking device, e.g. a radio or satellite collar, so that it can be tracked and found when appropriate [4]. Tracking intensely in real time with satellite or GSM (Global System for Mobile Communications) technique that send the locations automatically to the manager's computer often allows the manager to decide, in the office, by the pattern of the animal's movements when it has connected to other animals, saving time to visit the animal repeatedly to find this out in the field which has to be done with the VHF (Very high frequency) technique [12]. The VHF technique on the other hand has the advantage of much lighter transmitters that can be attached to much smaller animals, such as insects [10]. In some cases, it is also advantageous to mark the animal visibly, e.g. with paint or ear marks, not to harm the wrong animal e.g. when in a herd of other animals, if the disclosed animals are to be culled [2].

Judas animals are most effective when deployed in low density populations [4]. In high density populations most or all Judas animals will soon find other animals, they will get "trap saturated". At high population densities a lot of work is required to capture wild individuals and move Judas animals; efficiency is relatively small under such circumstances. If efficient alternative methods are limited, the captures due to the Judas animals can however make up a large proportion of all captured animals, although it will have little effect on the population as a whole. Using solely Judas animals is in practice not sufficient to stop the population from increasing once it has reached above a certain threshold level [12].

At medium densities Judas animals will find some new individuals. Some of these new animals will be animals that would have been hard to catch otherwise. In general, though, using only Judas animals will have limited effect on a medium sized population. If alternative measures, e.g. hunting, are reasonably effective, a rather small proportion of all captured animals will be captured by Judas animals [8, 12].

At low population densities, Judas animals will have larger effect on the population and make up a larger proportion of all captured animals. At very low densities it is almost impossible to find just a few remaining individuals, especially in a large area, with other methods, e.g. hunting dogs, with traps, with aircrafts or with game cameras. Judas animals on the other hand will constantly and continuously actively search for conspecifics every hour of every day [12].

Independent of the population density however, tagged animals may still be of great value in conservation projects to learn more about the population to be managed, e.g. social interactions, movement paths and preferred habitats, but then not solely with the purpose of finding undetected animals [16,17].

The Judas technique is typically used for social species that are aggregating into "groups". These "groups" can however be very variable in size, from pairs in monogamous species, such as the raccoon dog (*Nyctereutes procyonoides*) [18], to large herds in flocking species, such as goats [4]. The technique is, however, also used successfully for more solitary species, e.g. to find breeding sites once a year [10, 15], or temporal aggregations of other sorts [13].

Both sexes may be used as Judas animals, but the efficiency of either sex is highly dependent on the ecology of the species [16]. Judas animals may receive varying treatment before being released. The simplest and least invasive method is to immediately mark and release the animal upon capture. In other cases, the animals are systematically relocated to high-risk areas to become more efficient. Sterilization of Judas animals is quite common, to ensure that no reproduction can take place if the animal is lost. It is important to note that animals are sterilized, not castrated, to keep their libido, and thereby their will to search for conspecifics of the opposite sex intact. A standard practice is also to treat the Judas animals against diseases and parasites before releasing them, when relevant [12]. Even more elaborate methods include hormone therapy to increase the animal's attractiveness to others [4, 5, 19]. Farmed/domesticated animals may also be used as Judas animals, it may be an easy and cheap way to set up an early warning system for countries that do not yet have a specific invasive alien species in their country [12].

Culling/eradication is usually the main purpose of Judas animals (combined with other methods such as shooting), but they may also be useful for other purposes, e.g. as an early warning system to detect when a new individual has invaded or reinvaded an area [4, 12].

The use of Judas animals may need responsible national authorities' permission to capture and release wild animals in nature. Legislation may differ between species and countries. The use of Judas animals may need ethical approval from responsible authorities. Legislation may differ between species and countries.

2.1.b. Integration with other measures

Hunting dogs to put animals at bay for capture or culling, animal control stick if the animal is to be captured, shooting if the animal is to be culled. Sterilization is also often used to eliminate the chance of the Judas animal reproducing once released.

2.2.a. Availability - species and objectives

Objective	Unknown objective		Rapid Eradication		Management					
					Eradication		Control		Containment	
Species	Avail.	Ref(s).	Avail.	Ref(s).	Avail.	Ref(s).	Avail.	Ref(s).	Avail.	Ref(s).
Acridotheres tristis	P	34								
Alopochen aegyptiaca										
Callosciurus erythraeus										
Corvus splendens	P	35	P							
Herpestes javanicus	P									
Lepomis gibbosus										
Lithobates catesbeianus										
Muntiacus reevesi	P	36, 37			P		P		P	
Myocastor coypus	U	32								
Nasua nasua	P	26								
Nyctereutes procyonoides	A	12, 23, 24, 25, 42	A	12, 23, 24, 25, 42	A	12, 23, 24, 25, 42	A	12, 23, 24, 25, 42	A	12, 23, 24, 25, 42
Ondatra zibethicus	P	38								
Oxyura jamaicensis										
Perccottus glenii										
Plotosus lineatus										
Procyon lotor	U	21, 22								
Pseudorasbora parva										
Sciurus carolinensis										
Sciurus niger										
Tamias sibiricus										

Threskiornis aethiopicus	P	28								
Trachemys scripta	U?	31								

Here ‘Unknown’ objective refers to detection. (P) species have behavior where Judas animals should logically work, but where we have not found any reference backing this up.

2.2.b. Application – EU Member States and objectives										
Objective	**Unknown objective**		**Rapid Eradication**		**Management**					
					Eradication		**Control**		**Containment**	
Country	Avail.	Ref(s).	Avail.	Ref(s).	Avail.	Ref(s).	Avail.	Ref(s).	Avail.	Ref(s).
Austria										
Belgium										
Bulgaria										
Croatia										
Cyprus										
Czech Republic										
Denmark	X	25, 42								
Estonia										
Finland	X	23								
France										
Germany										
Greece										
Hungary										
Ireland										
Italy										
Latvia										
Lithuania										
Luxembourg										
Malta										
Netherlands										
Poland										
Portugal	X	31								
Romania										
Slovakia										
Slovenia										
Spain	X	31								
Sweden	X	24								
United Kingdom*										

* Not an EU Member State

3. Humaneness of the measure			
3.1. Welfare for all measures			
Measure type (if applicable): Judas animals	**Humaneness impact categories**		
Domain	**No impact**	**Mild-Moderate**	**Severe - Extreme**
1: Water deprivation, food deprivation, malnutrition	No negative impact for either the disclosed animals or the Judas animals, assuming good husbandry practices for when the Judas animal is in captivity. Judas animals may even be in better condition after captivity than when being captured. Raccoon dogs for example usually increase in weight while in captivity and are also routinely treated against diseases/parasites, improving their well-being, and most likely also their survival and thereby fitness, once released. After release they are also supplementary fed during harsh winters to keep the valuable animals in good condition and thereby increasing their chances of finding a mate. Although, a few individuals are in such poor condition or so ill from parasites or disease they cannot be treated. In such cases they are euthanized quickly and humanely [12, Unpublished data from the Swedish raccoon dog project].		
2: Environmental challenge		No impact for the disclosed animals if they are captured, tagged and released at the place of capture. If Judas animals are moved, or farmed/captive animals are used, there will be a change of environment for the animals which may initially affect their welfare negatively,	

		especially for farmed/captive animals that have no previous experience of living in the wild, e.g. finding food. If animals are to be sterilized, consideration should be taken to what is least invasive for the species. Most species can and should be brought to a sterile clinic for the sterilization, e.g. raccoon dogs or squirrels [29, 40]. Appropriate transportation arrangements must be considered. An appropriate facility should be available if the sterilization is made in a clinic, for keeping the animal before the surgery and letting it recover after the surgery, before being released back to the wild. Depending on the species, e.g. sensitivity of stress, consideration should be taken to the time between capture and surgery, and time of post-operative recovery [40].	
3: Injury, disease, functional impairment		The negative impact on Judas animals is mild-moderate once released back into the wild. Animals may sustain injuries during (re)capture/restraint (e.g. with the use of dogs or traps, see separate assessments). If these compromise their survival in the wild, the animals should be euthanized quickly and humanely. The collar must be fitted correctly to allow it to move up the tapered neck if the animal grows or gets fat. Ill-fitting collars	

		can also cause chafing or constriction. Adverse effects of wearing the collar should be monitored by looking for irritation or hair loss under the collar [41].	
4: Behavioural, interactive restriction		If animals get hormone treatment to enhance efficacy (Mata Hari animals [5]) there may be behavioral/physiological effects but this is largely unstudied. Autopsied Mata Hari raccoon dog females showed no negative effects with 200 day implants. It is well known however, that domestic dogs may develop uterus inflammation after the effect of the hormone implant decreases [30]. Repeatedly being isolated and having to find other conspecifics may cause fear and anxiety, particularly in highly social animals [41].	
5: Anxiety, fear, pain, distress, thirst, hunger etc.		Judas animals may be distressed when cohorts are killed. Also, the sound of gunshots and presence of people is likely to cause further fear and anxiety. Repeatedly being isolated and having to find other conspecifics may cause fear and anxiety, particularly in highly social animals. When the Judas animals are moved to another area, they need to be trapped and transported, which can cause stress, pain or suffering [41].	

3.2. Mode of death (if relevant)			
Measure type (if applicable):	**Immediate death (i.e. no suffering)**	**Not immediate death (mild - moderate suffering)**	**Not immediate death (severe - extreme suffering)**
Rationale:	N/A		

3.3. Humaneness summary	Capture of Judas animals, e.g. by darting from helicopter, dogs or traps cause stress of varying severity to the Judas animals (see separate assessment on these methods). Culling of disclosed animals, e.g. by shooting from long range, or culling with a hand gun after first being put at bay by a dog cause stress of varying severity to the Judas animals (see separate assessment on shooting). The welfare of Judas animals may also be affected by surgical sterilization, by being introduced in new areas, by being isolated and by having to find other conspecifics. The severity of the stress is exacerbated by the repetition of these actions and the cumulative effect on the animal should then be considered. This can also vary according to the social and behavioral characteristics of the different species. In general, the impact of the method on Judas animals is Mild-Moderate.

4. Costs and effectiveness of the measure	
General effectiveness of the measure	The Judas animal method is most cost-efficient at very low densities of the target population. When only few animals are left in a population up for eradication, Judas animals will often be the only way of finding them all. Similarly, based on the experience in the Nordic countries, it is effective as an early warning in countries (or geographical areas within countries) that do not yet have the invasive alien species, i.e. to detect immigrating individuals with Judas animals. Farmed or semi-domestic animals may also be an easy and cheap way to set up such an early warning system. In Sweden some Judas animals have been found to be very efficient while others have never paired up with a new mate. It is likely that there are individual differences in how efficient the animals are on this task. Ineffective animals should, if possible, be humanely disposed and replaced with new animals [12].

4.1. Case studies	
CASE STUDY #1	
Measure type (if relevant):	Judas Animals
Species:	Raccoon dog (*Nyctereutes procyonoides*)
Objective:	Finding animals for Eradication/Containment

Use of measure	Wild individuals of both sexes are captured, sterilized, treated against diseases and fitted with a satellite transmitter. The Judas animal's movements are observed in real time; the transmitter is sending positions to the manager. When the Judas animal stops for several days in the same area it is visited in the field and any partner is captured.	
Combined with other measure(s)	Hunting dogs to keep animals at bay, animal control stick if the animal is to be captured, shooting if an animal kept at bay are to be culled. Sterilization is also often used to eliminate the chance of the Judas animal reproducing once released.	
Country(ies) of application:	Sweden	
Geographic scale (km^2) and/or population size measure applied to:	150 000 Km2 50-100 Individuals distributed over the area.	
Time period:	Evaluated and tested 2008-2010, started actively 2010/2011, still ongoing 2020 [12, 24].	
Effort:	Difficult to give exact quantities and costs since the measure is integrated with other measures. One full time employee will manage about 20-25 Judas animals if working full time with that. In practice however, the employees manage the other methods at the same time as checking Judas animals, saving time and money on combining work in one travel. All six field personnel work with all measures, but together they may devote one full time to Judas animal work per year. Depending on the size of the management area, a minimum of ten Judas animals should always be active, preferably with an equal sex ratio since both sexes are equally likely to disperse into new areas. Judas animals pairing up with each other should be immediately separated to keep the system as efficient as possible.	
Costs:	Overall costs:	
	Personnel costs:	
	Capturing of animals is included in the cost of the full time employee. Sterilization will cost approximately 200 Euro per animal.	
	Equipment and infrastructure:	
	One GSM/Satellite collar costs approximately 2,000-2,500 Euros. Depending on the settings of the positioning the battery will last between 6 months to one year. A battery replacement will cost approximately 500 Euros. A collar can last up to five years if not physically damaged. Operator costs for GSM traffic may have to be added. In total, considering that collars are able to work for five years, the cost of collars will be between 1,000-1,500 Euros per collar/year. Triangulation equipment for locating the transmitter signal in the field costs about 1,000 Euros per unit.	
	Other, including overheads:	
	Additional costs include a place to keep raccoon dogs captured while waiting for sterilization, and when recovering after the surgery.	
Effectiveness:	As expected in a medium sized population other measures have been more efficient so far, but now the population is getting very small, and the Judas animals are showing signs to increase in relative efficiency in finding new animals compared with the other measures. During 2020 the Judas animals has delivered about 30% of the new individuals until September. Since 2010 the Judas animals has however also given invaluable	

	information about the ecology of the species in its dispersal front, helping to improve the whole management system of several integrated methods (e.g. efficiency of citizen science, game cameras, hunting dogs and lures).

4.2. Costs effectiveness summary	At low population densities Judas animals are very cost efficient, but much less so at high densities. However, the information they allow to be collated on dispersal, habitat selection and distribution hotspots may still be very useful for the management.

5. Side effects	
Non-target native species, their habitats and the broader environment:	Positive:
	Negative:
	There may be some added predation/herbivory/trampling from Judas animals, but very marginal.
Other invasive alien species:	Positive:
	Negative:
Public health and well-being:	Positive:
	Negative:
	No risk if the Judas animals are treated against diseases and parasites before release.
Economic:	Positive:
	Negative:

6. Conclusion
Overall assessment of the measure (qualitative)
Judas animals is a technique intense (quite expensive) and quite labor intensive measure. At high population densities its value is limited seen only as a mean to reduce the population. As a combined research and management measure it may, however, be well worth the cost and time also at high or medium sized population densities, to learn more about how to manage the population and become more efficient as a whole [12]. At very low population densities the measure has proven to be very valuable, sometimes necessary, to find and cull the last remaining individuals [3, 4, 5]. Judas animals can also be recommended as a very effective early warning system, to detect and find immigrating IAS in an area or on an island [12]. There are few, if any, side effects of the measure, but while disclosed wild animals can be culled humanly, the impact on the welfare of Judas animal is mild-moderate. People's perception of the ethical side of the measure may however differ between countries.

We have in 2.2a listed species as (p) where the Judas technique can potentially be useful, based on their biology/social behavior of the species.	
Assessor:	Fredrik Dahl and Per-Arne Ahlen
Reviewer 1:	Kevin Smith
Reviewer 2:	Ilaria Di Silvestre

7. References

1. Taylor, D., & Katahira, L. (1988). Radio telemetry as an aid in eradicating remnant feral goats. Wildlife Society Bulletin. 16, 297-299.
2. Wikipedia. Judas goat. https://en.wikipedia.org/wiki/Judas_goat. Downloaded 2020-06-26.
3. Campbell, K., Donlan, C., Cruz, F., & Carrion, V. (2004). Eradication of feral goats Capra hircus from Pinta Island, Galápagos, Ecuador. Oryx, 38(3), 328-333. doi:10.1017/S0030605304000572
4. Campbell, K., & Donlan, C. (2005). Feral Goat Eradications on Islands. Conservation Biology. 19, 1362 - 1374. Doi:10.1111/j.1523-1739.2005.00228.x.
5. Cruz, F., Carrion, V., Campbell, K., Lavoie, C., & Donlan, C. (2009). Bio-Economics of Large-Scale Eradication of Feral Goats From Santiago Island, Galapagos. Journal of Wildlife Management. 73, 191-200. doi:10.2193/2007-551.
6. Spencer, P. B., Hampton, J. O., Pacioni, C., Kennedy, M. S., Saalfeld, K., Rose, K., & Woolnough, A.P. (2015). Genetic relationships within social groups influence the application of the Judas technique: A case study with wild dromedary camels. Journal of Wildlife Management. 79, 102-111. doi:10.1002/jwmg.807.
7. Carrick, P., Thomson, D., & Calley, G. (1990). The use of radio transmitters for tracking and shooting feral buffalo. The Rangeland Journal. 12, 84-90.
8. Massei, G., Roy, S., & Bunting, R. (2011). Too many hogs? A review of methods to mitigate impact by wild boar and feral hogs. Human-Wildlife Interactions. 5, 79–99.
9. Woolnough, A. P., Lowe, T. J., & Rose K. (2006). Can the Judas technique be applied to pest birds?. Wildlife Research. 33, 449-455.
10. Moore, A., Barahona, D. C., Lehman, K. A., Skabeikis, D. D., Iriarte, I. R., Jang, E. B., & Siderhurst, M. S. (2017). Judas Beetles: Discovering Cryptic Breeding Sites by Radio-Tracking Coconut Rhinoceros Beetles, Oryctes rhinoceros (Coleoptera: Scarabaeidae). Environmental Entomology. 46 (1), 92–99. https://doi.org/10.1093/ee/nvw152.
11. Kennedy, P. J., Ford, S. M., Poidatz, J. et al. (2018). Searching for nests of the invasive Asian hornet (Vespa velutina) using radio-telemetry. Communications Biology. 1, 88. https://doi.org/10.1038/s42003-018-0092-9
12. Dahl, F., & Åhlén, P. A. (2017). Information on measures and related costs in relation to species included on the Union list: Nyctereutes procyonoides. Technical note prepared by IUCN for the European Commission.
13. Bajer, P. G., Chizinski, C. J., & Sorensen, P. W. (2011). Using the Judas technique to locate and remove wintertime aggregations of invasive common carp. Fisheries Management and Ecology. 18, 497-505. doi:10.1111/j.1365-2400.2011.00805.x
14. Kendrot, S. R. (2011). Restoration through eradication: protecting Chesapeake Bay marshlands from invasive nutria (Myocastor coypus). Pages 313-319 In: Veitch, C. R., Clout, M. N., & Towns, D. R. (eds.). (2011). Island invasives: eradication and management. IUCN, Gland, Switzerland. http://www.issg.org/pdf/publications/island_invasives/pdfhqprint/3kendrot.pdf. Downloaded 2020-06-30.
15. Smith, B. J., Cherkiss, M. S., Hart, K. M. et al. (2016). Betrayal: radio-tagged Burmese pythons reveal locations of conspecifics in Everglades National Park. Biological Invasions. 18, 3239–3250. https://doi.org/10.1007/s10530-016-1211-5
16. Herfindal, I., Melis, C., Åhlén, P. A., & Dahl, F. (2016). Lack of sex-specific movement patterns in an alien species at its invasion front – consequences for invasion speed. Ecology and Evolution. 6, 5570–5584.
17. Melis, C., Herfindal, I., Dahl, F., & Åhlén, P. A. (2015). Individual and Temporal Variation in Habitat Association of an Alien Carnivore at Its Invasion Front. PLoS ONE. 10(3). e0122492. https://doi.org/10.1371/journal.pone.0122492
18. Kauhala, K., Helle, E., & Taskinen, K. (1993). Home range of the raccoon dog (Nyctereutes procyonoides) in southern Finland. J. Zool., Lond. 231, 95-106.
19. Campbell, K. J., Baxter, G. S., Murray, P. J., Coblentz, B. E., Donlan, J. C., & Carrion, V. G. (2005). Increasing the efficacy of Judas goats by sterilization and pregnancy termination. Wildlife Research. 32, 737-743.
20. Dahl, F., Åhlén, P. A., & Granström, Å. (2010). The management of raccoon dogs (Nyctereutes procyonoides) in Scandinavia. Aliens. 30, 59-63.
21. García, J. T., García, F. J., Alda, F. et al. (2012). Recent invasion and status of the raccoon (Procyon lotor) in Spain. Biological Invasions. 14, 1305–1310. https://doi.org/10.1007/s10530-011-0157-x.
22. Gestión de la población de mapache (Procyon lotor L.) en la Comunidad de Madrid. http://secforestales.org/publicaciones/index.php/congresos_forestales/article/view/14467/14310.

23. Report of Raccoon Dog management in Finland for 2019. https://jagareforbundet.se/contentassets/f110ce2f2e8643d083259c1d1d24d7f2/report-of-raccoon-dog-management-in-finland-for-2019.pdf.
24. Swedish raccoon dog project, including additional tasks on raccoon (NV-03794-15), muskrat (NV-01089-18), water turtles (Trachemys scripta S.p) and Egyptian goose (NV08788-18), and Sibirian chipmunk (NV-02057-19). (2020). https://jagareforbundet.se/contentassets/f110ce2f2e8643d083259c1d1d24d7f2/arsrapport-svenska-mardhundsprojektet-2019.pdf
25. Forvaltningsplan for mink, mårhund og vaskebjørn i Danmark. (2020). https://mst.dk/media/191343/netversion-miljoestyrelsen_forvaltningsplan_2020-mink-maarhund-og-vaskebjoern.pdf
26. Hirsch, B. T. (2011.) Long term adult male sociality in ring-tailed coatis (Nasua nasua). Mammalia 75, 301–304.
27. Robertson, B. A., Ostfeld, R. S., Keesing, F. (2017). Trojan females and Judas goats: Evolutionary traps as tools in wildlife management. BioScience. 67 (11), 983-994. 10.1093/biosci/bix116
28. Johnson, S., & McGarrity, M. (2009). "Florida's Introduced Birds: Sacred Ibis (Threskiornis Aethiopicus)". EDIS 2009 (9). https://journals.flvc.org/edis/article/view/118150.
29. Arnemo, J. M., Åhlén P. A., & Dahl, F. Biomedical Protocol for Free-ranging Raccoon Dogs. Manuscript.
30. Hestvik, G. (2015). Patologisk utvärdering av ett östrogenimplantats effekter på mårdhundar. Statens Veterinärmedicinska Anstalt, Dnr. 2015/626.
31. Project LIFE+Trachemys (LIFE09 NAT/ES/000529). https://ec.europa.eu/environment/life/project/Projects/index.cfm?fuseaction=home.showFile&rep=file&fil=LIFE09_NAT_ES_000529_LAYMAN.pdf
32. Kendrot, S. R. (2014). Chesapeake Bay Nutria Eradication Project: Update 2009-2014. Proceedings of the Vertebrate Pest Conference. 26(26). 10.5070/V426110637. https://escholarship.org/uc/item/1q470719.
33. Hanson, C., Hall, T., DeNicola, A. J., Silander, S., Keitt, B., & Campbell, K. (2019). Rhesus macaque eradication to restore the ecological integrity of Desecheo National Wildlife Refuge, Puerto Rico. In: Island invasives: Scaling up to meet the challenge, International Union for Conservation of Nature and Natural Resources, 249-255.
34. Charlotte, A. M., Yap, S. N., & Brook, B. (2002). Roost Characteristics of Invasive Mynas in Singapore. The Journal of Wildlife Management, 66(4), 1118-1127. doi:10.2307/3802943.
35. Peh, K. S. H., & Sodhi, N. S. (2002). Characteristics of nocturnal roosts of house crow in Singapore. Journal of Wildlife Management. 66, 1128–1133.
36. Chapman, N. G., Furlong, M., & Harris, S. (1997). Reproductive strategies and the influence of date of birth on growth and sexual development of an aseasonally-breeding ungulate: Reeves' muntjac (Muntiacus reevesi). Journal of Zoology. 241, 551-570. doi:10.1111/j.1469-7998.1997.tb04847.x.
37. Yahner, R. H. (1979). Temporal Patterns in Male Mating Behavior of Captive Reeve's Muntjac (Muntiacus reevesi). Journal of Mammalogy. 60 (3), 560–567. https://doi.org/10.2307/1380097.
38. Ahlers, A. A., Heske, E. J., Schooley, R. L., & Mitchell M. A. (2010). Home ranges and space use of muskrats Ondatra zibethicus in restricted linear habitats. Wildlife Biology. 16(4), 400-408.
39. Matthew 26:47-48. 47 While he was still speaking, Judas, one of the Twelve, arrived. With him was a large crowd armed with swords and clubs, sent from the chief priests and the elders of the people. 48 Now the betrayer had arranged a signal with them: "The one I kiss is the man; arrest him.".
40. Scapin, P., Ulbano, M., Ruggiero, C., Balduzzi, A., Marsan, A., Ferrari, N., & Bertolino, S. (2019). Surgical sterilization of male and female grey squirrels (Sciurus carolinensis) of an urban population introduced in Italy. Journal of Veterinary Medical Science, 18-0319.
41. Sharp, T. (2012). Model code of practice for the humane control of feral goats. Code of Practice. PestSmart website. https://pestsmart.org.au/toolkit-resource/code-of-practice-feral-goats. Accessed 21-09-2020.
42. Forvaltningsplan for mink, mårhund og vaskebjørn i Danmark (2020). https://mst.dk/media/191343/netversion-miljoestyrelsen_forvaltningsplan_2020-mink-maarhund-og-vaskebjoern.pdf

Appendix 12. Stupefying bait

1. Measure name	
1.1. English:	Stupefying bait
1.2. Lethal or non-lethal:	Non-lethal if used as intended, but potentially lethal in overdose or if smaller non-target species are impacted.

1.3. Other languages (if available):

Bulgarian	Упойваща примамка	Italian	Esca con sostanze stupefacenti
Croatian	Omamljujući mamac	Latvian	Stulbinoša ēsma
Czech	Omamující návnady	Lithuanian	Mikdantys masalai
Danish	Bedøvende lokkemad	Maltese	
Dutch	Bedwelmend aas	Polish	Przynęty oszałamiające
Estonian	Uimastav sööt	Portuguese	Isco estupefaciente
Finnish	Tainnuttava syötti	Romanian	Momeală stupefiantă
French	Appâts stupéfiants	Slovak	Omamujúca návnada
German	Betäubungsköder	Slovenian	Vabe za omamljanje
Greek	Δόλωμα νάρκωσης	Spanish	Cebo con narcotizantes
Hungarian	Kábító csalétek	Swedish	
Irish			

2. Technical details of measure

2.1.a. Measure description

Stupefying baits are food items treated with chemicals that, when ingested in the correct quantity, render the target animal unconscious or incapable of escape, 'stupefied', so that it can be captured for humane dispatch or other purposes [1, 3, 4, 5, 6]. Stupefying baits can be fed to large numbers of animals simultaneously and allow more animals to be captured with lower operator effort than techniques such as shooting or live trapping [1, 2]. If correctly dosed, a non-target animal that takes the treated bait should eventually recover, which means that non-target species will, in theory at least, be at low risk. Unfortunately, controlling the quantity of treated bait that an animal consumes can prove very difficult in practice and death by overdose of a proportion of both target and non-target animals is common [6, 7). Other problems, involving the dispersal of semi-stupefied animals from the treatment site, and consequent injury, predation or public relations problems have also been encountered [8, pers. obs.].

At present there are no stupefying chemicals approved for general use in the EU. A number of stupefying chemicals have been evaluated in the past, both in the EU and around the world, for use against both the IAS listed in this study and other analogous species [10, 11]. Of these, only Alphachloralose has been regularly used in the EU in the past. Diazepam and quinalbarbitone (deer mix) have been used outside the EU to capture deer species [21] but significant problems with dosing and non-target species were encountered and it often proved difficult to retrieve the stupefied animals [22]. Alphachloralose was quite widely used in bird management, both in the EU and elsewhere, up to the 1990s [6], but its use in the EU is now restricted to rodenticide applications within buildings [12]. Despite not being approved for use in the EU, it has been occasionally permitted for use as a stupefying chemical under special license to help eradicate localized populations of IAS [13, 14]. It has been used more widely to control wildlife populations (both invasive and native) around the world, including some of the IAS being considered in this study [2, 16, 17]. This section, therefore, focusses on the use of Alphachloralose as a stupefying chemical for birds, using examples of work carried out within the EU on the listed IAS where possible. Where necessary, examples of the use of Alphachloralose on similar species, and/or from locations outside the EU are cited in order to inform the possible use of Alphachloralose under special license in the future.

When used as a stupefying chemical, Alphachloralose is normally offered as a powder delivered by adhesion to the surface of granular baits (rice, wheat or other grains) which are fed to the target animals by broadcasting on the ground [3, 18]. In some cases it can be offered on larger baits (pieces of bread, fish etc.) which can be fed individually to the target species [13, 14, 23]. Providing that the target animal consumes enough bait, it will become disorientated and eventually unconscious or semi-conscious in a matter of 10-30 minutes depending on the quantity ingested [18]. Alphachloralose acts by inducing a state of dissociation and then sleep, but the essential bodily functions remain unaffected [18]. Stupefied animals can be collected and humanely dispatched by an approved method. Provision needs to be made to ensure that stupefied animals can be easily collected (e.g. a boat may be needed if it is used on waterbirds [19]) and that facilities are available to prevent any non-target species that take the bait from becoming harmed (e.g. taken by predators) whilst they recover prior to release. Full recovery can take up to 24hours (pers. obs.) so means of holding the animals safely and provisioning with water and/or food may be required. When using Alphachloralose against birds, problems can be encountered with birds becoming frightened as the stupefying chemical takes effect or being disturbed by predators or people and flying away from the bait site. This can result in public relations problems if partially stupefied birds land on roads, gardens and other public places or possible injury or predation of the birds involved [8, pers. obs.]. To stop this happening Alphachloralose has, in the past, been combined with a second sedative, such as Secobarbitol, to keep the birds calm whilst the stupefying chemical took effect [20]. Secobarbitol is, however a barbiturate drug that can be abused by humans and is therefore subject to very strict controls making its use in general wildlife management difficult.

Despite the technical difficulties described above, Alphachloralose has been used as an effective wildlife management tool in the correct circumstances. In situations where the correct dose can be delivered, where the treated animals can be easily recovered, and where non-target species are either absent or can be prevented from taking the bait, Alphachloralose permits the capture of large numbers of animals in a short time with relatively low effort compared to trapping or shooting [23]. The fact that non-target animals can, with proper treatment, recover makes this technique more attractive than the use of toxicants in situations where non-target species are likely to take a bait, but recovery is at best uncertain [24] and it is far preferable to avoid the ingestion of treated bait by non-target species if at all possible. If non-target species in the area are likely to take the treated bait it may be better to use alternatives, such as live trapping, where non-target species can be released unharmed, or selective shooting, where non-target casualties can be avoided [7].

Most of the successful uses of Alphachloralose have been for localised control of small populations e.g. nuisance waterfowl on lakes or gull breeding colonies [23, 4, 5, 6] (see case studies 2 and 3 below). This technique has been less successful when considered for large scale control of IAS and in most cases had been abandoned in favour of shooting or trapping after limited evaluation [7] (see case study 1 below).

2.1.b. Integration with other measures

Stupefying baits are not suitable for use over large areas, but may be used, with the appropriate license, to remove IAS from locations where other techniques cannot be used and where the constraints around dosage, non-target species and recovery of stupefied animals described above can be overcome. For example, control of birds at a breeding colony has been successfully achieved for gulls where single baits with a known dosage of Alphachloralose can be placed on each nest with a very low chance of other species taking the bait [23, pers. obs.]. Examples of the control of IAS in the EU using Alphachloralose are limited, but it was successfully used to control Sacred Ibis in France where a known dose of the chemical was added to fish baits as part of a wider programme to eradicate the species [13, 14]. The use of Alphachloralose on selected sites allowed more Ibis to be captured more quickly and with less manpower than would have been the case if shooting or trapping had been used.

2.2.a. Availability - species and objectives

Note: Because there are no stupefacients licensed for use in the EU, several of the examples below are from studies carried out outside the EU or on species not in the list of IAS used in this study. Where this is the case, the availability code is bracketed e.g. (A)

Objective	Unknown objective		Rapid Eradication		Management					
					Eradication		Control		Containment	
Species	Avail.	Ref(s).	Avail.	Ref(s).	Avail.	Ref(s).	Avail.	Ref(s).	Avail.	Ref(s).
Acridotheres tristis					(U)	2, 7, 24, 25, 26, 27, 28	(U)	2, 7, 24, 25, 26, 27, 28		
Alopochen aegyptiaca							(P)	4, 5, 6, 8		
Callosciurus erythraeus										
Corvus splendens					(U)	15, 17	(U)	15, 17		
Herpestes javanicus										
Lepomis gibbosus										
Lithobates catesbeianus										
Muntiacus reevesi	(P)	21								
Myocastor coypus										
Nasua nasua										
Nyctereutes procyonoides										
Ondatra zibethicus										
Oxyura jamaicensis										
Perccottus glenii										
Plotosus lineatus										
Procyon lotor										
Pseudorasbora parva										
Sciurus carolinensis										
Sciurus niger										
Tamias sibiricus										
Threskiornis aethiopicus					A	13, 14				
Trachemys scripta										

2.2.b. Application – EU Member States and objectives

Note: Because there are very few examples of stupefacients being used against IAS in the EU, several of the examples below are from studies carried out on species not in the list of IAS used in this study. Where this is the case, the application code is bracketed e.g. (X)

Objective	Unknown objective		Rapid Eradication		Management					
					Eradication		Control		Containment	
Country	**Avail.**	**Ref(s).**	**Avail.**	**Ref(s).**	**Avail.**	**Ref(s).**	**Avail.**	**Ref(s).**	**Avail.**	**Ref(s).**
Austria										
Belgium										
Bulgaria										
Croatia										
Cyprus										
Czech Republic										
Denmark										
Estonia										
Finland										
France						X	13,14			
Germany										
Greece										
Hungary										
Ireland										
Italy										
Latvia										
Lithuania										
Luxembourg										
Malta										
Netherlands										
Poland										
Portugal										
Romania										
Slovakia										
Slovenia										
Spain						(X)	23			
Sweden										
United Kingdom*						(X)	29			

* Not an EU Member State

3. Humaneness of the measure			
3.1. Welfare for all measures			
Measure type (if applicable):	**Humaneness impact categories**		
Domain	**No impact**	**Mild-Moderate**	**Severe - Extreme**
1: Water deprivation, food deprivation, malnutrition		Animals that are stupefied and then quickly dispatched will not suffer significantly, but for non-target species where recovery is the desired outcome there may be issues. Depending on the dose received, animals may be unconscious for a number of hours. In hot weather dehydration may become a welfare issue [pers. obs.]. As the animal is unconscious, suffering is limited, but on recovery there is the possibility that the animal may suffer as the result of being deprived of food and, in particular, water, for several hours.	
2: Environmental challenge	None		
3: Injury, disease, functional impairment	If correct dose is applied and animal is quickly recovered there should be no injuries sustained.		Stupefied animals that are not recovered may suffer accidents if they attempt to fly away from the treatment site whilst partially stupefied e.g. collisions with trees or buildings or being hit by cars.
4: Behavioural, interactive restriction		Whilst an animal is stupefied it will experience disorientation and consequent stress prior to becoming unconscious.	If the dosage is not correct, suffering is unlikely to be severe in itself, but a stupefied animal may remain partially conscious for a considerable time before recovering, and during this time, it will be unable to perform normal behaviours to avoid or escape from predators or other

			threats. If an animal is threatened but cannot escape or avoid the threat, it will experience considerable stress. If the animal is actually killed or eaten by a predator whilst conscious but unable to escape or defend itself the stress will be especially severe. The long term physiological effects of treatment with Alphachloralose are not well understood.
5: Anxiety, fear, pain, distress, thirst, hunger etc.		A successful operation will involve correctly dosed animals being quickly retrieved and humanely dispatched. A level of stress will be experienced as the animal becomes stupefied, but unconsciousness quickly follows so suffering is low.	An unsuccessful operation can result in partially stupefied animals being difficult to retrieve, possibly suffering injury or predation and non-target species needing to be looked-after pending recovery. In this case the humaneness issues can be severe.

3.2. Mode of death (if relevant)			
Measure type (if applicable):	**Immediate death (i.e. no suffering)**	**Not immediate death (mild - moderate suffering)**	**Not immediate death (severe - extreme suffering)**
Rationale:		If the animal is retrieved quickly and humanely dispatched time to death will be around 20 to 60 minutes. If correctly dosed the animal will become unconscious in around 20 minutes and will not suffer further prior to dispatch.	An incorrectly dosed animal may remain conscious but unable to coordinate its movements for a period of 1-2 hours after ingesting the treated bait. During this period, it will at least be very stressed and may injure itself or be predated. If an animal remains conscious but unable to behave normally for a longer period it may suffer from lack of food or, particularly, water which may also cause suffering.

3.3. Humaneness summary	The humaneness of treatment with Alphachloralose depends critically on the operator's ability to quickly retrieve the animals that have taken the bait and humanely dispatch them and ensure the welfare of non-target species pending recovery. This, in turn, is dependent on dosage of the stupefacient received by each animal, which may be hard to control, and the extent to which the treated animals disperse. A worst-case scenario could involve significant humaneness issues, but if used successfully in the correct locations humaneness issues will be low.

4. Costs and effectiveness of the measure	
General effectiveness of the measure	The effectiveness of alphachloralose treatments depends on the ability to deliver the correct dose of the bait to the target animal so that stupefication occurs quickly and the animal does not move away from the treatment area so that it is hard to find and humanely dispatch. At the same time, risks to non-target species need to be managed, either by ensuring that only target species take the bait, or by ensuring that the dose received by non-target species permits them to recover on site or to be collected and housed until they recover and then released. The three case studies below illustrate how, in some cases, the risk to non-target species was too great and Alphachloralose use was discontinued (case study 1). In other cases, a level of both target and non-target deaths were encountered due to difficulties with accurate dosing but tolerated (case study 2). In a third case the method of baiting permitted a high dose of stupefacient to be used because non-target species did not take the bait and so an effective control programme was carried out (case study 3). Neither of case studies 2 and 3 involved IAS, but in similar situations it may be possible to use stupefying baits successfully under special license as part of a broader control programme as in case study 4.

4.1. Case studies	
CASE STUDY #1	
Measure type (if relevant):	Use of Alphachloralose as part of a programme to eradicate the Common Myna (*Acridotheres tristis*) from the Seychelles
Species:	*Acridotheres tristis*
Objective:	Eradication
Use of measure	At the beginning of this project a field trial of several techniques to capture Common Mynas was carried out. Alphachloralose, offered on cooked rice, was trialed both to assess its effectiveness against Common Mynas and to evaluate possible non-target species issues on Fregate Is. in the Seychelles [24]. At the time, Fregate Is. was the only site supporting the critically endangered Seychelles Magpie Robin, and the removal of the introduced Mynas was a key step in the recovery programme for this species. Treated bait was distributed close to the communal roosting site used by the Mynas. The baited area was watched continuously to prevent any Magpie Robins from accessing the site. Stupefied Mynas were collected and humanely dispatched and uneaten bait was removed or buried after the treatment was completed. Although no

	Magpie robins were harmed during the trial, a number of Ground Doves and Turnstones were stupefied and several failed to recover. Because of the presence of several critically endangered species, the risk of using stupefying baits on Fregate Is. and elsewhere in the Seychelles was deemed too great and the use of Alphachloralose was abandoned in favour of shooting and trapping [7, 25, 26, 27, 28].
Combined with other measure(s):	Because of risks to non-target species, the use of stupefying baits was discontinued. Control by shooting and trapping, plus the use of avicides where appropriate was used in a multiyear programme which was eventually successful in eradicating Mynas and some other introduced species from a number of the smaller islands in the Seychelles group [7].
Country(ies) of application:	Seychelles Is.
Geographic scale (km^2) and/or population size measure applied to:	Single site trial
Time period:	The stupefacient application, plus recovery of non-target birds took 3 staff approximately 2 days to complete.
Effort:	3 staff 2 days
Costs:	Overall costs:
	Not determined
	Personnel costs:
	3 staff 2 days, bait preparation, baiting, observation to deter non-target species, bait removal, care and retrieval of non-target species
	Equipment and infrastructure:
	Cages for non-target species
	Other, including overheads:
	Cost of Alphachloralose powder
Effectiveness:	The use of stupefying baits was not effective at all because of non-target species risks and dosage issues. Shooting was used to eradicate the birds instead.

CASE STUDY #2	
Measure type (if relevant):	Use of Alphachloralose to capture nuisance Canada Geese
Species:	Canada Geese (*Branta canadensis*) analogous to Egyptian goose (*Alopochen aegyptiacus)*
Objective:	Capture relocation and release of Canada Geese causing a public nuisance
Use of measure	A series of field trials and practical applications of the use of Alphachloralose were carried out by the United States Department of Agriculture to assess the use of stupefying baits as a technique for waterfowl management in the USA [4, 5, 6, 8]. Once an appropriate dosing rate had been established, this technique was deemed effective against a variety of species, although some non-target casualties were encountered and, in some cases, stupefied birds needed to be collected from up to 3 miles away. Nevertheless, the public reaction to the operations was regarded as preferable to the response to the use of shooting or toxicants and the technique was recommended for wider use in these situations.

Combined with other measure(s):	Round-up of flightless moulting birds was carried out during the immediate post breeding season.
Country(ies) of application:	USA
Geographic scale (km^2) and/or population size measure applied to:	Localised management on specific water bodies up to $5km^2$
Time period:	Each action lasted up to 48 hours
Effort:	Not given
Costs:	Overall costs:
	Not provided
	Personnel costs:
	Not provided
	Equipment and infrastructure:
	Not provided
	Other, including overheads:
	Not provided
Effectiveness:	The method was effective in capturing and either dispatching or relocating nuisance waterfowl. But a low level of non-target species deaths (up to 5%) was encountered. This was deemed acceptable by the operators.

CASE STUDY #3	
Measure type (if relevant):	Use of Alphachloralose to control breeding colonies of Yellow-legged Gulls
Species:	Yellow-legged Gull (*Larus caccinans*)
Objective:	Reduction or eradication of a breeding colony to manage public nuisance and potential conservation impacts.
Use of measure	Alphachloralose was used over a number of years to reduce the size of a breeding colony of Yellow-legged Gulls in Spain. The action was carried out to control public nuisance and possible conservation issues [23]. Alphachloralose was offered on pieces of bread coated in margarine. A single piece of bread was placed on each nest and was eaten by the incubating bird when it returned after being disturbed when the baits were laid. The incubating birds ate the bread and then settled down on the nest so there were no issues with birds departing from the site, nor with possible non-target species eating the bait as there were no other species present in the colony. This enabled a high dose of stupefacient to be used, effectively causing it to act as a toxicant, because none of the birds recovered even if they were not captured and dispatched. Other work in the UK using the same technique to remove gull colonies from airfields to preserve flight safety resulted in the total eradication of breeding colonies within 3-4 years [pers. obs.].

Combined with other measure(s):	No
Country(ies) of application:	Spain
Geographic scale (km^2) and/or population size measure applied to:	Individual breeding colonies of several thousand pairs of gulls
Time period:	Repeated applications over several years. Each individual application lasted one day.
Effort:	Not provided
Costs:	Overall costs:
	Not known
	Personnel costs:
	Not known. In other work on airfields in the UK, 3 staff were able to apply Alphachloralose to a site with around 500 pairs of nesting gulls in 1 day [pers. obs.].
	Equipment and infrastructure:
	None needed
	Other, including overheads:
	Negligible
Effectiveness:	Significant reduction in the colony size was achieved after 3 annual applications.

CASE STUDY #4	
Measure type (if relevant):	Use of Alphachloralose to capture Sacred Ibis
Species:	*Threskiornis aethiopicus*
Objective:	Eradication
Use of measure	Alphachloralose was used to capture a number of free-flying Sacred Ibis in a zoological park. Fish baits dosed with Alphachloralose were fed to the Ibis and captured birds were allowed to recover before being returned to captivity.
Combined with other measure(s):	This formed a small part of a much larger programme involving shooting and nest and egg destruction.
Country(ies) of application:	France
Geographic scale (km^2) and/or population size measure applied to:	Alphachloralose use was limited to a small site in a zoological park. The overall programme was very large covering hundreds of square kilometres.
Time period:	Two visits were made with a total of 38 Ibis (the entire population) removed over the 2 years.

Effort:	The effort involved a small number of staff over 2 days. Far fewer than would have been required to trap the Ibis.
Costs:	Overall costs:
	The cost of Alphachloralose use was €39 per bird captured.
	Personnel costs:
	Not stated
	Equipment and infrastructure:
	Negligible
	Other, including overheads:
	Not stated
Effectiveness:	The method was effective in removing all the Ibis from the site involved. Some Ibis were reported to have died but the number is not provided.

4.2. Costs effectiveness summary	Stupefying baits can be highly cost-effective when used in a situation that minimises the issues of dosage and non-target species. They require little or no equipment, just the bait and the Alphachloralose powder plus the manpower, vehicles etc. needed to deliver the bait to the target animals. Where dosage problems or non-target species issues may occur, additional manpower may be required to deal with the problems that may arise, but, purely in cost effectiveness terms, stupefying baits are still likely to be more cost effective than techniques such as shooting or trapping, especially when large numbers of target animals are involved.

5. Side effects	
Non-target native species, their habitats and the broader environment:	Positive:
	If correctly dosed, non-target species will recover and can be released back into the wild. Provision may need to be made to ensure that non-target species are collected and kept safe from predators, harmful accidents and, possibly, dehydration.
	Negative:
	In practice, controlling the dosage of alphachloralose administered is difficult, and most studies where non-target species have ingested the bait have involved a level of casualties. Particular care should be taken if contemplating the use of any stupefacient where non-target species of high conservation concern may be impacted.
Other invasive alien species:	Positive:
	None significant
	Negative:
	None significant
Public health and well-being:	Positive:
	None significant, although anecdotal evidence from the USA suggests that stupefying animals may be more acceptable than shooting or poisoning [4, 5, 6].
	Negative:
	If target or non-target species disperse from the treatment site, they may be found by the public who will not necessarily understand why the animal is exhibiting unusual behaviour. Stupefied animals may also be predated or suffer accidents that the public may find distressing.
Economic:	Positive:
	Where the issues around dosage and non-target species can be overcome, stupefying baits can be deployed over a period of a few hours by a small number of staff. A large number of birds can be removed at a single visit, without the disturbance and learned avoidance that can happen when techniques such a shooting or trapping are used [9].
	Negative:
	None significant

6. Conclusion

Overall assessment of the measure (qualitative)

The use of stupefying baits, and in particular Alphachloralose, have posed problems in the past. The delivery of the correct dosage to target species and impacts on non-target impacts were the main issues encountered. Public relations problems, with both target and non-target species dispersing from the capture site, have also caused problems. Where the correct dosage can be delivered to the target species, and where non-target species can either be avoided or managed to keep any casualties to an acceptable level, stupefying baits are a very cost effective way of capturing large numbers of animals with comparatively low levels of effort.

For IAS management, it is unlikely that stupefacients would be the only technique used to control or eradicate a population, although there could be cases where a rapid eradication of IAS from a single site could be accomplished using stupefacients. It is more likely that stupefacients could be used on particular sites as part of a larger programme which employed other techniques at sites where the issues often encountered with stupefacients could not be overcome.

Assessor:	John Allan
Reviewer 1:	Riccardo Scalera
Reviewer 2:	Sandro Bertolino

7. References

[1] Ridpath, M. G., Thearle, R. J. P., McCowan, D., & Jones, F. J. S. (1961). Experiments on the value of stupefying and lethal substances in the control of harmful birds. *Annals of Applied Biology*, *49*(1), 77-101.
[2] Yap, C. A., & Sodhi, N. S. (2004). Southeast Asian invasive birds: ecology, impact and management. *Ornithological Science*, *3*(1), 57-67.
[3] McLeod, L. & Saunders, G. (2013). *Pesticides used in the management of vertebrate pests in Australia: A review*. NSW Department of Primary industries.
[4] Belant, J. L., Tyson, L. A., & Seamans, T. W. (1999). Use of alpha-chloralose by the Wildlife Services program to capture nuisance birds. *Wildlife Society Bulletin*, 938-942.
[5] O'Hare, J. R., Eisemann, J. D., Fagerstone, K. A., Koch, L. L., & Seamans, T. W. (2007). Use of alpha-chloralose by USDA Wildlife Services to immobilize birds. *Proceedings of the 12th Wildlife Damage Management Conference*, 103-113.
[6] Woronecki, P. P., Dolbeer, R. A., & Seamans, T. W. (1990). Use of alpha-chloralose to remove waterfowl from nuisance and damage situations. *Proceedings of the Vertebrate Pest Conference*, 14(14), 343-349.
[7] Millett, J., Climo, G., & Shah, N. J. (2004). Eradication of common mynah *Acridotheres tristis* populations in the granitic Seychelles: successes, failures and lessons learned. Advances in Vertebrate Pest Management, 3, 169-183.
[8] Crider, E. D., & McDaniel, J. C. (1967). Alpha-chloralose used to capture Canada geese. The Journal of Wildlife Management, 31, 258-264.
[9] Roy, S., Ridley, R., Sandon, J., Allan, J. R., Robertson, P. S., & Baxter, A. (2016). Adapting strategies to maintain efficiency during a cull of yellow-legged gulls. Human–Wildlife Interactions, 10(1), 11.
[10] Schafer , E. W., & Cunningham, D. J. (1972). *An evaluation of 148 compounds as avian immobilizing agents* (No. 150-165). US Fish and Wildlife Service, Bureau of Sport Fisheries and Wildlife.
[11] Schafer, E. W., Bowles, W. A., & Hurlbut, J. (1983). The acute oral toxicity, repellency, and hazard potential of 998 chemicals to one or more species of wild and domestic birds. *Archives of Environmental Contamination and Toxicology*, *12*(3), 355-382.
[12] EU (2008) Directive 98/8/EC concerning the placing biocidal products on the market. Inclusion of active substances in Annexe 1 or 1A to Directive 98/8/EC. Assessment Report Alphachloralose Product type 14 (Rodenticide).
[13] Sarat, E., Dutartre, A., & Mazaubert, E. (2015). Invasive alien species in aquatic environments. Practical information and management insights. Volume 2. Management Insights. Onema. *Véronique Barre (Onema, Research and development department).*
[14] DRUNAT É., LE NEVÉ A. & CADIOU B. (Coord.) 2006 - Sternes de Bretagne – Observatoire2005. Contrat Nature «oiseaux marins» 2003-2006. Bretagne Vivante – SEPNB / Conseil régionalde Bretagne / Conseil général des Côtes d'Armor / Conseil général du Finistère. 36 pages.

[15] Lim, H. C., & Sodhi, N. S. (2009). Space use and habitat selection of house crows in a tropical urban environment: a radio-tracking study. The Raffles Bulletin of Zoology, 57(2), 561-568.
[16] Shek, C. T., Chan, C. S., & Wan, Y. F. (2007). Camera trap survey of Hong Kong terrestrial mammals in 2002-06. *Hong Kong Biodiversity*, *15*, 1-11.
[17] Feare, C. J., & Mungroo, Y. (1990). The status and management of the House Crow *Corvus splendens* (Vieillot) in Mauritius. *Biological Conservation*, *51*(1), 63-70.
[18] Nelson, P. C. (1994). Bird control in New Zealand using alpha-chloralose and DRC1339. Proceedings of the Vertebrate Pest Conference, 16(16), 260-264.
[19] Davies, S. J., Jordaan, M. S., Karsten, M., Terblanche, J. S., Turner, A. A., van Wilgen, N. J., .& Measey, J. (2020). Experience and lessons from alien and invasive animal control projects in South Africa. In *Biological Invasions in South Africa* (pp. 629-663). Springer, Cham.
[20] Cyr, A., & Brunet, R. (1992). Anesthetization of captive red-winged blackbirds with mixtures of alpha-chloralose and secobarbital, 56(4), *The Journal of wildlife management*, 806-809.
[21] Hampton, J. O., Finch, N. A., Watter, K., Amos, M., Pople, T., Moriarty, A. & Mitchell, J. (2019). A review of methods used to capture and restrain introduced wild deer in Australia. *Australian Mammalogy*, *41*(1), 1-11.
[22] Huntington, P. J., Chapman, C. B., & Dyer, R. (1993). Nervous disorders caused by accidental ingestion of a deer catch mix in 18 horses. *The Australian Equine Veterinarian*, *1993*(2), 67-69.
[23] Bosch, M., Oro, D., Cantos, F. J., & Zabala, M. (2000). Short-term effects of culling on the ecology and population dynamics of the yellow-legged gull. *Journal of Applied Ecology*, *37*(2), 369-385.
[24] Feare, C.J. and Allan, J.R. (1993). Seychelles Magpie Robin Recovery Programme: Reduction of Interference from Common Mynas. Report to International Council for Bird Protection (unpublished).
[25] Saavedra, S. (2010). Eradication of invasive mynas from islands. Is it possible. Aliens Invasive Species Bull, 29, 40-47.
[26] Canning, G. (2011). Eradication of the invasive common myna, *Acridotheres tristis*, from Fregate Island, Seychelles. *Phelsuma*, *19*, 43-53.
[27] Feare, C. J., van der Woude, J., Greenwell, P., Edwards, H. A., Taylor, J. A., Larose, C. S., .& Raines, K. (2017). Eradication of common mynas *Acridotheres tristis* from Denis Island, Seychelles. *Pest management science*, *73*(2), 295-304.
[28] Bunbury, N., Haverson, P., Page, N., Agricole, J., Angell, G., Banville, P., ... & Melton-Durup, E. (2019). Five eradications, three species, three islands: overview, insights and recommendations from invasive bird eradications in the Seychelles. *Island invasives: Scaling up to meet the challenge. Occasional Paper SSC*, (62), 282-288.
[29] Wanless, S. & Langston, D.R. (1984) The effects of culling on the Abbeystead and Mallowdale gullery. Bird Study 30: 17-23.

Appendix 13. Chemical treatment of habitats

1. Measure name			
1.1. English:	Chemical treatment of habitats		
1.2. Lethal or non-lethal:	Lethal		
1.3. Other languages (if available):			
Bulgarian	Химическа обработка на местообитанията	Italian	Trattamento chimico degli habitat
Croatian	Kemijska obrada staništa	Latvian	Dzīvotņu ķīmiskā apstrāde
Czech	Chemické ošetření prostředí	Lithuanian	Buveinių apdorojimas chemikalais
Danish	Kemisk behandling af habitater	Maltese	
Dutch	Behandeling van habitats met chemicaliën	Polish	Chemiczne oczyszczanie siedlisk
Estonian	Elupaikade keemiline töötlemine	Portuguese	Tratamento químico do habitat
Finnish	Elinympäristöjen kemiallinen käsittely	Romanian	Utilizarea tratamentelor chimice în natură
French	Traitement chimique des habitats	Slovak	Chemické ošetrenie biotopov/prostredia
German	Chemische Behandlung von Habitaten	Slovenian	Vnos strupov in drugih snovi v habitate
Greek	Χημικός χειρισμός των ενδιαιτημάτων	Spanish	Tratamiento químico del hábitat
Hungarian	Élőhelyek vegyszeres kezelése	Swedish	Kemisk behandling av vattendrag, Rotenon
Irish			

2. Technical details of measure
2.1.a. Measure description
This assessment is looking exclusively at toxins applied directly to habitats for the purposes of eradication, control and containment, and therefore it excludes active substance used in bait, and used as euthanasia once captured (which are covered by separate assessments), and those substances used only as a repellent. The application of toxins directly to habitats is regulated within the EU by the Biocidal Products Regulation (BPR, Regulation (EU) 528/2012), which came in to force in September 2013, repealing the Biocidal Products Directive (BPD, Directive 98/8/EC). Any biocidal product requires authorization before it can be used, and the active substances contained in that biocidal product also need to be approved under the BPR (excluding products in the review programme, or active substances under assessment). All biocidal products containing approved active substances are evaluated for safety and efficacy under the BPR before they are allowed to be sold in the EU. The approval of active substances takes place at the Union level, and the subsequent authorization of products at Member State (MS) level, which can be extended to other MS by mutual recognition (though there is the option of Union level authorization). All active substances and products are categorized (and assessed/authorized) according to their targeted application into 22 Product Types (PT) grouped into four categories. Within the group 'Pest Control' there are four PTs relevant to this assessment: PT 14 Rodenticide, PT 15 Avicide, PT 17 Piscicide, and PT 20 Control of other vertebrates. Based on the information provided in the European Chemical Agencies (ECHA) 'Information on biocides' database (https://echa.europa.eu/information-on-chemicals/biocidal-active-substances) which provides

information on all active substances for which an application for approval for a specific biocidal product-type has been submitted, and all biocidal products authorized on the EU/EEA market, in accordance with the BPR or BPD.

There is only one active substance approved for use in the EU, **aluminium phosphide releasing phosphine** which has biocidal products authorised for use in 23 separate EU Member States. Aluminium phosphide releasing phosphine (APP) is authorized under the BPR for the outdoor control of pest rodents (PT 14): the terrestrial form of the aquatic vole (*Arvicola terrestris*) and Norway rat (*Rattus norvegicus*), and other burrowing vertebrates (PT 20), moles (*Talpa europaea*) and rabbits (*Oryctolagus cuniculus*), to reduce their damage to areas of interest (e.g. agricultural areas, railway embankments, dams, flood dikes etc.) [1,2]. APP products (tablets/pellets) are laid out in burrow systems which are then sealed, for example with soil, the phosphide reacts with moisture in soil and air and releases the toxic gas, phosphine [2,3]. While it is not authorised in the EU for use on any of the vertebrate IAS of Union concern, nor could any evidence of its application to these species be found, it could be applied to ***Myocastor coypus*** but its efficacy has not been scientifically demonstrated against this species [4]. Note that zinc phosphide concentrate, which releases phosphine as aluminium phosphide, is the only toxicant registered in the US for use on aquatic rodents (EPA Reg. No. 56228-6 – though it needs registering at the State level also) including for muskrat [5]. In addition, its use as part of an IAS management programme with the objective of eradication, control, or containment is unknown, as it is currently only used to remove animals that may cause damage to a site (i.e. objective of asset protection).

There is only one active substance for use as a piscicide (PT 17) listed in the ECHA database, **rotenone**, this is listed as 'under assessment' (and its authorization application (by UK) has been 'cancelled'). However, below we discuss the key piscicides that are in use across the world as they have been shown to be effective in eradications in certain situations, including for some species of Union concern, and could potentially be applied in the EU if authorization and approval were sought under the BPR.

Rotenone - Rotenone is a general piscicide most commonly used to eradicate invasive fish and control fish pathogens and has been used in countries including South Africa, United States of America, UK and Norway [6], and Spain [7]. There is also limited evidence of its use to control populations of invasive alien fishes [8]. Rotenone was withdrawn from use in the European Union in 2008 [9], but derogations are probably possible in some MS [10]. A standard operating procedure (SOP) has been produced by Finlayson et al. [11] which is designed to provide fishery managers and others with procedures needed for carrying out restoration projects with rotenone in an effective and safe manner. It includes information relevant for targeting invasive alien species, including strategies for eliminating under-treatment and over-treatment of target species, guidance on conducting bioassays and designing treatments using effective pest management techniques, and strategies for implementing selective treatments and biomanipulation. According to the SOP the selected treatment rate is based on the response of target fish (or surrogate species). Rotenone has been used to successfully eradicate populations of ***P. parva*** in the UK [12,13], and eradication of ***P. glenii*** populations using rotenone is probably possible in small closed systems (like aquaculture ponds and oxbows) [10]. Rotenone has also been used for the management of unauthorized stocking of ***L. gibbosus*** in the USA [14]. The use of rotenone (with permethrin) has also been found to be lethal to ***L. catesbeianus*** but application is through dermal spray and not broadcast to habitats [15,16].

Chlorine - Chlorine is a non-specific toxin and will kill most aquatic organisms. Although it is lethal to fish in the correct dosage, sub-lethal chlorine gradients can be detected and avoided by numerous fish species [17]. There is evidence of its experimental application to remove ***P. glenni*** [18]. All aquatic species of Union concern should be susceptible to treatments of chlorine at the correct dosage.

Ammonia - Ammonia is a natural product of fish metabolism that is naturally present in the environment at low levels and is broken down by naturally occurring bacteria through the nitrification cycle, yet it is known to be toxic to most aquatic species at high concentrations (>2 ppm) [19]. Ammonium sulphate can be combined with lime treatment to take advantage of the fact that ammonia is much more toxic to aquatic organisms at higher pH values [20]. Ammonia has been found to be lethal against ***L. castebeianus*** tadpoles (and other aquatic IAS including the crayfish

Orconectes virilis) in two experimental ponds in Arizona USA, however the two adult ***T. scripta elegans*** individuals that were also present in the ponds survived [19]. As ammonia is lethal to most aquatic species, all fish species of Union concern should be susceptible to treatments.

Antimycin A – Antimycin A is toxic to all organisms that depend on mitochondrial respiration, however, there is a large divergence in toxicity, even between closely related species [21]. It is particularly toxic to scaled fishes, but less toxic to channel catfish (*Ictalurus punctatus*), which has led to its use by catfish farmers to remove unwanted scaled fishes and invertebrates from large production ponds [20]. In addition, unlike rotenone, fish do not avoid Anitmycin, which may be important if the area under treatment had escape routes or areas of low concentrations [22]. No evidence could be found of its application to aquatic IAS of Union concern.

Carbon dioxide (CO2) – CO2 dissolved into water, displaces the dissolved oxygen and thereby decreasing its concentration affecting aquatic species, such as fish [20]. Treanor et al. [23] have assessed the use and its potential application in fisheries and aquatic invasive species management. They state that the addition of CO2 into water can be accomplished in several ways, including diffusion of CO2 gas, introduction of sodium bicarbonate NaHCO3, followed by the addition of sulfuric acid (H2SO4) to form carbonic acid and dissolved CO2, or through the addition of dry ice. It has been shown to be effective against a number of species through experimental applications (see [23]) including to larvae of ***L. castebeianus* [24]**. Treanor et al. [23] conclude that the use of CO2 may be preferable to chemical pesticides, as the absorption efficiency of CO2 in some aquatic settings can be very high, and in large aquatic systems the diffusion of CO2 to the atmosphere following its introduction is sufficiently low that losses to the atmosphere can be considered negligible. They recommend that further investigation of the use of CO2 to manage aquatic invasive species is needed.

2.1.b. Integration with other measures

Animals need to be removed from the environment (e.g. netting) once they have died to prevent risks to human and animal health and as an environmental pollutant. In addition monitoring post eradication is required, for example using eDNA in aquatic systems, or trapping, to confirm success of measures application.

2.2.a. Availability - species and objectives

Objective	Unknown objective		Rapid Eradication		Management					
					Eradication		Control		Containment	
Species	Avail.	Ref(s).	Avail.	Ref(s).	Avail.	Ref(s).	Avail.	Ref(s).	Avail.	Ref(s).
Acridotheres tristis										
Alopochen aegyptiaca										
Callosciurus erythraeus										
Corvus splendens										
Herpestes javanicus										
Lepomis gibbosus			P (Rotenone)	[14]	P (Rotenone)	[14]	P (Rotenone)			
Lithobates catesbeianus	P (Rotenone & CO2)	[16]								
Muntiacus reevesi										

Myocastor coypus	P (Aluminium phosphide)	[4]								
Nasua nasua										
Nyctereutes procyonoides										
Ondatra zibethicus			P (Aluminium phosphide)	[5]						
Oxyura jamaicensis										
Percottus glenii			P (Rotenone)	[10]	P (Rotenone)	[10]	P (Rotenone)			
Plotosus lineatus										
Procyon lotor										
Pseudorasbora parva			A (Rotenone)	[13]	A (Rotenone)	[13]	P (Rotenone)			
Sciurus carolinensis										
Sciurus niger										
Tamias sibiricus										
Threskiornis aethiopicus										
Trachemys scripta										

2.2.b. Application – EU Member States and objectives										
Objective	**Unknown objective**		**Rapid Eradication**		**Management**					
					Eradication		**Control**		**Containment**	
Country	Avail.	Ref(s).	Avail.	Ref(s).	Avail.	Ref(s).	Avail.	Ref(s).	Avail.	Ref(s).
Austria										
Belgium										
Bulgaria										
Croatia										
Cyprus										
Czech Republic										
Denmark										
Estonia										
Finland										
France										
Germany										
Greece										
Hungary										

Ireland										
Italy										
Latvia										
Lithuania										
Luxembourg										
Malta										
Netherlands										
Poland										
Portugal										
Romania										
Slovakia										
Slovenia										
Spain					X	[7]				
Sweden										
United Kingdom*					X	[13]				

* Not an EU Member State

3. Humaneness of the measure			
3.1. Welfare for all measures			
Measure type (if applicable): Aluminium phosphide	**Humaneness impact categories**		
Domain	**No impact**	**Mild-Moderate**	**Severe - Extreme**
1: Water deprivation, food deprivation, malnutrition	No impact as time of death (based on *Oryctolagus cuniculus*) not sufficiently long to lead to water or food deprivation [25]. Burrows are sealed so this may have a mild impact if application does not lead to 100% mortality. However, it is assumed the animal can burrow out relatively easily.		
2: Environmental challenge		Mild impact as there is the potential for slight temperature increase in sealed burrow due to presence of phosphine vapour, and lightly reduced ability for animals to thermoregulate due	

		to respiratory effects of phosphine [25].	
3: Injury, disease, functional impairment		Moderate impact as defined functional respiratory impairments for mammals, however apparent full recovery from sub-lethal exposure in humans and rabbits [25].	
4: Behavioural, interactive restriction		Mild impact as reduction in movement due to sealed burrows, effects on respiration likely to result in short-term reductions in movement [25].	
5: Anxiety, fear, pain, distress, thirst, hunger etc.		Mild impact as no signs of distress are seen in rabbits, first behavioural change was increased activity as mean of 235 minutes after exposure [25].	

Measure type (if applicable): Aquatic toxins	**Humaneness impact categories**		
Domain	**No impact**	**Mild-Moderate**	**Severe - Extreme**
1: Water deprivation, food deprivation, malnutrition	All aquatic toxins - no impact.		
2: Environmental challenge	All aquatic toxins - no impact.		
3: Injury, disease, functional impairment		Rotenone – Moderate impact as cellular oxygen deprivation occurs but this would reverse if treatment discontinued, especially before buoyancy lost [25]. Death results from tissue anoxia, especially cardiac and neurological failure [26].	For some of the aquatic toxins (chlorine, ammonia, and antimycin) evidence of the reversibility of the impacts could not be found therefore their impacts may in fact be classed as Severe. In addition, if a non-lethal dose is provided, the resulting injuries would likely reduce survival if the animal were to be

		Chlorine – Moderate impact as chlorine is a strong oxidizer that attacks the gill tissue of fish (Westers 2001 in [20]), however the degree of reversibility is unknown, so the impact could be severe if non-lethal doses applied. Ammonia – Moderate impact as interferes with osmoregulation at the gills and disrupts the blood chemistry, so assumed impact similar to rotenone, however the degree of reversibility is unknown, so the impact could be severe if non-lethal doses applied. Antimycin – Moderate impact as Antimycin is an inhibitor of mitochondrial respiration [21], however reversibility is unknown. CO2 – Moderate impact as increased CO2 leads to reduces blood and hemolymph pH which results in hypoxia [23]. It is assumed that if the treatment was discontinued hypoxia would be reversed.	released and therefore will have 'Severe' impacts.
4: Behavioural, interactive restriction		Rotenone – assessed as moderate as fish swim erratically and forced to the surface in search of oxygenated water [25]. Chlorine – Evidence could not be found on behavioural changes due to chlorine exposure. Assumed to be similar therefore to rotenone. Ammonia – assumed mild impact, as Ward et al. [19] observed little erratic movement	

		and struggling of fish after treating ponds with ammonia. Antimycin – Assessed as mild as fish do not avoid antimycin (unlike rotenone) and according to Holden [22] no behavioural abnormalities occurred in cases where ultimate death did not occur. CO2 – Assessed as moderate as fish that cannot escape elevated CO2 levels exhibit behavioural changes including increased fin beats, listing, increased attempts to surface, erratic swimming, impaired sensory systems, and loss of lateralization. Sustained exposure leads to a loss of reflex and opercular activity and loss of equilibrium [23].	
5: Anxiety, fear, pain, distress, thirst, hunger etc.		Rotenone – Moderate impact as the assumption is that the erratic swimming indicates mild distress, but loss of buoyancy is at least moderate [25]. Chlorine – Mild/Moderate impact, as death occurs within 1 hour, however distress is unknown. Ammonia – Mild/moderate impact as the assumption is that little erratic swimming observed indicates mild distress. Antimycin – Mild/moderate as the assumption is that as fish do not avoid it and no behavioural abnormalities were observed where applications did not lead to lethal outcomes, that distress may be mild.	As noted above, in cases of non-lethal doses, application could lead to severe injuries to the animals.

		CO_2 – Moderate impact as elevated CO_2 leads to increased plasma glucose and adrenaline (indicators of environmental stress) and altered hematocrit and cortisol levels [23], combined with loss of equilibrium indicates moderate stress impact.	

3.2. Mode of death (if relevant)			
Measure type (if applicable): Aluminum phosphide	**Immediate death (i.e. no suffering)**	**Not immediate death (mild - moderate suffering)**	**Not immediate death (severe - extreme suffering)**
Rationale:		Moderate impact based on affect to *Oryctolagus cuniculus*. Mean time to death 225 minutes, and assumed to be conscious until soon before death, however the mean time between first symptoms and death was 29 minutes. Moderate respiratory irritation considered likely [25].	

Measure type (if applicable): Aquatic toxins	**Immediate death (i.e. no suffering)**	**Not immediate death (mild - moderate suffering)**	**Not immediate death (severe - extreme suffering)**
Rationale:		Chlorine – Assessed as mild-moderate as chlorine will kill most fish species after as little as 1 h of exposure (Westers 2001 in [20]) however distress is unknown. Ammonia – Assessed as mild, as according to Ward et al. [19] fish showed little erratic swimming after treatment and began dying within 20 min of dosing in experimental ponds.	Rotenone – Severe impacts due to prolonged time to death. Loss of consciousness is not immediate and there is aversion behaviour and likely moderate suffering. However, it usually kills fish within 24–36 h [25]. Allen et al. [27] state that for field application on *P. parva* a dosage of 0.150 mg/litre rotenone will result in 100% mortality of topmouth gudgeon over a 2 hour exposure time. The time required to cause

		Antimycin – Assessed as mild due to the lack in avoidance behaviour, however time to death is unknown.	100% mortality decreases approximately 2 to 3-fold for each five-degree rise in temperature (Gilderhus 1972 in [26]). CO_2 – Assessed as severe due to potential prolonged time to death. Treanor et al. [23] reports that 70% mortality of common carp was observed during exposure to elevated CO_2 after 10 hours. However moderate impacts are seen in changes in behavior, and elevated cortisol/ adrenaline levels.

3.3. Humaneness summary	Aluminium phosphide (phosphine) has a moderate impact upon the humaneness of the animals (based on studies on rabbits), with the animals displaying no behavioural changes until close to death, which occurs on average 225 minutes after exposure. However, this cannot be automatically assumed to be the same if the measure was used for any of the Union of concern (e.g. *Myocastor coypus*). Ammonia and Antimycin, seem to lead to limited distress in fish due to the observed lack of erratic swimming and behavioural changes, and time to death using ammonia seems to begin c. 20 mins. Chlorine leads to death relatively quickly (c. 1 hour) however the injury and distress impacts are unknown. Rotenone leads to observable behavioural stress and death occurs between 24-36 hours (though time to death is heavily affected by water temperature). CO_2 leads to elevated distress in fish, and based on 1 study time to death is c. 10 hours.

4. Costs and effectiveness of the measure	
General effectiveness of the measure	**Aluminium phosphide** – Is effective at killing moles, rabbits, rats and voles [1,2]. However, there is no evidence of being used to kill any of the vertebrate species of Union concern. It may have potential as a control agent for *M. coypus*, and *O. zibethicus* but its efficacy has not been scientifically demonstrated on this species [4]. In addition, the measure has only been proposed/used for removal of animals for the purposes of damage control, and not part of an eradication, control or containment program. In terms of costs, the products that contain AP are relatively cheap, e.g. a flask of 160 pellets of Talunex is c. €28 (GBP26) and the applicator, case and dibber is €119 (GBP108) [28]. While the application of all the piscicides can be completed over a short period of time, it is important that monitoring before and after application is undertaken, with post application monitoring potentially lasting several years [6]. Monitoring post eradication using eDNA which can detect presence at low densities, is more

	effective than conventional trapping methods. Robinson et al. [29] used eDNA to detect the presence of *P. parva* at ponds in South Wales (Millenium ponds) where the species was previous thought to be eradicated. **Rotenone** - The use of piscicides, such as rotenone, is most effective in enclosed water bodies, including ponds, lakes and reservoirs [6]. A systematic review undertaken by Rytwinskli et. al. [8] found that rotenone was reported effective in eradicating non-native fish in 75% of data sets, though two studies required two applications. Also that the majority of rotenone treatments occurred in lakes (41%), followed by ponds (25%), creeks (19%), rivers (6%), reservoirs (6%), and lagoons (3%). The actual treatment rate and concentration needed to kill a target species varies widely, depending on the type of water, environmental factors including pH, temperature, depth, turbidity, and organic-loading, and sensitivity of target species [30]. Other factors include the availability of other underwater refuges, for example root masses, algal mats and undercut banks, and ground water recharge [31]. Rotenone was used to eradicate five populations of *P. parva* from lakes in the UK between 2005-2008, summarized in the case study section below [13]. Eradication of *P. glenii* populations using chemicals, such as rotenone, is probably possible in small closed systems (like aquaculture ponds and oxbows) [10]. Other examples exist of successful eradication of alien fish species using rotenone, for example the centrarchid *Micropterus dolomieu* smallmouth bass was eradicated from the lower reaches (4km) of a river in South Africa [32]. Witmer et al. [16] found that after a dermal spray-application of 4 ml of rotenone with permethrin at 5–10 % concentrations in water was 100 % lethal for adult *L. catesbeianus*, and also fast acting with time-to-death <2 h. Costs of eradication using rotenone can be high, for example the eradication of *Cyprinus carpio* from a 38 hectare lake in Spain cost approximately €600,000 (= €15,789/ha) [7], the eradication of P. parva from 5 UK lakes (all <5 ha) cost c. €206,000 (GBP136,350) (c. <€8,300/ha) [13], and the application of rotenone to eradicate Micropterus dolomieu in 4 km of stream in South Africa cost c. €321,166 (R3.3 million) (= c. €80,279/km) [32]. **Chlorine** – Chlorine used at a concentration of 5 mg/L will kill most fish species after as little as 1 h of exposure (Westers 2001 in [20]). Bogutskaya and Naseka [18] found that lime chloride at 0.3 g/l with exposure of not less than 6 hours will result in 100% death of *P. glenni*. In terms of costs, the treatment of a hypothetical 8,000m3 pond at an application rate of 10 mg/L would cost c. €500 (NZ$800) for the piscicide [20]. **Ammonia** – Ward et al. [19] tested the effects of Ammonia in 2 ponds at concentrations of 29.8 and 33.9 ppm, upon a number of alien invasive aquatic species including *L. castebeianus* tadpoles, *Orconectes virilis*, and *Lepomis cyanellus*, and two adult red-eared sliders *Trachemys scripta elegans*. Fish started dying after 20 minutes, and after 49 days both ponds were drained and all individuals of all species were dead, apart from the two adult red-eared sliders *Trachemys scripta elegans* (and hatchling mud turtles that were also present). Ward et al. [19] conclude that a single dose of ammonium hydroxide (30–34 ppm) was sufficient to completely kill the aquatic organisms, and recommend that ammonia may be an effective tool for management of invasive aquatic species in pond locations where rotenone treatments have previously been attempted but were unsuccessful. Clearwater et al. [20] recommend that for use as a piscicide on a range of freshwater pest fish species (in New Zealand), the required total ammonia concentration should be 100–200 mg NH4–N/L at pH 10, and that 0.1–0.3 kg/m3 of lime would be sufficient to raise the pH to 10. In terms of

	costs, the treatment of a hypothetical 8,000-m3 pond at 100–200 mg NH4–N/L and pH 10, would cost c. €945-1,891 (NZ$1509–3018) for the ammonium sulphate, and c. €137-413 (NZ$220–660) for the hydrated lime (to raise the pH) [20]. The high solubility of ammonia allows it to spread throughout the water column without spraying or mixing, and liquid ammonia is relatively inexpensive (c. €152 / US$200 for a 208-L drum) and readily available, although gaseous (anhydrous) ammonia may be even more cost effective for very large-scale applications [19]. **Antimycin** - Depending on the fish species, antimycin is up to 10 times more toxic and requires shorter contact times than rotenone (6 h versus 18 h), and fry and fingerlings are more sensitive that juvenile and adult fish (Finlayson et al. 2002 in [20]). Effectiveness also depends upon water temperature and pH, with reduced toxicity in cold water and at higher pH (8.5-9.5) (Marking 1992 in [20]). The systematic review undertaken by Rytwinskli et. al. [8] found antimycin was reported effective in eradicating a non-native fish in 89% of cases, and that 78% applies more than 1 round of application (ranged from 1 to 3). According to Clearwater et al. [20] treatment of a hypothetical 8,000-m3 pond with an application of 20 µg/L to eradicate catfish would cost c. €1,277 (NZ$2024) for the antimycin (Fintrol). **CO2** – The effectiveness of CO2 depends upon on the duration of the exposure, the level and constancy of the dissolved oxygen concentration, and water temperature [20]. However, little is currently known about threshold values that lead to acute mortality among fish species and more research and field trails is needed (Treanor et al. 2017). Abbey –Lambertz et al. [24] based on laboratory trails estimated that the 24-h 50% and 99% lethal concentration (LC50 and LC99) values for *L. castebeianus* (Gosner stages 26–42), were 371 and 549 mg CO2/L, respectively, and that hibernating juvenile and adult bullfrogs may also be susceptible to increased CO2. In terms of field applications, Treanor et al. [23] suggest that short-term, small-scale applications, high-pressure gas cylinders may serve as a viable, cost-effective option for administering CO2. However, for larger projects, it may be more likely that the volumes of CO2 necessary to successfully treat an isolated lentic system would require bulk CO2 delivery systems (e.g., pumper trucks or large stainless storage tanks that are capable of holding more than 3,000 L of compressed CO2). In terms of costs, Treanor et al. [23] also estimate that to treat a 0.4 ha pond for eradication of common carp and channel catfish the total cost would be c. €1,822 (US$2,055) for 6 tanks of CO2 over a 5-day period, however this does not cover the mechanism of delivery.

4.1. Case studies

CASE STUDY #1	
Measure type (if relevant):	Rotenone
Species:	*P. parva*
Objective:	Eradication
Use of measure	Application of rotenone to 5 lakes in England, UK (Cumbria, N. Yorkshire, Surrey, Devon, & Berkshire).
Combined with other measure(s):	An additional lake (*P. parva* eradication) was treated by drawdown and disinfection.

Country(ies) of application:	England, UK
Geographic scale (km²) and/or population size measure applied to:	Each lake was <5 ha.
Time period:	Each lake had one application, during February or March between 2005-2008.
Effort:	One application.
Costs:	Overall costs:
	GBP 136,350 for the lakes where rotenone application was undertaken (average cost per lake = GBP 27,270, note that the additional lake where drawdown and disinfection was undertaken cost GBP 50,800). Costs relate to the directly incurred costs of the operation, for example, the purchase of rotenone and associated equipment for its application.
	Personnel costs:
	Equipment and infrastructure:
	Other, including overheads:
Effectiveness:	Effective [13,31].

CASE STUDY #2	
Measure type (if relevant):	Rotenone
Species:	*Cyprinus carpio*
Objective:	Eradication
Use of measure	Application in a lake within the Laguna de Zóñar protected area in southern Spain to eradicate *Cyprinus carpio*.
Combined with other measure(s):	None
Country(ies) of application:	Spain
Geographic scale (km²) and/or population size measure applied to:	Zóñar lake is 38 ha, depth max of 16m.
Time period:	It was estimated that the rotenone concentration was lethal to fish for approximately 15 days (10–24 July 2006). The mean persistence of the rotenone was estimated at some six days due to the weather and water conditions (pH 9.0, water temperature ca 28°C, maximum air temperature ca 40°C, 14 h of sunlight per day).
Effort:	Two phases, the first (on 10–12 July 2006) with greater concentration (90 ppb) than the second (ca 50 ppb), which was applied six days later (17–18 July 2006).

Costs:	Overall costs:	
	Approx. 600,000 €	
	Personnel costs:	
	Equipment and infrastructure:	
	Other, including overheads:	
Effectiveness:	Effective [7].	

4.2. Costs effectiveness summary	Based on the data gathered, the piscicides assessed are effective, or have the potential to be, to eradicate aquatic vertebrate species of Union concern in small lentic systems. However, application costs will increase with size of the spatial area being treated, so there will likely be a point where its effective application in relation to minimizing its indirect adverse impacts becomes impossible with available technology [31]. In relation to ammonium phosphide, it is clearly an effective measure for the removal of rodents and other burrowing vertebrate individuals to prevent damage, but its practicality and effectiveness for application as part of an IAS management programme with the objective of eradication, control or containment is unknown.

5. Side effects	
Non-target native species, their habitats and the broader environment:	Positive:
	Negative:
	Aluminium phosphide - According to the USDA [3] ecological and human health risk assessment for APP there is a risk to terrestrial vertebrates and invertebrates that are in burrows and exposed to phosphine gas during treatment. However, there is no secondary risk to non-target species because phosphine rapidly dissipates and does not accumulate in target animals. According to the Standing Committee on Biocidal Products [2] it may theoretically pose a risk for carnivorous and scavenging terrestrial vertebrates that feed on intoxicated animals, however in organisms phosphine is metabolised to non-toxic phosphates therefore there is no risk of secondary poisoning. Despite of the high aquatic toxicity, there is no risk for the aquatic compartment (incl. sediment) from the professional use according to the intended application. The fumigant causes also no risk to the atmosphere.

	All of the chemical piscicides and **CO2** will affect many, if not all of native and alien aquatic species present (e.g. fishes, benthic invertebrates, amphibians) based on their tolerance to the concentrations applied. However, collateral damage may be reduced where individuals can be removed prior to application [31]. **Rotenone -** A principal advantage of rotenone application is that it degrades relatively rapidly through pathways involving photolysis and hydrolysis, which allow affected native communities to recover and/or recolonise relatively quickly post-treatment, especially where adjacent waters are untreated [6]. In addition, should more rapid degradation be required potassium permanganate can be used to achieve this [6]. However, studies have shown that rotenone has some negative impacts on non-target groups, at least in the short term. For example, Dalu et al. [33] showed that planktonic invertebrates were particularly sensitive to rotenone even at very low concentrations, and Skaar et al. [34] found a light reduction in the abundance of mayflies, stoneflies, and caddisflies immediately following rotenone treatments abundance increased to pre-treatment levels within one year. **Chlorine –** Chlorine deteriorates rapidly, and usually loses its toxicity after 1 day at this concentration, and it can easily be neutralised with the application of sodium sulphite. Therefore, one-off use of chlorine as a piscicide would be unlikely to produce significant long-term environmental effects [20]. **Ammonia** - The addition of ammonia might cause post-treatment eutrophication of the water body. Ammonia also degrades slowly, Ward et al. [19] found that it took 38-45 days for the nitrification cycle to complete for ammonia, nitrite and nitrate levels to return to pre-treatment levels. Although it might be possible to reduce the post-treatment ammonia toxicity by adding acid to the treatment [20]. **Antimycin** – Antimycin degrade rapidly, and at concentrations used to control pest fish populations in the USA, antimycin has minimal effects on other aquatic organisms such as invertebrates (Finlayson et al. 2002 in [20]). **CO2** – According to Treanor et al. [23] all aquatic life will be affected due to deoxygenation due to CO2, however it may be preferable to chemical piscicides as it does not persist in the environment. Also, CO2 introductions in water will reduce the pH of a target system, which will likely alter the solubility of metals and other constituents in water [23].
Other invasive alien species:	Positive:
	All of the piscicides will affect many, if not all of native and alien aquatic species present (e.g. fishes, benthic invertebrates, amphibians) based on their tolerance to the concentrations applied.
	Negative:
Public health and well-being:	Positive:
	Negative:

	Aluminium phosphide – Phosphine gas is toxic to humans, however the risk to human health is low because inhalation exposure is reduced due to underground applications [3]. The use of **chemical piscicides** such as rotenone is controversial, and has become a concern to a variety of stakeholder groups [13,30,10]. **Rotenone** – Rotenone has historically been widely used as an insecticide both agriculturally and in home gardens, and there has been concern regarding the impacts upon human health, including in the development of Parkinsons Disease (PD)., However Finlayson et al. [11] state that while there is little doubt that rotenone, given excessive and unrealistic exposure, may cause specific damage to nerve cells, inducing symptoms of neutrotoxicity similar to those associated with PD. They conclude that the risk of developing PD-like symptoms as a result of rotenone exposure from use in fisheries management is negligible because with recommended care, rotenone exposure has been effectively eliminated. **Chlorine** - Short-term exposure to high doses of chlorine and chlorine compounds may be fatal, however risks can be effectively managed by the use of protective equipment and clothing, and implementation of safety procedures [20]. **Ammonia –** According to Clearwater et al. [20] the use of anhydrous ammonia or ammonia solutions is inadvisable in field situations as liquid ammonia is an extremely hazardous substance, while ammonium sulphate is relatively safe to handle, but irritates respiratory surfaces and skin with prolonged exposure. Hydrated lime is caustic to human skin and respiratory surface. **Antimycin** - Conjunctivitis is a potential consequence when applying antimycin, therefore the use of safety glasses when handling the product is recommended (Finlayson 2002 in [20]).
Economic:	Positive:
	Negative:
	All of the **chemical piscicides** will affect the economic and recreational use of the water systems, but the length of time is dependent on the time it takes for the toxins to be at safe levels. **Rotenone** - The recreational use of treated waters is also impacted in the short-term (as it degrades rapidly), leading to negative social and economic effects [6].

6. Conclusion	
Overall assessment of the measure (qualitative)	
Aluminium phosphide – APP is already licensed in the EU, however not for the application to any of the species of Union concern. It is effective against rabbits, moles, voles, and rats, has mild to moderate humaneness impacts, and is relatively cheap. However, its effectiveness against species of Union concern, in particular ***M. coypus*** or ***O. zibethicus,*** which is the likely potential target for this toxin, is unknown. In addition, its effectiveness and practicality as part of an IAS eradication, control or containment programme is unknown. **Chemical piscicides** and **CO2** – One key drawback is that none of these measures are currently approved for use under the BPR. They are all effective in killing fish and other freshwater species, but the only evidence for the specie of Union concern is for rotenone (***P. parva, L. catesbeianus***), chlorine (***P. glenni***), ammonia (***L. catesbeianus***) and CO2 (***L. catesbeianus***), however they are likely to be effective for the other freshwater species if applied at the correct rates. They are most cost-effective in small lentic (enclosed) systems, though there is evidence of their effective use in larger lakes and in lotic systems. In terms of their humanness, ammonia and antimycin seem to lead to mild distress, and ammonia has a relatively quick time to death. Rotenone and CO2 both lead to visible behavioural changes and distress, with longer time to death. In terms of side effects, all measures negatively affect/kill native species, however they are all (apart from ammonia) quick to degrade.	
Assessor:	Kevin Smith
Reviewer 1:	Riccardo Scalera
Reviewer 2:	Sandro Bertolino

7. References

[1] Standing Committee on Biocidal Products. (2008). Inclusion of active substances in Annex I or IA to Directive 98/8/EC Assessment Report Aluminium phosphide releasing phosphine Product-type 14 (Rodenticides)

[2] Standing Committee on Biocidal Products. (2013). Evaluation of active substances Assessment Report Aluminium phosphide releasing phosphine Product-type 20 (Control of other vertebrates).

[3] USDA. (2017). Human Health and Ecological Risk Assessment for the Use of Wildlife Damage Management Methods by USDA-APHIS-Wildlife Services Chapter IX The Use of Aluminum Phosphide in Wildlife Damage Management.

[4] Wildlife Damage Management. (2019). Nutria damage management. https://wildlife-damage-management.extension.org/nutria-damage-management/ [Accessed 16/07/2020]

[5] USDA. (2018). Muskrats. Wildlife Damage Management Technical Series. U.S. Department of Agriculture Animal & Plant Health Inspection Service Wildlife Services. https://www.aphis.usda.gov/wildlife_damage/reports/Wildlife%20Damage%20Management%20Technical%20Series/Muskrat-WDM-Technical-Series.pdf

[6] Britton, R. (2019). Information on measures and related costs in relation to species included on the Union list: *Pseudorasbora parva*. Technical note prepared by IUCN for the European Commission.

[7] Ferreras-Romero, M., Marquez-Rodriguez, J., & Fernandez-Delgado, C. (2016). Long-time effect of an invasive fish on the Odonata assemblage in a Mediterranean lake and early response after rotenone treatment. *Odonatologica,* 45(1/2): 7-21.

[8] Rytwinski, T., taylor, J. J., Donaldson, L. A., Britton, J. R., Browne, D. R., Gresswell, R. E., Lintermans, >, Prior, K. A., Pellat, M. G., Vis, C., & Cooke, S. J. (2018). The effectiveness of non-native fish removal techniques in freshwater ecosystems: a systematic review. *Environmental Reviews*, 27(1), 71-94. https://doi.org/10.1139/er-2018-0049

[9] OJEU - Official Journal of European Union. (2008). Commission Decision of 8 December 2008 concerning the non-inclusion of certain active substances in Annex I to Council Directive 91/414/EEC and the withdrawal of authorisations for plant protection products containing these substances. L 335/91.13.12.2008. (2008c).

[10] Verreycken, H. (2019). Information on measures and related costs in relation to the species included on the Union list: Perccottus glenii. Technical note prepared by IUCN for the European Commission.

[11] Finlayson, B., Schnick, R., Skaar, D., Anderson, J., Demong, L., Duffield, D., Horton, W., Steinkjer, J., & VanMaaren, C. (2012). Rotenone Use in Fish Management and Parkinson's Disease: Another Look, Fisheries, 37:10, 471-474. http://dx.doi.org/10.1080/03632415.2012.723963
[12] Britton, J., & Brazier, M. (2006). Eradicating the invasive topmouth gudgeon, *Pseudorasbora parva*, from a recreational fishery in northern England. *Fisheries Management and Ecology*, 13, 329-335
[13] Britton, J. R., Davies, G. D., & Brazier, M. (2010). Towards the successful control of the invasive *Pseudorasbora parva* in the UK. *Biological Invasions*, 12:125-131 DOI 10.1007/s10530-009-9436-1
[14] State of Washington Department of Fish and Wildlife. (2002). Addendum 19-031 to final supplemental environmental impact statements. https://wdfw.wa.gov/sites/default/files/2019-05/19031dns.pdf
[15] Snow, N. P. & Witmer, G. (2010). American Bullfrogs as Invasive Species: A Review of the Introduction, Subsequent Problems, Management Options, and Future Directions. Proceedings of the Vertebrate Pest Conference, 24(24). DOI 10.5070/V424110490
[16] Witmer, G. W., Snow, N. P., & Moulton, R. S. (2015). Efficacy of potential chemical control compounds for removing invasive American bullfrogs (*Rana catesbeiana*). *Springer Plus*, 4, 497. DOI 10.1186/s40064-015-1319-6
[17] Noatch, M. R., & Suski, C. D. (2012). Non-physical barriers to deter fish movements. *Environmental Reviews*, 20(1), 71-82. https://doi.org/10.1139/a2012-001
[18] Bogutskaya, N. G. & Naseka, A. M. (2002). *Perccottus glenii* Dybowski, 1877. Freshwater Fishes of Russia. Zoological Institute Russian Academy of Science.
[19] Ward, D. L., Morton-Starner, R., & Hedwall, S. J. (2013). An Evaluation of liquid ammonia (ammonium hydroxide) as a candidate piscicide. *North American Journal of Fisheries Management*, 33:2, 400-405. http://dx.doi.org/10.1080/02755947.2013.765528
[20] Clearwater, S. J., Hickey, C. W., & Martin, M. L. 2008. Overview of potential piscicides and molluscicides for controlling aquatic pest species in New Zealand. Science for conservation 283. Department of Conservation, New Zealand.
[21] Kenneke, J., & T. Sack. (2016). Environmental and Metabolic Transformations of the Piscicide Antimycin A. SETAC Europe 26th Annual Meeting, Nantes, France, May 22 - 26, 2016. https://cfpub.epa.gov/si/si_public_record_report.cfm?Lab=NERL&dirEntryId=322558
[22] Holden, A. V. (1980). Chemical methods, Chapter 8. In Backiel, T. and R. L. Welcomme (eds), Guidelines for sampling fish in inland waters. EIFAC 1980 Tech.Pap., (33):176 p. http://www.fao.org/3/AA044E00.htm#TOC
[23] Treanor, H. B., Ray, A. M., Layhee, M., Watten, B. J., Gross, J. A., Gresswell, R. E., & Webb, M. A. H. (2017). Using Carbon Dioxide in Fisheries and Aquatic Invasive Species Management. *Fisheries*, 42(12), 621-628. DOI:10.1080/03632415.2017.1383903
[24] Abbey-Lambertz, M., Ray, A., Layhee, M., Densmore, C., Sepulveda, A., Gross, J., & Watten, B. (2014). Suppressing bullfrog larvae with carbon dioxide. *Journal of Herpetology*, 48(1) 59-66. DOI: http://dx.doi.org/10.1670/12-126
[25] Landcare Research. 2010. How humane are our pest control tools? (09-11326) MAF Biosecurity New Zealand Technical Paper No: 2011/01 Prepared for MAFBNZ Operational Research by Landcare Research, Lincoln, New Zealand.
[26] Ling, N. (2003). Rotenone. A review of its toxicity and use for fisheries management. Science for Conservation 211. Department of Conservation, New Zealand
[27] Allan, Y., Kirby, S., Copp, G. H., & Brazier, M. (2006). Toxicity of rotenone to topmouth gudgeon *Pseudorasbora parva* for eradication of this non-native species from a tarn in Cumbria, England. *Fisheries Management and Ecology*, 13, 337–340.
[28] Pest Magazine. 2011. Talunex or Phostoxin? November & December 2011. Page 21 https://www.pestmagazine.co.uk/media/245810/21-talunex-or-phostoxin.pdf
[29] Robinson, C. V., de Leaniz, C. G., Rolla, M., & Consuegra, S. (2019) Monitoring the eradication of the highly invasive topmouth gudgeon (*Pseudorasbora parva*) using a novel eDNA assay. Environmental DNA, 1, 74-85 DOI: 10.1002/edn3.12
[30] Finlayson, B., Skaar, D., Anderson, J., Carter, J., Duffield, D., Flammang, M., Jackson, C., Overlock, J. Steinkjer, J., & Wilson, R. (2018). Planning and standard operating procedures for the use of in fish management— rotenone SOP manual, 2nd edition. American Fisheries Society, Bethesda, Maryland rotenone.
[31] Britton, J. R., Gozlan, R. E., & Copp, G. H. (2011). Managing non-native fish in the environment. *Fish and Fisheries*, DOI: 10.1111/j.1467-2979.2010.00390.x
[32] Impson, N. D., Van Wilgen, B. W., & Weyl, O. L. F. (2013). Coordinated approaches to rehabilitating a river ecosystem invaded by alien plants and fish. South African Journal of Science, 109(11/12). http://dx.doi. org/10.1590/sajs.2013/a0041
[33] Dalu, T., Wasserman, R. J., Jordaan, M., Froneman, W. P., & Weyl, O. L. F. (2015). An Assessment of the Effect of Rotenone on Selected Non-Target Aquatic Fauna. *PLoS ONE* 10(11): e0142140. doi:10.1371/journal.pone.0142140
[34] Skaar, D. R., Arnold, J. L., Koel, T. M., Ruhl, M. E., Skorupski, J. A., & Treanor, H.B. (2017). Effects of Rotenone on Amphibians and Macroinvertebrates in Yellowstone. Yellowstone Science 25(1): 28-34

Appendix 14. Poisons and toxins in bait

1. Measure name	
1.1. English:	Poisons and toxins in bait
1.2. Lethal or non-lethal:	Lethal

1.3. Other languages (if available):

Bulgarian	Примамка с отровни и токсични вещества	Italian	Esca con veleni o tossine
Croatian	Otrovi i toksini u mamcu	Latvian	Indes un toksīni ēsmā
Czech	Jedy a toxiny v návnadě	Lithuanian	Užnuodyti masalai
Danish	Gift og toksiner i lokkemad	Maltese	
Dutch	Vergiftigd aas	Polish	Trucizny i toksyny w przynęcie
Estonian	Mürkide ja toksiinidega sööt	Portuguese	Venenos e toxinas no isco
Finnish	Myrkyt syöteissä	Romanian	Momeli cu otravă și toxine
French	Poisons et toxines dans les appâts	Slovak	Jedy a toxíny v návnade
German	Köder mit Gift und Toxinen	Slovenian	Zastrupljene vabe
Greek	Δηλητηριασμένο δόλωμα	Spanish	Venenos y tóxicos en cebo
Hungarian	Mérgek és méreganyagok a csaliban	Swedish	Bekämpningsmedel, gift
Irish			

2. Technical details of measure

2.1.a. Measure description

The measures using poison and toxins in bait reflect the different active substances (AS) that might be incorporated into baits for delivery to invasive alien species (IAS) of Union concern. In this assessment each such AS or group of AS with a common mode of action, such as the anticoagulant rodenticides (ARs), represents a distinct Measure Type. These substances are collectively known as Biocides which are regulated and approved in the EU under the Biocidal Products Directive (Directive 98/8/EC) or the Biocidal Products Regulation (Regulation (EU) No 528/2012). Biocides are categorised according Product Type (PT) as described at:
https://echa.europa.eu/regulations/biocidal-products-regulation/product-types.

Biocides with approvals in the EU that are either current, expired or in progress are listed at:
https://echa.europa.eu/information-on-chemicals/biocidal-active-substances

This assessment considered the following four PTs covering substances that could potentially be delivered in baits to IAS of Union concern: PT14 Rodenticides, PT15 Avicides, PT17 Piscicides and PT20 Other vertebrates.

Vertebrate toxicants have also been authorized for use in the EU as plant protection products i.e. pesticides under EU directive 91/414 replaced in 2009 by Regulation (EC) No 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC https://eur-lex.europa.eu/eli/reg/2009/1107/oj consolidated in 2019 at https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX:02009R1107-20191214). Eisemann et al. [21] states that the rodenticides bromadiolone, chlorophacinone and difenacoum were, at least at that time, approved for use in plant protection in the EU. However, searches of the current active substance database for regulation 1107/2009 show that chlorophacinone and difenacoum are no longer approved, and bromadiolone expired on 31/05/2021. The only other vertebrate toxicants identified as currently approved as plant protection products are aluminium phosphide and carbon dioxide with expiration dates of 31/08/2022 and 31/08/2020 respectively (https://ec.europa.eu/food/plant/pesticides/eu-pesticides-database/public/?event=activesubstance.selection&language=EN).

According to the Bern Convention Appendix IV, the use of poison (as other means of indiscriminate capture and killing) is prohibited to kill mammal, bird and freshwater fish species protected by the Convention. The same prohibition has been transposed in the EU Habitats Directive (92/43/EEC) and in the Birds Directive (2009/147/EC). The prohibition extends to the use of poison that may cause the local disappearance of species protected under the Birds and Habitats Directives, even if primarily targeting other species (like IAS). However, derogation from the required protection can be permitted under licence, e.g. via Article 16 of the Habitats Directive with respect to activities that would otherwise be illegal under Article 12, for reasons including prevention of serious damage, or being in the interests of public health and public safety, or for other imperative reasons of overriding public interest, including those of a social or economic nature.

Biocides with approvals in the EU that are either current, expired or in progress
PT14 – Rodenticides

A total of 16 AS are listed for this PT. Thirteen of these are currently approved, two have expired approvals and one has an approval application in progress. Rodenticides are the most extensively used vertebrate toxicants worldwide, mainly to control three species of commensal rodent, namely the House mouse *(Mus musculus domesticus*), Norway or Brown rat (*Rattus norvegicus)* and Ship or Black rat (*Rattus rattus*).

Anticoagulant rodenticides (ARs)

The ARs have dominated rodenticide use since 1948 when warfarin was first recognized as a rodenticide and registered in the US for this purpose [1,2]. The major advantage of ARs over prior existing fast-acting rodenticides, such as zinc phosphide, is the delay in the onset of symptoms post-ingestion reducing the likelihood of a conditioned taste aversion (CTA) to treated bait developing after consumption of a sub-lethal dose. Hence, learned bait aversion or "bait shyness" preventing consumption of a lethal dose is not considered likely with ARs [3]. ARs have been widely used in the EU mainly as biocidal products against commensal rodents but also as plant protection products against other species.

ARs can be classified by chemical type into the indandiones, including chlorophacinone and diphacinone, pindone (Pival) and the hydroxycoumarins, including brodifacoum, bromadiolone, coumatetralyl, difenacoum, difethialone, flocoumafen and warfarin. However, the inandiones and hydroxycoumarins have similar toxic profiles and all share a common mode of action which targets the Vitamin K-dependent epoxide reductase enzyme [4]. This reduces the recycling of Vitamin K to its active form thus inhibiting hepatic post-ribosomal carboxylation of essential clotting factors. The production of clotting factors is thus compromised and, once the existing factors have been depleted, the clotting mechanism fails and haemorrhaging begins. Because the mechanism is common to all ARs, there is no difference between them in the average time to death once the enzyme is blocked. Typically, the onset of poisoning symptoms and death is delayed, while the depletion of vitamin K–dependent clotting factors occurs, for up to 7 days after initial bait consumption. Furthermore, again due to their common mode of action, ARs do not differ in the sites at which the lethal haemorrhages occur. The antidote to poisoning common to all ARs is administration of Vitamin K_1 [5]. Given these commonalities all ARs are considered in this assessment as a single Method Type (1).

The so called “first-generation” anticoagulants (FGARs), chlorophacinone, diphacinone, pindone, coumafuryl, coumachlor, coumatetralyl, difenacoum and warfarin all required consumption of multiple-doses over several days to deliver a lethal dose and thus they are known as “chronic” poisons. Furthermore, from the early 1960s onwards physiological resistance began to emerge towards all FGARs amongst all three commensal target species [6,7,8,9,10]. The emergence of resistance to the FGARs led to the development of more potent molecules that became known as the second-generation anticoagulants (SGARs). These are derivatives of 4-hydroxycoumarins and include bromadiolone, brodifacoum, difenacoum, difethialone and flocoumafen. SGARs have a greater affinity for binding sites in the liver and consequently are more potent than the FGARs but also exhibit greater persistence. Nakayama et al. [11] provides comparisons of the toxicities and elimination half-lives for FGARs and SGARs across a number of target and non-target species. The concentrations of active substances in rodenticidal baits varies with their potency. For instance, typically a warfarin bait formulation would contain 0.025% of the active substance while the initial formulations of brodifacoum contained 0.005% [12]. The toxicity of the more potent SGARs means that individuals could potentially consume a lethal dose during a single feed which led to the concept of “pulsed baiting” at approximately weekly intervals, in contrast to the continuous or “surplus” baiting strategy employed with FGARs [13]. The more potent SGARs brodifacoum, difethialone and flocoumafen are thus sometimes referred to as “single-feed” anticoagulants.

Because of the relatively high toxicity and persistence of SGARs their potential adverse effects on non-target species have been a particular cause for concern [11]. Routes of exposure of non-target species can be primary, via the consumption of toxic bait [e.g. 14, 15], or secondary, via the consumption of poisoned prey or carcasses [16,17]. In studies carried out around the world, reviewed by Nakayama et al. [11]., residues of brodifacoum, bromodiolone and difenacoum were detected in 31%, 30% and 26% respectively of the non-target individuals examined. The importance of the secondary route of exposure is apparent in these data with AR residues detected in 56.8% and 56.6% of carnivore and raptor individuals respectively, with the exposure of raptors probably posing greater risk of toxicosis and death due to the potentially lethal range for SGARs in raptors being considered relatively low [e.g. 18]. These and other data have led to the classification of all ARs as persistent, bioaccumulative and toxic (PBT) substances and thus they do not meet the environmental and public health safety criteria required by the European Union (EU) for use as biocidal products and consequently ARs meet both the exclusion and substitution criteria of the Regulation, i.e., Article 10 (1), (a) and (e) and Article 5 (1), (c) and (e), [e.g. 19]. Nevertheless, it is still currently considered that no sufficient substitution for anticoagulant substances has yet been found, which results in periodic renewals of approval of these active substances such as the EU Regulation of 25 July 2017 [20]. Furthermore, the Commission accepted the recommendation of the European Chemical Agency (ECHA) for the reclassification of currently used anticoagulant rodenticides (Commission Regulation (EU) 2016/1179; applied from 1 March 2018). Consequently, a Specific Concentration Limit (SCL) has been set at 0.003% in formulated bait, equivalent to 30ppm or 30mg/kg, for all ARs and only products containing lower concentrations can now be labelled for use by amateurs [21].

Despite the concerns regarding non-target poisoning ARs have been used to eradicate invasive rodent species from islands around the world [e.g. 22, 23] including in the EU [e.g. 24, 25].

EU approvals of ARs as biocides are restricted to use “in and around buildings”, as defined by European Commission (2009). This constraint will compromise the potential effective use of ARs under biocides approvals against target IAS of Union concern.

The following provides a summary of each of the ARs with approvals as Biocides.

Brodifacoum
EC number: 259-980-5, CAS number: 56073-10-0
Brodifacoum is a SGAR and is considered a "single-feed" AR [9]. Brodifacoum has been used successfully in commensal rodent eradications on islands in the conservation context of protecting ground nesting birds [e.g. 27]. Brodifacoum has also been used for the control of possums in New Zealand [28, 29].
The ECHA substance information can be found at:
https://echa.europa.eu/substance-information/-/substanceinfo/100.054.509
The data and assessments associated with the approval can be found at:
https://echa.europa.eu/information-on-chemicals/biocidal-active-substances/-/disas/factsheet/11/PT14

Bromadiolone
EC number: 249-205-9, *CAS number:* 28772-56-7
Bromadiolone is a SGAR [30]. It has been used in the EU to control two IAS of Union concern, namely coypu (*Myocastor coypus*) [31] and muskrat (*Ondathra zibethicus*) [32]. However, the effectiveness of such use would be compromised if restricted under the Biocides approval to application only "in and around buildings". Its use as an approved Plant Protection Product also expired on 31/05/2021.
The ECHA substance information can be found at:
https://echa.europa.eu/substance-information/-/substanceinfo/100.044.718
The data and assessments associated with the approval can be found at:
https://echa.europa.eu/information-on-chemicals/biocidal-active-substances/-/disas/factsheet/12/PT14

Alpha-bromadiolone
This active substance is an analogue of bromadiolone and is thus be a SGAR. This approval is currently under assessment.
The ECHA substance information can be found at:
https://echa.europa.eu/substance-information/-/substanceinfo/100.044.718

Chlorophacinone
EC number: 223-003-0, *CAS number:* 3691-35-8
Chlorophacinone is a FGAR [33]. It has been used in the EU to control two IAS of Union concern, namely coypu (*Myocastor coypus*) [31] and muskrat (*Ondathra zibethicus*) [34, 35]. However, the effectiveness of such use would be compromised if restricted under the Biocides approval to application only "in and around buildings". Effective use might be possible through an approval as a Plant Protection Product. However, although Eisemann et al. [21] state that chlorophacinone was approved for use in plant protection in the EU, this approval is no longer current.
The ECHA substance information can be found at:
https://echa.europa.eu/substance-information/-/substanceinfo/100.020.912
The data and assessments associated with the approval can be found at:
https://echa.europa.eu/information-on-chemicals/biocidal-active-substances/-/disas/factsheet/14/PT14

Coumatetralyl
EC number: 227-424-0, *CAS number:* 5836-29-3
Coumatetralyl toxicity is intermediate between FGARs, such as warfarin, and the more potent SGARS, such as brodifacoum, difethiolone and flocoumafen [36] It is considered to be a SGAR [21].
The ECHA substance information can be found at:

https://echa.europa.eu/substance-information/-/substanceinfo/100.024.931
The data and assessments associated with the approval can be found at:
https://echa.europa.eu/information-on-chemicals/biocidal-active-substances/-/disas/factsheet/18/PT14

Difenacoum
EC number: 259-978-4 , *CAS number:* 56073-07-5
Difenacoum is a SGAR [37, 38, 39]. It has been used in the EU to control two IAS of Union concern, namely coypu (*Myocastor coypus*) [31] and muskrat (*Ondathra zibethicus*) [40]. However, the effectiveness of such use would be compromised if restricted under the Biocides approval to application only "in and around buildings". Effective use might be possible through an approval as a Plant Protection Product. However, although Eisemann et al. [21] state that difenacoum was approved for use in plant protection in the EU, this approval is no longer current.
The ECHA substance information can be found at:
https://echa.europa.eu/substance-information/-/substanceinfo/100.054.508
The data and assessments associated with the approval can be found at:
https://echa.europa.eu/information-on-chemicals/biocidal-active-substances/-/disas/factsheet/26/PT14

Difethialone
EC number: 600-594-7, CAS number: 104653-34-1
Difethialone is a SGAR and is considered a "single-feed" AR [41].
The ECHA substance information can be found at:
https://echa.europa.eu/substance-information/-/substanceinfo/100.118.383
The data and assessments associated with the approval can be found at:
https://echa.europa.eu/information-on-chemicals/biocidal-active-substances/-/disas/factsheet/27/PT14

Flocoumafen
EC number: 421-960-0, *CAS number:* 90035-08-8
Flocoumafen is a SGAR and is considered a "single-feed" AR [42].
The ECHA substance information can be found at:
https://echa.europa.eu/substance-information/-/substanceinfo/100.102.053
The data and assessments associated with the approval can be found at:
https://echa.europa.eu/information-on-chemicals/biocidal-active-substances/-/disas/factsheet/34/PT14

Warfarin
EC number: 201-377-6, *CAS number:* 81-81-2
Warfarin is a FGAR and has been used to control an IAS of Union concern in the UK, namely the grey squirrel (*Sciurus carolinensis*). The "chronic" LD50 of warfarin in the Norway rat is approximately 5 daily doses of 1 mg/kg [12] and the maximum daily food consumption of the Norway rat is approximately 10% of its body weight [43]. Hence, a high proportion of the rat's diet would need to be bait in order to consume a lethal dose at the regulatory concentration limit of 0.003%. Hence, Eisemann et al. [21] suggest that "effective FGAR products are implausible with active ingredient concentrations <30 ppm". Similarly, the baits used for grey squirrel control contained 0.02% warfarin [44]. Hence, restricting the formulation to be less than 0.003% might well constrain effectiveness although it has been suggested, in a laboratory study, that "....baits should be effective at a level as low as 0.003%" [45].
The ECHA substance information can be found at:

https://echa.europa.eu/substance-information/-/substanceinfo/100.001.253
The data and assessments associated with the approval can be found at:
https://echa.europa.eu/information-on-chemicals/biocidal-active-substances/-/disas/factsheet/56/PT14

Warfarin sodium
Warfarin sodium is a FGAR, However, this approval has expired.
EC number: 204-929-4, CAS number: *129-06-6*
The ECHA substance information can be found at:
https://echa.europa.eu/substance-information/-/substanceinfo/100.004.483
The data and assessments associated with the approval can be found at:
https://echa.europa.eu/information-on-chemicals/biocidal-active-substances/-/disas/factsheet/57/PT14

Non- Anticoagulant rodenticides

Alphachloralose
EC number: 240-016-7, *CAS number*: 15879-93-3
Although this AS has an approval as a rodenticide it is primarily used as a stupefying agent rather than a poison in bait. Its use is thus not considered in this assessment.
The ECHA substance information can be found at:
https://echa.europa.eu/substance-information/-/substanceinfo/100.036.363
The data and assessments associated with the approval can be found at:
https://echa.europa.eu/information-on-chemicals/biocidal-active-substances/-/disas/factsheet/3/PT14

Aluminium phosphide
EC number: 244-088-0, *CAS number:* 20859-73-8
The approval of this AS releasing phosphine as a rodenticide is as a burrow fumigant thus it is not considered in this assessment of poisons and toxins in bait.
The ECHA substance information can be found at:
https://echa.europa.eu/substance-information/-/substanceinfo/100.040.065

Carbon dioxide
EC number: 204-696-9, CAS number: 124-38-9
The approval of this AS as a rodenticide is as a burrow fumigant thus it is not considered in this assessment of poisons and toxins in bait.
The ECHA substance information can be found at:
https://echa.europa.eu/substance-information/-/substanceinfo/100.004.271

Cholecalciferol
EC number: 200-673-2, *CAS number*: 67-97-0
Cholecalciferol (vitamin D_3) was developed in the 1980s as a non-anticoagulant rodenticide [46, 47]. The primary mode of action appears to be heart failure [48, 49]. It has been used in New Zealand as an alternative to sodium monofluroacetate for control of possums and rodents because of the relatively low risk of secondary poisoning of dogs and birds [50].

The ECHA substance information can be found at:
https://echa.europa.eu/substance-information/-/substanceinfo/100.000.612
The data and assessments associated with the approval can be found at:
https://echa.europa.eu/information-on-chemicals/biocidal-active-substances/-/disas/factsheet/1443/PT14

Hydrogen cyanide (HCN)
EC number: 200-821-6, *CAS number:* 74-90-8
The approval of HCN as a rodenticide is as a burrow fumigant it thus is not considered in this assessment of poisons and toxins in bait. However, it is of note that cyanide toxicosis, as described by Gregory et al. [51], is considered to have the lowest negative welfare impact of all the commonly used vertebrate toxicants [52, 53, 54].
The ECHA substance information can be found at:
https://echa.europa.eu/substance-information/-/substanceinfo/100.000.747

Powdered corn cob
EC number: None, CAS number: None
There are only limited data in the public domain demonstrating efficacy of powdered corn cob as a rodenticide. Schmolz [55] reported low palatability and no mortality during bait choice studies. Zhelev et al. [56] reported mortality in no-choice studies but low palatability in choice and field experiments. These findings are consistent with the EU approval that states that "….the data package is definitely not suitable to support a product authorisation (as further data, including laboratory palatability and confirmatory field data against larger rodent populations will be required)": Directive 98/8/EC Assessment Report Powdered Corn Cob Product-type PT 14 (Rodenticides) (2012)
https://echa.europa.eu/documents/10162/44bfef85-fb69-79f0-c0d7-87f0779ffe2a)
Furthermore, the approval assessment also states under "Elements to be taken into account by Member States when authorising products" that "Further efficacy studies regarding the biocidal products containing corn cob powdered should be submitted".

With respect to welfare impacts the approval assessment states that "None of the symptoms appeared to be noticeably severe, although this is not necessarily indicative of an humane end-point". In this context Zhelev et al. [56] reported, with respect to morbidity, general body dehydration, severe caecal obstruction and extremely enlarged faecal balls in the bowel lumen. These observations indicate potentially negative welfare consequences.

Unless additional data emerges in the future it is not recommended to consider the possible use of powdered corn cob against any IAS of Union concern, given these potential issues regarding both efficacy and welfare associated with its use as a rodenticide.

The ECHA substance information can be found at:
https://echa.europa.eu/substance-information/-/substanceinfo/100.240.911
The data and assessments associated with the approval can be found at:
https://echa.europa.eu/information-on-chemicals/biocidal-active-substances/-/disas/factsheet/1278/PT14

PT 15 - Avicides

Carbon dioxide
EC number: 204-696-9, CAS number: 124-38-9

The approval of this gas for use as an avicide is not appropriate for consideration in this assessment of poisons and toxins in bait.
The ECHA substance information can be found at:
https://echa.europa.eu/substance-information/-/substanceinfo/100.004.271

PT 17 - Piscicides (see Toxins to habitats assessment for more details)

Rotenone (2R,6aS,12aS)-1,2,6,6a,12,12a-hexa hydro-2-isopropenyl-8,9-dimethoxychro meno[3,4-b]furo[2,3-h]chromen-6-one)
EC number: 201-501-9, CAS number: 83-79-4
The striped eel catfish (*Plotosus lineatus*) is susceptible to rotenone poisoning following system wide application [57]. However, the approval of rotenone has been cancelled and it is thus not currently available for use against fish in the EU. Dermal application of 1% rotenone was found to be 100 % lethal for adult bullfrogs [58]. However, there is no system available for the delivery of this toxin in bait nor did this assessment find evidence of anything suitable being developed. It is thus not recommended to consider further the possible use of rotenone against the bullfrog given these regulatory and practical issues.
The ECHA substance information can be found at:
https://echa.europa.eu/substance-information/-/substanceinfo/100.001.365
The data and assessments associated with the approval can be found at:
https://echa.europa.eu/information-on-chemicals/biocidal-active-substances/-/disas/factsheet/1375/PT17

PT 20 - Other vertebrates

Aluminium phosphide ***(see Toxins to habitats assessment for more details)***
EC number: 244-088-0, *CAS number:* 20859-73-8
This approval for the control of other vertebrates by releasing phosphine is not appropriate for consideration in this assessment of poisons and toxins in bait.
The ECHA substance information can be found at:
https://echa.europa.eu/substance-information/-/substanceinfo/100.040.065

Other Biocides without current approvals in the EU
Several vertebrate biocides with potential to be delivered in baits to IAS of Union concern have been used elsewhere in the world or in the EU prior to current biocides and plant protection product regulation. These are considered here as some might have potential use in the future, subject to appropriate approvals being obtained. However, any such approval will be costly, for instance, Knight & Cooke [59], quoted by Buckle et al. [60], estimated that, on average, each active substance reviewed under the BPD would, in 2002, require expenditure (combined cost of studies and resources of regulatory affairs personnel) of € 2.74 million.

Bromethalin
Bromethalin is neurotoxin and single-dose rodenticide formulated in baits containing 0.01% of the AS. It is registered in the USA [61] but has never had an approval in the EU. Bromethalin and its main metabolite desmobromethalin uncouple oxidative phosphorylation. This results in intra-myelin fluid accumulation, leading to long nerve demyelination and intra-myelin cerebral edema. The net result is cerebral and spinal edema and

increased CSF pressure, leading to neurologic dysfunction [62]. There is no known antidote [63] which is an operator safety and regulatory concern [64].

Diphacinone
This a FGAR that has been used historically as a rodenticide in the EU [65]. It has since been used outside the EU in baits to control the mongoose (*Herpestes auropunctatus*) [66,67]. It has no current approval in the EU.

Ergocalciferol
A form of Vitamin D, ergocalciferol (also called calciferol) was used in EU prior to the approval of cholecalciferol (Vitamin D3) as a PT-14 rodenticide. These materials act in the same way in terms of mode of action, toxicity, symptoms of toxicosis and environmental fate [68].

Indomethacin
Indomethacin is a non-steroidal anti-inflammatory drug (NSAID) that has been reported to have high toxicity in commensal rodents [69, 70]. The data available because of its use as a therapeutic drug might help facilitate development of a data package for an approval submission for use as a Biocidal product against rodents and perhaps other vertebrates. However, nothing is known about potential environmental fate and effects of using such a chemical as a vertebrate toxicant. Furthermore, the clinical symptoms of toxicosis described by Zhelev et al. [71], including severe gastrointestinal tract haemorrhages along with signs of severe respiratory distress for a few hours before death, indicate potentially severe negative welfare consequences.

Norbomide
Norbormide is selectively toxic to rodents of the genus *Rattus*, e.g. *Rattus norvegicus, R. hawaiiensis* and *R. rattus*, and thus potentially offers reduced risks to non-target species [72]. This is attractive from a regulatory perspective but less so with respect to commercial interests. The compound selectively acts on rat microvasculature as a vaso-constrictor and calcium channel blocker [72, 73]. Its selective toxicity arises from opening the permeability transition pores in rat mitochondria [74]. It is relatively fast acting and is also relatively unpalatable [75]. Microencapsulation formulation has thus been advocated to both mask flavour and delay onset of symptoms to reduce the potential for learned aversion developing and preventing consumption of a lethal dose. There has recently been renewed interest in this AS as a relatively rat-specific toxicant with consequent reduced hazard to non-target species, for instance in the context of island rat eradication [76]. However, this specificity means that it is unlikely candidate for use against any of the IAS of union concern.

PAPP
A relatively new vertebrate pesticide containing the active ingredient para-aminopropiophenone is known as PAPP, the characteristics of which are detailed in Eason et al. [77]. PAPP was originally studied as protection from the effects of radiation and then for the treatment of cyanide poisoning. It is particularly toxic to carnivores, with birds and humans being less sensitive [78, 79]. The reasons for this specificity reflect differences in methaemoglobin reductase systems, as described by Eason et al. [77]. The mode of action leads to reduced blood oxygen-carrying capacity inducing lethargy, unconsciousness and death within a few hours of exposure [80]. The negative animal welfare impact of PAPP toxicosis is considered less than commonly used toxicants such as 1080 and, in particular, the ARs [54], although some symptoms in some individuals do indicate suffering including seizures [81]. Methylene Blue reverses the effects of PAPP and can be administered as an antidote [79]. PAPP has been shown to kill a range of carnivores (Canids, Felids and Mustelids) in Australia and New Zealand [82, 78, 50]. It has been registered for use in New Zealand for feral cat (*Felis domesticus*) and stoat (*Mustela erminea*) control [77] and also in Australia [83]. In the United States, PAPP has been investigated as a tool for coyote control [80]. The IAS of Union concern that might potentially be controlled by this relatively carnivore-specific toxicant are the mongoose (*Herpestes auropunctatus*), and also perhaps the raccoon dog (*Nyctereutes procyonoides*) and the coati (*Nasua nasua*). Sugihara et al. [84] demonstrated 100% mortality in *H. auropunctatus* using PAPP formulated in bait. Ruell et al. [81] acknowledged that PAPP had several substantive advantages over other

toxicants for mongoose control, in particular, relative carnivore specificity and relatively low negative welfare impacts. However, they also acknowledge the substantial registration costs in the USA, of between US$ 810,000 and US$ 5,8000,000, despite the extensive relevant data already available from the New Zealand and Australian regulatory packages. There is no reason to suggest that the costs of an EU approval would be any less than these estimates. Furthermore, there is unlikely to be commercial interest in seeking such an approval thus the costs of would probably need to be borne by the public sector.

Pindone
Pindone, also known as "Pival" is a FGAR [85]. It has been used to control muskrats [86]. It has also been used extensively to kill rabbits (*Oryctolagus cuniculus*). The welfare of such use was evaluated by Fisher et al. [87] who concluded that use caused severe to extreme welfare compromise in rabbits primarily through functional impairments, although there was no suggestion that this represented any more or less negative welfare impact than other ARs.

Sodium monofluoroacetate
Sodium monofluoroacetate emerged as a vertebrate toxicant in the 1950s with the proprietary name of "1080" [88]. Sodium monofluoroacetate is metabolised to fluorocitrate which inhibits cellular energy production by inhibiting enzymes responsible for the conversion of citrate and succinate in the tricarboxylic acid cycle [88]. It is a fast-acting poison with symptoms of toxicosis appearing between 0.5 and 3 hours after ingestion. The most extensive use of sodium monofluoroacetate has been in New Zealand and Australia, primarily against marsupials, and it has also been used in Israel and the USA [89]. Sherley [90] reviewed the humaneness of sodium fluoroacetate and considered claims that it is a humane poison were inappropriate and that research into alternative control methods and/or improving the humaneness of sodium monofluoroacetate baits should be a priority. Although this view was subsequently challenged [91], concerns about its humaneness have driven attempts to find alternative toxicants [92].

Sodium nitrite
Sodium nitrite is a food preservative and ingredient in commercial fertilizers. It can, like PAPP, in some species, induce methemoglobemia and consequent death through hypoxia [92]. Feral pigs are particularly sensitive to this AS and a patented bait formulation of sodium nitrite has been developed to control this species in Australia [93, 94]. Such use has also been explored against feral pigs in the USA using a bait hopper designed to restrict access by non-target species [95]. Witmer [96] evaluated sodium nitrite as a rodenticide indicating that it was insufficiently toxic to be a useful rodent control tool. Given the similar mode of act to PAPP, the negative animal welfare impact of sodium nitrite toxicosis is considered less than commonly used toxicants such as 1080 and, in particular, the ARs [54], although some symptoms in some individuals do indicate suffering including seizures [81]. None of the IAS of Union concern are known to be particularly susceptible to this potential toxicant. Furthermore, the costs of seeking regulatory approval in the EU as a biocide would be considerable, despite its use a food preservative.

Starlicide
Starlicide (DRC-1339 or 3-chloro-p-toluidine hydrochloride) is an avicide [97], that has higher toxicity for certain species that are sometimes considered overabundant, such as starlings (*Sturnus vulgaris*), some North American blackbirds (Icteridae), crows (Corvidae) and gulls (Laridae), than for some other avian taxa, especially raptors [98], thereby exhibiting a degree of specificity. Furthermore, its toxicity to mammals is relatively low [99, 100]. DRC-1339 is readily absorbed into the circulatory system [101] and is rapidly metabolized in the liver, with the target species dying around three hours after consuming bait [102]. The mode of action of DRC-1339 involves the build-up of uric acid in the kidneys and blood vessels which causes necrosis and circulatory impairment, resulting in death from uremic toxicosis and congestion of major organs [101]. Because DRC-1339 is rapidly metabolized it apparently does not accumulate in tissues and is thus considered to have limited potential for secondary poisoning hazard [99]. However, the level of suffering associated with its mode of death has been assessed as "severe" [52]. It has been registered as an avicide in the USA [201] but not in the EU. It

has been used outside the EU to manage two IAS of Union concern namely the Common myna (*Acridotheres tristis)* [103, 98] and the House crow (*Corvus splendens*) [e.g. 104, 105, 106, 107].

Strychnine
Strychnine is historically one of the most widely used vertebrate toxicants. In 1947 it accounted for 85% of the rodenticide products registered in the USA [21]. It acts directly on the central nervous system causing interference with postsynaptic inhibition in the spinal cord and medulla [108]. The principal symptoms of strychnine poisoning are convulsive seizures, commonly appearing minutes after ingestion, and death occurs from a tetanic arrest of respiration during a major convulsion. Most recent use in Europe was against burrow dwelling animals such as the European mole (*Talpa talpa*) in the UK [109]. Strychnine is considered to be one of the least humane vertebrate toxicants [53, 54]. No strychnine products have been available in the EU since 2006 when no dossier was submitted for review to meet regulatory requirements [110, 111].

Zinc phosphide
Zinc phosphide is a fast-acting rodenticide that is converted into phosphine post-ingestion which inhibits cytochrome oxidase. The onset of intoxication is rapid and symptoms can appear prior to consumption of a lethal dose, with consequent development of a conditioned taste aversion inhibiting further bait consumption. Hence, zinc phosphide rodenticides are typically applied after a period of pre-baiting with unpoisoned baits [61]. Zinc phosphide is the only toxicant currently registered in the USA for use on aquatic rodents, where it is used to control an IAS of Union concern, namely the Muskrat *(Ondatra zibethicus)* [112]. Although historically widely used in Europe there is no current approval in the EU.

Literature Searches on the Use of Poisons and Toxins in Bait to Manage IAS of Union Concern
An online literature search was undertaken using Google Scholar of each IAS of Union concern with the search terms: “Common name” and “Specific Name” plus “Poison” “Poison+Bait” “Bait” “Toxin” “Toxicant”; plus “Anticoagulant” “Calciferol” “PAPP” “1080” “Strychnine” “Zinc phosphide” for mammals; plus “Starlicide” “DRC-1339” for birds; plus “Rotenone” for fish, amphibia and reptiles. The following provides summaries of the results of these searches for each IAS.

Acridotheres tristis **Common myna**
The toxicant Starlicide (DRC-1339) has been used in baits against the Common myna outside the EU, mainly on islands in New Zealand, St Helena, Ascension Island, American Samoa, and the Seychelles [e.g. 113, 114, 103, 98, 115). Variable outcomes have been reported, particularly regarding bait acceptance thus Avery & Eismann [116] concluded that Starlicide use alone would not eradicate mynas from American Samoa, while Parkes [117] recommended development of a strategy that incorporates several techniques. In any case, Starlicide does not have a current EU approval and development of the necessary regulatory package would be costly, with limited commercial interest with respect to funding such a development Given the variable efficacy of Starlicide in this species, the “severe” level of suffering caused by its the mode of death [52], the lack of a current approval and the absence of other candidate avicides for delivery via bait, the recommendation of this assessment is that the use of poisons and toxins in bait to manage the Common myna is– unsuitable for a modern control program.

Alopochen aegyptiaca **Egyptian goose**
No relevant literature identified except, for alphachloralose used as a stupefying agent in Canada goose.

Callosciurus erythraeus **Pallas's squirrel**
Warfarin has been used to control this species in conifer plantations in Taiwan [118] but with negative effects on non-target species [119]. It is possible that the current EU approval of the AR bromadiolone, as a plant protection product, could permit use in this species in a similar way to the historical use of warfarin against the Grey squirrel in the UK [120]. However, the current approval of bromadiolone will expire on 31/05/2021, unless a dossier is

successfully submitted for review. Furthermore, such use would need to be evaluated and justified with respect to potential negative non-target and welfare impacts, which is likely to be difficult given that successful eradication of local populations of this species is possible via trapping [121].

Corvus splendens **House crow**

Starlicide has been used to control the House Crow in several countries outside the EU, including Mauritius, Aden and Kenya, [104, 106, 107] and can be used to substantially reduce local populations [105]. However, such use would need to be evaluated and justified with respect to potential negative non-target and welfare impacts, which may be difficult given that successful eradication of local populations of this species is possible via shooting [e.g. 107].

Starlicide does not have a current EU approval and development of the necessary regulatory package would be costly, with limited commercial interest with respect to funding the development of such a package Given the “severe” level of suffering caused by its the mode of death [52], the lack of a current approval and the absence of other candidate avicides for delivery via bait, the recommendation of this assessment is that the use of poisons and toxins in bait to manage the House Crow is unsuitable for a modern control program.

Herpestes javanicus **Small Asian mongoose**

The Small Asian or Javan mongoose (*Herpestes javanicus*) is closely related to the Small Indian mongoose (*Herpestes auropunctatus*). The taxonomy of the two has been fluid as to whether they are one or two species [122]. The majority of the literature relevant to this assessment relates to *H. auropunctatus*. However, given that the two species are closely related it seems reasonable to apply direct read-across from these studies to *H. javanicus*.

Several poisons and toxicants have been used in attempts to control mongooses, including non-anticoagulant rodenticides such as sodium monofluoroacetate, strychnine, thalium sulfate, and zinc phosphide [123, 124, 125, 126]. Mongooses are susceptible to the FGAR diphacinone [127]. An extensive series of studies were undertaken of its effectiveness against the mongoose in Hawaii by the USDA [128, 129, 130, 131, 132, 133, 134]. Diphacinone was found to be lethal at relatively low doses and diphacinone formulated in raw hamburger meat, at a concentration of 0.00025%, placed in bait stations 125–250 m apart, killed a high proportion of radio-collared mongooses [134, 135, 81]. In 1991, a Special Local Needs registration was approved in Hawaii for 0.1% diphacinone concentrate to be mixed into raw hamburger to make a 0.00025% diphacinone bait to be applied in specially designed bait stations. This technique proved effective but expensive due to a variety of factors, including bait cost, labor costs associated with bait preparation, bait station construction, logistics involved with ensuring fresh bait was available over a 12–15 day period in remote areas. Given these constraints, it was impractical to apply to large conservation areas [66]. Furthermore, block bait formulations of diphacinone, that would be easier and less costly to distribute, produced mixed results [66, 67, 84]. Due to these issues the special registration was allowed to expire [66]. The approach is thus also unlikely to be viable for controlling the mongoose in the EU.

More recently studies have focused on the potential use of PAPP to control the mongoose. PAPP is known to be relatively toxic to mustelids [50], and Barun et al. [136] suggested that the mongoose would likely be susceptible to PAPP. Preliminary results with formulated microencapsulated PAPP delivered in fresh minced chicken have been promising for mongoose [135, 84]. Effective baiting systems have been developed for the delivery of rabies vaccines to the mongoose [137, 138] and these could potentially be adapted to deliver toxicants. Ruell et al. [81] evaluated the registration prospects for diphacinone, PAPP, bromethalin and sodium nitrite to control invasive mongooses in the USA. Despite acknowledging the advantages of PAPP over diphacinone, with respect to lower negative environmental and welfare costs, they concluded that the lower cost of registering diphacinone, due to an existing approval in the USA, outweighed these advantages. This argument would not apply in the EU where there is no current approval for diphacinone. In the EU it is thus PAPP that offers the best prospects for developing an effective product that could potentially be relatively safe and humane. However, the registration costs of PAPP as a novel AS in the EU would be considerable and currently unrealistic. Given the absence of a viable currently approved AS to use against this species in the EU, and the experience of using diphacinone in Hawaii indicating that the approach is not viable in some circumstances, the recommendation of this assessment is that the use of poisons and toxins in bait to manage the mongoose is unsuitable for a modern control program. However, this assessment might be revisited if a cost-effective means of generating an approval for PAPP in the EU emerged.

Lepomis gibbosus **Pumpkinseed**
No relevant literature was identified on the use of poison and toxins in bait against this species. The main constraint on the control of invasive fish species is the lack of species-specific eradication techniques [139]. Methods have been developed to deliver baits to fish [202]. In the future toxicants, such as antimycin-a, suitable for delivery to fish in baits may emerge [140]. However, while there appears to be some variation between species in susceptibility to such toxins, it is unclear how their delivery might be made target species-specific. Bajer et al. [141] pointed out, in their review of the control of invasive fish species, that the only use of poison has involved system-wide application of toxin. With respect to the cost of system-wide poison intervention to eradicate freshwater non-native fish in Europe, local eradication of Topmouth gudgeon (*Pseudoasbora parva)* in England using Rotenone cost approximately £2 per m^2 of water area treated [142].

Lithobates catesbeianus **Bullfrog**
No relevant species-specific literature on the use of poisons and toxins in bait was identified for the bullfrog. The use of poisons and toxins in baits was not one of the options for bullfrog management identified by Snow & Witmer [143], nor has the approach been proposed for the control of other invasive amphibian species elsewhere [e.g. 144]. Several toxicants have been found to be lethal to bullfrogs via dermal application, including a 1% solution of Rotenone in water [58]. However, there is currently no known species-specific means of delivering this toxicant to the bullfrog. Furthermore, the EU approval for Rotenone has been cancelled. Given the absence of both a currently approved AS to use against this species in the EU and a method of delivery via bait, the recommendation of this assessment is that the use of poisons and toxins in bait to manage the bullfrog is unsuitable for a modern control program.

Muntiacus reevesi **Reeves' muntjac**
No relevant species-specific literature on the use of poisons and toxins in bait was identified for the Reeves' muntjac. Poisoning via baits has been considered successful for controlling local populations of several species of deer (not including muntjac) in Australia, although typically being used to support other methods such as shooting [145]. Baits containing sodium monofluoroacetate have also been used to control deer in New Zealand [146], including aerial broadcasting of carrot baits containing sodium monofluoroacetate [147]. Sodium monofluoroacetate has also been incorporated into a gel and smeared on leaves as a deer toxin in New Zealand [148]. Sodium monofluoroacetate does not have an EU approval nor is there currently any realistic prospect of an approval being given for use of any vertebrate toxicant in baits against any species of deer.

Myocastor coypus **Coypu**
Poison and toxins in bait have historically been used to control coypu in Europe and elsewhere. Zinc phosphide has been used in the USA against coypu [149]. Typically a concentrate of the toxicant was mixed with a carrier such as carrots, sweet potatoes, watermelon rind, and/or apples, generally several pre-baiting visits were required before use of toxic bait but efficacy could exceed 95% at suitable sites [150]. However, the cost of large-scale use is considered prohibitive [151]. Morin *et al.* [152] demonstrated that bromadiolone is lethal to Coypu with both single-dose (acute) and multi-dose (chronic) exposure. Bromadiolone baits have been used in the EU against Coypu, particularly in France [32], where, in 2003, a decree was issued that, for a period of three years, gave the chief administrative officers representing authority in a *département* power to authorise the use of chemicals, to complement other pest control methods and in very 'strict conditions of supervision', to control the spread of coypu and the muskrat [153]. However, the use of bromadiolone against coypu was implicated in secondary toxicity to two rare aquatic mustelids, the European mink (*Mestela lutreola*) and European otter (*Lutra lutra*). Consequently, Barrat et al. [154] report that the method is no longer used in France against coypu and nor is it in Belgium

[31]. It is possible that the current EU approval of bromadiolone, as a plant protection product, could, in principle, still permit use in this species. However, the current approval of bromadiolone will expire on 31/05/2021, unless a dossier is successfully submitted for review. Furthermore, such use would need to be evaluated and justified with respect to potential negative non-target and welfare impacts, which is likely to be difficult given that successful eradication of local populations of this species is possible via other methods such as trapping [155 156].

Nasua nasua **Coati**
No relevant species-specific literature identified. Coati are potentially susceptible to the relatively carnivore specific toxicant PAPP, which is also considered to cause relatively low negative animal welfare impact. However, the registration costs of PAPP as a novel AS in the EU would be considerable and currently unrealistic. Furthermore, no species-specific bait delivery system is currently available for the coati.

Nyctereutes procyonoides **Raccoon dog**
No relevant species-specific literature was identified. The raccoon dog is potentially susceptible to the relatively carnivore specific toxicant PAPP, which is also considered to cause relatively low negative animal welfare impact. However, the registration costs of PAPP as a novel AS in the EU would be considerable and currently unrealistic. Baits can be effectively delivered to the raccoon dog, as demonstrated in studies of the delivery of oral rabies vaccine [157].

Ondatra zibethicus **Muskrat**
Zinc phosphide and the pindone have been used to control the muskrat in the USA [158, 159]. Chlorophacinine has been used in New Zealand [35]. The use of poison baits against muskrats in Europe has mainly involved the FGAR chlorophacinone, and the SGARs difenacoum and bromadiolone [33, 34, 160, 40, 32].

Moens & Colin [33] reported that an initial chlorophacinone control exercise required 70 person-days to distribute 12,057 baits across a 240 km^2 area, and 52 person-days were required for the distribution of 8,681 baits during a follow-up exercise. Tuyttens & Stuyck [35] found that more time was required for a similar scale exercise, highlighting that other factors, such as the geography, topography, and accessibility of the terrain, the density of watercourses in the area, and the density of the target muskrat population, can greatly influence the effort required. They thus questioned whether the approach was viable at a large-scale, with realistic estimates of available resources suggesting that it would only be applicable to sporadic control efforts aimed at particularly intractable local populations. They also recognised the need to take into account criteria other than just efficacy when choosing the most appropriate approach, such as the need to inflict as little suffering as possible on the target species.

Poison baiting has never been part of the strategy to control or eradicate muskrats in either the Netherlands or the UK [161, 162] and no successful eradications of muskrat are known to have been achieved using chemical control [163]. In the Loire Atlantique department in France no poison has been used for the control of muskrats since 2003 [164], and in Flanders the use of rodenticides was abandoned at the start of the millennium [165]. Evidence of the effectiveness of eradication through trapping is provided by several successful muskrat eradication campaigns in Britain and Ireland [155, 162]. Muskrats have also been successfully removed by trapping alone from several regions and small islands in the Netherlands [166, 167] and large parts of Flanders, Belgium [168], without the use of poison [161, 168]. Given these successes, using techniques with relatively low negative welfare costs, there seems no current need to resort to the use of poisons, such as ARs, in bait against this species with the inevitable imposition of higher welfare costs [e.g. 54] and also potential hazard to non-target wildlife.

Oxyura jamaicensis **Ruddy duck**
No relevant literature was identified for the use of poisons and toxins in bait against the ruddy duck. The species is potentially susceptible to Starlicide but no bait delivery system available.

Percottus glenii **Amur sleeper**
No relevant literature was identified for the use of poisons and toxins in bait against this species. The main constraint on the control of invasive fish species is the lack of species-specific eradication techniques [169]. *P. glenii* is probably susceptible to piscicides, such as rotenone, but such toxicants would not discriminate between *P. glenii* and native species [170]. There is no currently available method for delivering such toxicants to fish via baits, although see assessment of Pumpkinseed (*Lepomis gibbosus*) for generic discussion of the use of poison and toxins in bait against fish.

Plotosus lineatus **Striped eel catfish**
No relevant literature was identified for the use of poison and toxins in bait against this species. The main constraint on the control of invasive fish species is the lack of species-specific eradication techniques [169]. The species is susceptible to Rotenone poisoning following system wide application [57], but there is no currently available method for delivering such toxicants to fish via baits, although see assessment of Pumpkinseed (*Lepomis gibbosus*) for generic discussion of the use of poison and toxins in bait against fish.

Procyon lotor **Raccoon**
No relevant literature was identified for the use of poisons and toxins in bait against this species, except that the species is susceptible to bromethalin [62] and baits containing rabies vaccine can be delivered successfully to raccoons [171]. The raccoon is potentially susceptible to the relatively carnivore specific toxicant PAPP, which is also considered to cause relatively low negative animal welfare impact. However, the registration costs of PAPP as a novel AS in the EU would be considerable and currently unrealistic.

Pseudorasbora parva **Topmouth gudgeon**
No relevant literature was identified for the use of poison and toxins in bait against this species. The main constraint on the control of invasive fish species of fish is the lack of species-specific eradication techniques [169]. See assessment of Pumpkinseed (*Lepomis gibbosus*) for generic discussion of the use of poison and toxins in bait against fish.

Sciurus carolinensis **Grey squirrel**
Warfarin has been used to control the grey squirrel in the UK, primarily to protect forestry interests [120]. While this was a common management practice in the UK it has been little used elsewhere in Europe [172]. Warfarin baits were delivered to grey squirrels via hoppers designed to reduce primary hazard to non-target species, although initial hopper designs allowed access to passerine birds and other rodents, such as wood mice (*Apodemus sylvaticus*) [173]. The hoppers were subsequently modified to further reduce bait consumption by non-target species [174,175], and practices for minimizing the amounts of warfarin used were recommended [176]. Nevertheless, the protection afforded to non-target species by the hoppers was deemed insufficient to allow use in areas where the native red squirrel (*Sciurus vulgaris*) was present [175, 177].

With respect to the cost- effectiveness of warfarin use, Mayle & Broome [178] reported that, based on survey data, the costs of squirrel damage to forestry fell below € 57 ha^{-1} (€ 1.14: £1 in 2011) when around € 23 ha^{-1} $year^{-1}$ was spent on control (at 1998 prices). They also suggested that warfarin use was the most cost- effective approach, in terms of number squirrels culled per € 1.14 spent, i.e. 0.5–2 for warfarin, compared with 0.02–0.14 for trapping or 0.01–0.34 for shooting. However, this was based on an assumption that every 200–250 g of warfarin bait taken led to the death of one squirrel, rather than an empirical assessment of the actual numbers of squirrels killed per unit spend on this method. The estimated cost of using poison and/or trapping to remove 5 squirrels ha^{-1} was reported as £9-30 (Mayle pers. comm. in Williams et al. [179]). At the national level, Williams et al. [179] estimated that the annual loss to forestry interests in Britain caused by squirrel damage was £684,802, while cost of control was estimated to be £5,412,518. In Italy two LIFE projects for the control of grey squirrels in north (2010-2015) and central Italy (2014-2018) cost € 1,930,00 and € 1,433,241 respectively.

Poisoning was considered to be the least humane and acceptable management approach in a survey of public attitudes to grey squirrel control in the UK [180] although disapproval was greater in the enthusiast than the professional group of respondents. More recently, 67% of respondents to a similar survey considered warfarin use to be unacceptable for the control of the grey squirrel in the UK [181]. Despite such concerns the use of warfarin became the preferred management tool used in most UK commercial woodland situations to mitigate grey squirrel damage, in the absence of red squirrels, putatively because of its relative cost- effectiveness compared to other methods. However, the EU approval of warfarin as a plant protection product for use against the grey squirrel bait lapsed in 2014 due to no commercial or public sector sponsor submitting an application dossier for renewal, with the estimated cost of the review exercise reported to have been a minimum of €460,000 [182]. Subsequently, there has been no approved poison product available for grey squirrel control in the EU [183]. Trapping is now the mainstay of grey squirrel control in Europe and has been successfully used to eradicate local mainland and island populations [184, 185, 186].

It is possible that the current EU approval of the AR bromadiolone, as a plant protection product, could permit its use against the grey squirrel in a similar way to the historical use of warfarin against this species in the UK. However, the current approval of bromadiolone will expire on 31/05/2021, unless a dossier is successfully submitted for review. It seems unlikely that such a dossier will be submitted with respect to the grey squirrel, given the high costs involved relative the potential scale of use. Furthermore, such use would need to be evaluated and justified with respect to potential negative non-target and welfare impacts. Hence, given the likely future absence of an approved AS for potential use against this species in the EU, the recommendation of this assessment is that the use of poisons and toxins in bait to manage the grey squirrel is unsuitable for a modern control program.

Sciurus niger **Fox squirrel**
No relevant species-specific literature identified except absence in USA of EPA registered toxicants for use against tree squirrels being noted by Frey et al [187]. The species is potentially susceptible to rodenticides, such as ARs, but no species-specific bait delivery system is available. It is possible that the current EU approval of the AR bromadiolone, as a plant protection product, could permit use in this species in a similar way to the historical use of warfarin against the Grey squirrel in the UK [120]. However, the current approval of bromadiolone will expire on 31/05/2021, unless a dossier is successfully submitted for review. Furthermore, such use would need to be evaluated and justified with respect to potential negative non-target and welfare impacts.

Tamias sibiricus **Siberian chipmunk**
No relevant species-specific literature was identified for the use of poisons and toxins in bait against this species. Several rodenticides have been used against ground squirrels in the USA, such as strychnine, zinc phosphide, sodium monofluoroacetate and the ARs chlorophacinone and diphacinone [188], and strychnine and chlorophacinone in Canada [189, 190] but with negative consequences in terms of secondary poisoning of predatory wildlife [191]. In the EU none of the current approvals of rodenticides as Biocides would be useful against this species as their use is limited to in and around buildings. It is possible that the current EU approval of bromadiolone, as a plant protection product, could, in principle, allow its use against this species. However, the current approval of bromadiolone will expire on 31/05/2021, unless a dossier is successfully submitted for review. Furthermore, such use would need to be evaluated and justified with respect to potential negative non-target and welfare impacts.

Threskiornis aethiopicus **Sacred ibis**
No relevant literature was identified for the use of poison and toxins in bait against this species. No control methods involving the use of poison or toxins against this species were identified by Smits et al. [192]. Potentially susceptible to Starlicide but no bait delivery system available.

Trachemys scripta **Red-eared slider**
No potential management tools using poison or toxins in bait were identified for use against invasive freshwater turtles by Lacomba [194]. A generic review of the exotoxicology of a range of pesticides in reptiles, including piscicides and vertebrate pest control agents, was undertaken by Pauli & Money [105]. There are currently no realistic prospects for the use of poisons and toxins in bait against this species.

2.1.b. Integration with other measures

Historically poisons and toxins in bait have been used to complement other lethal control methods, for instance poison bait being deployed to bring a population rapidly down followed by trapping and/or shooting to control the residual population e.g. grey squirrel [178].

2.2.a. Availability - species and objectives

Objective			**Management**					
	Rapid Eradication		**Eradication**		**Control**		**Containment**	
Species	Avail.	Reference(s)	Avail.	Reference(s)	Avail.	Reference(s)	Avail.	Reference(s)
Acridotheres tristis								
Alopochen aegyptiaca								
Callosciurus erythraeus								
Corvus splendens								
Herpestes javanicus	P	[134]	P	[134]	P	[134]	P	
Lepomis gibbosus								
Lithobates catesbeianus								
Muntiacus reevesi								
Myocastor coypus								
Nasua nasua								
Nyctereutes procyonoides								
Ondatra zibethicus					P	[164]		
Oxyura jamaicensis								
Percottus glenii								
Plotosus lineatus								
Procyon lotor								
Pseudorasbora parva								
Sciurus carolinensis			A	[120]	A	[120]		
Sciurus niger								
Tamias sibiricus								
Threskiornis aethiopicus								
Trachemys scripta								

2.2.b. Application – EU Member States and objectives								
Objective			Management					
	Rapid Eradication		Eradication		Control		Containment	
Country	Avail.	Reference(s)	Avail.	Reference(s)	Avail.	Reference(s)	Avail.	Reference(s)
Austria								
Belgium					X	[31]		
Bulgaria								
Croatia								
Cyprus								
Czech Republic								
Denmark								
Estonia								
Finland								
France					X	[32]		
Germany					X			
Greece								
Hungary								
Ireland								
Italy								
Latvia								
Lithuania								
Luxembourg								
Malta								
Netherlands								
Poland								
Portugal								
Romania								
Slovakia								
Slovenia								
Spain								
Sweden								
United Kingdom*					X	[120]		

* Not an EU Member State

3. Humaneness of the measure

Over the past 25 years there has been increasing recognition that methods for the scientific assessment of animal welfare can be applied to management practices targeting wildlife. Such assessments could potentially facilitate cost- benefit analyses, where the adverse effects caused by the target species are set against any poor welfare imposed by the management technique, although it may be considered that the negative effects of some management tools on animal welfare are so extreme that their use can never be justified [195].

The extensive world-wide use of rodenticides against commensal rodents initiated the first comparative reviews of the relative welfare impacts and humaneness of different rodent control techniques [68]. This was followed by the development of general principles for the humane management of vertebrate wildlife [196, 197, 198, 199], comparative reviews of the humaneness of vertebrate control techniques [52, 200, 54] and a model for assessing the relative humaneness of vertebrate control methods [53].

In a review by Mason & Littin [68], the humaneness of rodent pest control methods used in the UK and USA was assessed based on the following criteria: the degree of pain, discomfort or distress caused; the length of time for which individuals are conscious and displaying clinical signs of poisoning; and the effect on any individual that escapes and survives. This approach was developed into a model for comparative welfare assessment of management techniques by Sharp & Saunders [53]. This model has two parts. Part A is an assessment of overall welfare impact, based on the five domains of potential welfare impact while Part B considers the 'actual mode of death' and the 'extent and duration of suffering caused'. For methods involving toxic baits it is likely that there will be no welfare impact prior to the animal ingesting the bait, thus only the Part B assessment is required for such methods [53]. The scoring matrix for the Part B assessment is shown in Table 1.

Sufficient data are available for several of the AS considered in this task to facilitate assessment with respect to this scoring matrix and the results are summarised in Table 2.

Table 1. Scoring matrix for Part B. assessment of mode of death [53].

Level of suffering	Time until insensibility				
	Immediate/ seconds	Minutes	Hours	Days	Weeks
Extreme	E	F	G	H	H
Severe	D	E	F	G	H
Moderate	C	D	E	F	G
Mild	B	C	D	E	F
No impact	A	A	A	A	A

Table 2. Toxicant assessments according to Part B of the Sharp & Saunders [53] model for assessing the relative humaneness of vertebrate control methods.

Active substance	Welfare impact of the method of killing/death		
	Time to insensibility	**Level of suffering**	**Score**
Cyanide [54]	Minutes [54]	Mild/Moderate [54]	B-C*
PAPP [81, 77]	Minutes/Hours [81] <45 minutes [77]	Mild/Extreme [81]	C-G*
Sodium nitrite [53, 81]	Minutes/Hours [53, 81]	Mild/Extreme [53, 81]	D [53]
Rotenone (fish) [52]	Hours [52]	Moderate [52]	E [52]
Zinc phosphide [54]	Hours [54]	Moderate/Severe[54]	E-F [54]
Sodium monofluoroacetate [53, 54]	Hours [53, 54]	Severe [53, 54]	E-F [54]
Cholecalciferol[54]	Days [54]	Severe [54]	G*
Starlicide (birds) [52]	Days [52]	Severe [52]	G [52]
Strychnine [53]	Hours [53]	Extreme [53]	G [53]
Anticoagulants [53,54, 81]	Days [54, 81]	Moderate/Severe [54] Severe/Extreme [81]	F-H [54] G-H [53]
Bromethalin [81]	Days [81]	Severe/Extreme [81]	G-H*

*Score based on reported duration and level of suffering.

3.1. Welfare for all measures

As described by Sharp & Saunders [53] for methods involving toxic baits it is likely that there will be no welfare impact prior to the animal ingesting the bait and thus Part A (Section 3.1 of this assessment) is not relevant, and only the Part B (section 3.2 of this assessment) is required for such methods.

Measure type (if applicable):	**Humaneness impact categories**		
Domain	**No impact**	**Mild-Moderate**	**Severe - Extreme**
1: Water deprivation, food deprivation, malnutrition			
2: Environmental challenge			
3: Injury, disease, functional impairment			
4: Behavioural, interactive restriction			
5: Anxiety, fear, pain, distress, thirst, hunger etc.			

3.2. Mode of death (if relevant)			
Measure type (if applicable): Cyanide	**Immediate death (i.e. no suffering)**	**Not immediate death (mild - moderate suffering)**	**Not immediate death (severe - extreme suffering)**
Rationale:		Time to insensibility: Minutes Level of suffering: Mild/Moderate Score: *B-C [54]	

Measure type (if applicable): PAPP	**Immediate death (i.e. no suffering)**	**Not immediate death (mild - moderate suffering)**	**Not immediate death (severe - extreme suffering)**
Rationale:		Time to insensibility: Minutes/Hours Level of suffering: Mild/Extreme Score: *C-G [77, 81]	

Measure type (if applicable): Sodium nitrite	**Immediate death (i.e. no suffering)**	**Not immediate death (mild - moderate suffering)**	**Not immediate death (severe - extreme suffering)**
Rationale:		Time to insensibility: Minutes/Hours Level of suffering: Mild/Extreme Score: *D [53, 81]	

Measure type (if applicable): Rotenone (fish only – not assessed for amphibians or reptiles)	**Immediate death (i.e. no suffering)**	**Not immediate death (mild - moderate suffering)**	**Not immediate death (severe - extreme suffering)**
Rationale:		Time to insensibility: Hours Level of suffering: Moderate Score: E [52]	

Measure type (if applicable): Zinc phosphide	**Immediate death (i.e. no suffering)**	**Not immediate death (mild - moderate suffering)**	**Not immediate death (severe - extreme suffering)**
Rationale:			Time to insensibility: Hours Level of suffering: Moderate/Severe Score: *E-F [54]

Measure type (if applicable): Sodium monofluoroacetate	**Immediate death (i.e. no suffering)**	**Not immediate death (mild - moderate suffering)**	**Not immediate death (severe - extreme suffering)**
Rationale:			Time to insensibility: Hours Level of suffering: Severe Score: *E-F [53, 54]

Measure type (if applicable): Cholecalciferol	**Immediate death (i.e. no suffering)**	**Not immediate death (mild - moderate suffering)**	**Not immediate death (severe - extreme suffering)**
Rationale:			Time to insensibility: Days Level of suffering: Severe Score: *G [54]

Measure type (if applicable): Starlicide	**Immediate death (i.e. no suffering)**	**Not immediate death (mild - moderate suffering)**	**Not immediate death (severe - extreme suffering)**
Rationale:			Time to insensibility: Days Level of suffering: Severe Score: *G [52]

Measure type (if applicable): Strychnine	**Immediate death (i.e. no suffering)**	**Not immediate death (mild - moderate suffering)**	**Not immediate death (severe - extreme suffering)**
Rationale:			Time to insensibility: Hours Level of suffering: Extreme Score: *G [53]

Measure type (if applicable): Anticoagulant rodenticides	**Immediate death (i.e. no suffering)**	**Not immediate death (mild - moderate suffering)**	**Not immediate death (severe - extreme suffering)**
Rationale:			Time to insensibility: Days Level of suffering: Moderate/Severe/Extreme Score: *F-H [53, 54, 81]

Measure type (if applicable): Bromethalin	Immediate death (i.e. no suffering)	Not immediate death (mild - moderate suffering)	Not immediate death (severe - extreme suffering)
Rationale:			Time to insensibility: Days Level of suffering: Severe/Extreme Score: *G-H [81]

3.3. Humaneness summary	The ARs have the greatest negative welfare impact of the vertebrate toxicants with current approvals in the EU (Table 2). This is unsurprising, given that the UK's pesticides regulator in 1997, the Pesticides Safety Directorate, concluded that all anticoagulant rodenticides are "markedly inhumane" [203]. Hence, there remains the welfare anomaly that the most extensively used vertebrate toxicants, world-wide and in the EU, impose substantial negative welfare impacts [68]. The need to incorporate welfare into the development of eradications of invasive species has become increasingly recognised in the context of island rat eradications, which are heavily reliant on extensive use of markedly inhumane rodenticides [204, 205]. The inclusion of animal welfare in such vertebrate management decision making can lead to disagreement over the balancing of different concerns, such as protecting human interests, animal health and biodiversity, against negative welfare impacts on target species [206]. Therein lies a paradox as, from an economic perspective, animal welfare relates primarily to human demand or preferences and not to the experience of the animal [207]. Fundamentally, this is because benefits, such as enhanced biodiversity, are measured in different currencies from negative welfare costs. Hence, a cost-benefit approach to incorporating animal welfare into wildlife management policy making will always be open to potential challenge until there are objective means of evaluating benefits, such as enhanced biodiversity, against negative welfare impacts. Until then perceived welfare and sociocultural considerations will determine public perceptions and acceptance of wildlife management practices [144]. From an EU regulatory perspective, the principle of the Biocides Directive was to implement the highest possible standards of protection of human health and the environment and included the exclusion clause requiring that ".....when properly used for the purpose intended, they have no unacceptable effect on the target organisms............ such as........, in the case of vertebrate animals, unnecessary suffering and pain," [208]. However, provisions exist for the derogation of such exclusion if "the active substance is essential to prevent or control a serious danger to human health, animal health or the environment" or "not approving the active substance would have a disproportionate negative impact on society when compared with the risk to human health, animal health or the environment arising from the use of the substance." and one or both of these derogations are considered to apply to all approved ARs for use throughout the EU [21]. When such derogations are invoked, the relevant AS are named 'candidates for substitution'. These are then subject to a review process every 5 years, which involves a public consultation. The consultation on ARs conducted by ECHA at the first renewal finished in 2018. The consultation determined that no equally effective, more humane, and safer alternatives to the candidates for substitution had become available and the ECHA came to the conclusion that all AR active substance approvals were renewed for a further 5 years [21]. Hence, these substances, with a regulatory status of being "markedly inhumane", are still approved for use as biocides in the EU. Consequently, the paradox, pointed

	out by Paparella [209], "that the legal intention of the Biocidal Products Directive 98/8/EC to phase out vertebrate control products that cause unnecessary suffering and pain (98/8/EC Article 5.1. specified by Annex VI Article 91) cannot be translated into regulatory practice," still remains unresolved. There are emerging AS with potentially lower negative welfare impacts such as PAPP. However, the costs of gaining an approval for the use of such a novel AS in the EU would be substantial, e.g. estimated at € 2.74 million in 2002 [59].

4. Costs and effectiveness of the measure

4.1. Case studies	
CASE STUDY #1	
Measure type (if relevant):	Warfarin (0.02%) in whole wheat bait delivered via squirrel-specific feeding hoppers
Species:	*Sciurus carolinensis* Grey squirrel
Objective:	Control – mainly to reduce damage to forestry interests.
Combined with other measure(s):	Was used in combination with trapping and shooting, largely dependent on available resources.
Country(ies) of application:	UK
Geographic scale (km^2) and/or population size measure applied to:	9,700 km^2 of high forest vulnerable to grey squirrel damage in 1995-1998.
Time period:	1989-2015
Effort:	
Costs:	Overall costs:
	Costs have been estimated in terms of the number of squirrels killed per € 1.14 spent to be 0.5–2 for warfarin use, compared with 0.02–0.14 for trapping and 0.01–0.34 for shooting. However, the estimate for warfarin use was based on an assumption that every 200–250 g of warfarin bait taken led to the death of one squirrel, rather than an empirical assessment of the actual numbers of squirrels killed per unit spend on this method. The cost of using poison and/or trapping to remove five squirrels ha^{-1} has been estimated to be £9-30.
	Personnel costs:
	NA
	Equipment and infrastructure:
	NA
	Other, including overheads:
	NA

Effectiveness:	Cost-effectiveness data with respect to reducing damage to forestry are limited. However, the cost of damage to forestry reportedly fell to below € 57 ha^{-1} (€ 1.14: £1 in 2011) when around € 23 ha^{-1} $year^{-1}$ was spent on control (at 1998 values).
Reference(s):	[120, 178]

CASE STUDY #2	
Measure type (if relevant):	Diphacinone (0.00025%) in hamburger meat baits delivered at bait stations
Species:	*Herpestes sp.* Mongoose
Objective:	Local control & potentially eradication
Combined with other measure(s):	NA
Country(ies) of application:	Hawaii, USA
Geographic scale (km^2) and/or population size measure applied to:	Local populations targeted with baits placed at 125-250m intervals throughout area with mongoose present.
Time period:	Baiting maintained for 12-15 days
Effort:	Labour required for bait station construction, and daily bait delivery.
Costs:	Overall costs:
	Considerable effort required, particularly logistics associated with ensuring fresh bait available daily in remote areas.
	Personnel costs:
	Equipment and infrastructure:
	Formulated bait, Bait stations
	Other, including overheads:
Effectiveness:	Locally effective in terms of reducing local population rapidly in suitable areas. However, expensive due to both material and labor costs. Given these constraints, it was considered impractical to apply to large conservation areas.
Reference(s):	[134, 66, 135, 84, 81]

4.2. Costs effectiveness summary	Neither of the case studies offered strong evidence that the poison bait approach was cost-effective as a large-scale management tool for these species.

5. Side effects	
Non-target native species, their habitats and the broader environment:	Positive:
	Negative:
	Adverse effects on non-target native species caused by poisons and toxins through primary exposure, via the consumption of toxic bait [e.g. 14, 15], or secondary exposure, via the consumption of poisoned prey or carcasses [e.g. 16, 17].
Other invasive alien species:	Positive:
	Negative:
	Unintended adverse effects on other invasive alien species caused by poisons and toxins through primary exposure, via the consumption of toxic bait [e.g. 14, 15], or secondary exposure, via the consumption of poisoned prey or carcasses [e.g. 16, 17].
Public health and well-being:	Positive:
	Negative:
	The use of poison in large scale control programmes may represent a risk to public health. Poisoning was considered to be the least humane and acceptable management approach in a survey of public attitudes to grey squirrel control in the UK [180]. More recently, 67% of respondents to a similar survey considered warfarin use to be unacceptable for the control of the grey squirrel in the UK [181].
Economic:	Positive:
	Negative:
	Costs of management can be very high. For example, for *Sciurus carolinensis* Grey squirrel the annual cost to forestry in Britain of squirrel damage has been estimated as £684,802, while the cost of control was estimated to be £5,412,518. Hence, control costs seem substantially greater than the apparent total damage costs. Furthermore, surveys indicate that there may be little correlation between control effort and damage [178, 179].

6. Conclusion

Overall assessment of the measure (qualitative)

There are currently only very limited prospects for the use of poisons or toxins in bait to contribute to the management of IAS of Union concern. The PT-14 approvals of rodenticides as Biocides are all limited to use in and around buildings, which means they offer no realistic prospects as viable tools for any of the target IAS. The current approval of bromadiolone for use as a plant protection product has potential to permit use for the control of the rodent IAS, namely coypu, fox squirrel, grey squirrel, muskrat, Pallas's squirrel and Siberian chipmunk. However, a comprehensive risk-assessment of such use would need to be undertaken with respect to both primary and secondary hazards for non-target wildlife and only if a net benefit appeared likely, relative to other management options, could such an approach be justified. Furthermore, such use would accrue substantial negative welfare costs associated with materials whose mode of death is markedly inhumane. Again, a comparative evaluation would need to be undertaken of the expected benefits derived from the management of the IAS against the inevitable welfare costs incurred. Such consideration may, in any case, become redundant because the current approval of bromadiolone expires on 31/05/2021, unless a dossier is successfully submitted for review. There has been extensive and successful use of ARs to eradicate invasive species (none of them of Union Concern) from islands so the use of these materials, with associated major environmental and welfare concerns, has historically been considered justified in these contexts. However, transparent justification would be more challenging in mainland ecosystems. Use of such techniques in these contexts, without convincing justifications from both environmental and welfare perspectives, risks undermining public support for the overall ambition of reducing the negative impacts of the IAS of Union concern.

The future may bring AS with lower negative environmental and welfare consequences such as PAPP, particularly with respect to the carnivore IAS, namely mongoose, raccoon and raccoon dog. However, the costs of regulatory approval of such an AS would be substantial and unlikely to be borne by commercial interests and, hence, would require public sector investment. This assessment thus concludes that there are currently no method types, involving the use of poisons and toxins in bait, available to contribute to managing any IAS of Union concern. Hence, until such time that regulatory approval of a substance with relatively low non-target and welfare impacts emerges, the overall recommendation of this assessment is that the use of poisons and toxins in bait to manage IAS of Union concern is unsuitable for a modern control program.

Assessor:	David Cowan
Reviewer 1:	Ilaria Di Silvestre
Reviewer 2:	Riccardo Scalera

7. References

[1] O'Connor, J.A. (1948). The use of blood anticoagulants for rodent control. *Research London, 1*, 334-336.

[2] Link, K. P., (1959). The discovery of dicumarol and its sequels. *Circulation*, *19*(1), 97–107.

[3] Buckle, A. P., Odam, E. M., & Richards, C. J. G. (1987). Chemical bait markers for the study of bait uptake by Norway rats. In C. G. J. Richards & T. Y. Ku (Eds.), *Control of Mammal Pests* (pp. 199-213). Taylor and Francis, London.

[4] Silverman, R. B. (1980). A model for the molecular mechanism of anticoagulant activity of 3-substituted 4-hydroxycoumarins. *Journal of the American Chemical Society*, *102*, 5421-5423.

[5] MackIntosh, C. G., Laas F. J., Godfrey, M. E. R., & Turner. K. (1988). Vitamin K1 treatment of brodifacoum poisoning in dogs. *Proceedings of the Vertebrate Pest Conference, 13*, 86-90.

[6] Boyle, M. (1960). A case of apparent resistance of *Rattus norvegicus* to anticoagulant poisons. *Nature*, *4749*, 519.

[7] Rowe, F. P., & Redfern, R. (1965). Toxicity tests on suspected warfarin-resistant house mice (*Mus musculus*). *Journal of Hygiene, Cambridge, 63*, 417-425.
[8] Greaves, J. H., & Ayres, P (1969a). Heritable resistance to warfarin in rats. *Nature, 224*,284-285.
[9] Hadler, M. R., & Shadbolt, R. S. (1975). Novel 4- hydroxycoumarin anticoagulants active against resistant rats. *Nature, 253*, 275-277.
[10] Greaves, J. H., Rennison, B. D., & Redfern, R. (1976). Warfarin resistance in the Ship rat in Liverpool. *International Pest Control* 15,17.
[11] Nakayama, S. M. M., Morita, A., Ikenaka, Y., Mizukawa, H., & Ishizuka, M. (2019). A review: Poisoning by anticoagulant rodenticides in non-target animals globally. *The Journal of Veterinary Medical Science, 81*, 298–313. doi: 10.1292/jvms.17-0717
[12] Hadler M. R., & Buckle, A. P. (1992). Forty Five Years of Anticoagulant Rodenticides-Past, Present and Future Trends. *Proceedings of the Vertebrate Pest Conference, 15*, 149-155.
[13] Dubock, A. C. (1982). Pulsed baiting: a new technique for high potency. slow acting rodenticides. *Proceedings of the Vertebrate Pest Conference, 10*, 123-136.
[14] Sanchez-Barbudo, I. S., Camarero, P. R., & Mateo, R. (2012). Primary and secondary poisoning by anticoagulant rodenticides of non-target animals in Spain. *Science of the Total Environment, 420*, 280–288.
[15] Shore R. F. (2018a). Primary exposure and effects in non-target animals. In N. van den Brink, J. Elliott, R. Shore, & B. Rattner (Eds.), *Anticoagulant rodenticides and wildlife. Emerging topics in ecotoxicology (principles, approaches and perspectives), Vol 5* (pp. 135-137-318). Springer, Switzerland.
[16] Shore, R. F. (2018b). Rodenticides: the good, the bad, and the ugly. In D. A. DellaSala & M. I. Goldstein (Eds), *The encyclopedia of the anthropocene, volume 5.* (pp. 144-160). Elsevier, Oxford, UK. doi: https://doi. org/10.1016/B978-0- 12-809665-9.09993-6
[17] Shore, R. F., Potter, E. D., Walker, L. A., Pereira, M. G., Chaplow, J. S., Jaffe, J. E., Sainsbury, A. W., Barnett, E. A., Charman, S., Jones, A., Giela, A., Senior, C., & Sharp, E. A. (2018). The Relative Importance of Different Trophic Pathways for Secondary Exposure to Anticoagulant Rodenticides. *Proceedings of the Vertebrate Pest Conference, 28*, 322-328.
[18] Thomas, P. J., Mineau, P., Shore, R. F., Champoux, L., Martin, P. A., Wilson, L. K., Fitzgerald, G., & Elliott, J. E. (2011). Second generation anticoagulant rodenticides in predatory birds: Probabilistic characterisation of toxic liver concentrations and implications for predatory bird populations in Canada. *Environment International, 37*, 914–920.
[19] BPC (2016). Biocidal Products Committee (BPC) opinion on the application for renewal of the approval of the active substance: Brodifacoum, Product type: 14, Document # ECHA/BPC/113/2016, https://echa.europa.eu/documents/10162/b85dfd6e-177b-43df-809c-180bc025b612
[20] Frankova, M., Stejskal, V., & Aulicky, R. (2009). Efficacy of rodenticide baits with decreased concentrations of brodifacoum: Validation of the impact of the new EU anticoagulant regulation. *Scientific Reports, 9*, Article number 16779. https://doi.org/10.1038/s41598-019-53299-8
[21] Eisemann, J. D., Fisher, P. M., Buckle, A., & Humphrys, S. (2018). An international perspective on the regulation of rodenticides. In N. van den Brink, J. Elliott, R. Shore, & B. Rattner (Eds.), *Anticoagulant rodenticides and wildlife. Emerging topics in ecotoxicology (principles, approaches and perspectives), Vol 5* (pp. 287-318). Springer, Switzerland.
[22] Courchamp, F., Chapuis, J. L., & Pascal. M, (2003). Mammal invaders on islands: impact, control and control impact. *Biological Reviews, 78*, 347–383. doi: 10.1017/s1464793102006061
[23] Howald, G., Donlan, C. J., Galvan, J. P., Russell, J. C., Parkes, J., Samaniego, A., Wang, Y. W., Veitch, D., Genovesi, P., Pascal, M., Saunders, A., & Tershy, B. (2007). Invasive rodent eradication on islands. *Conservation Biology, 21*, 1258–1268.
[24] Pascal, M., Lorvelec, O., Bretagnolle, V., Culioli, J-M. (2008) Improving the breeding success of a colonial seabird: a cost-benefit comparison of the eradication and control of its rat predator. *Endangered Species Research 4*, 267–276.
[25] Bell, E., Boyle, D., Floyd, K., Garner-Richard, P., Swann, B., Luxmoore, R., Patterson, A., Thomson, R. (2011) The ground based eradication of Norway rats (*Rattus norvegicus*) from the Isle of Canna, Inner Hebrides, Scotland. In C.R. Veitch, M. N. Clout, D. R. Towns, (Eds.), *Island Invasives: Eradication and Management* (pp. 269-274). IUCN: Gland, Switzerland.
[27] Towns, D. R., & Broome, K.G. (2003). From small Maria to massive Campbell: forty years of rat eradications from New Zealand islands. *New Zealand Journal of Zoology 30*, 377–398.
[28] Littin, K. E., O'Connor, C . E., Gregory, N. G., Mellor, D. J., & Eason, C. T. (2002). Behaviour, coagulopathy and pathology of brushtail possums (*Trichosurus vulpecula*) poisoned with brodifacoum. *Wildlife Research, 29*, 259–267.
[29] Eason, C., Henderson, R., Hix, S., MacMorran, D., Miller, A., Murphy, E., Ross, J., & Ogilvie, S. (2010a). Alternatives to brodifacoum and 1080 for possum and rodent control—how and why? *New Zealand Journal of Zoology, 37*, 175-183. DOI: 10.1080/03014223.2010.482976
[30] Grand, M. (1976). Some experiments on a new anticoagulant rodenticide: bromadiolone. *Phytiarie-Phytopharmacie 25*,69-88.
[31] Robert, H., Lafontaine, R-M., BeudelsJamar, R. C., Delsinne, T., & Baiwy, E. (2013). Risk analysis of the Coypu Myocastor coypus (Molina, 1792). Risk analysis report of non-native organisms in Belgium. DOI: 10.13140/RG.2.2.33552.20484

[32] Fournier-Chambrillon, C., Berny, P. J., Coiffier, O., Barbedienne, P., Dassé, B., Delas, G., Galineau, H., Mazet, A., Pouzenc, P., Rosoux, R., & Fournier, P. (2004). Evidence of secondary poisoning of free-ranging riparian mustelids by anticoagulant rodenticides in France: implications for conservation of European mink (*Mustela lutreola*). *Journal of Wildlife Diseases, 40*, 688–95.
[33] Moens, R., & Colin, G. (1971). La lutte contre le rat musqué au moyen d'appats empoisonnés à la chlorophacinone: expériences effectuées dans le bassin de l'Eau Blanche. *Mededelingen Fakulteit Landbouwwetenschappen Gent*, *36*, 1088–1097.
[34] Giban, J. (1974). Use of chlorophacinone in the struggle against the common vole *Microtus arvalis*, Pallas, and against the muskrat *Ondatra zibethica* L. *Proceedings of the Vertebrate Pest Conference, 6*, 262–271.
[35] Tuyttens, F. A. M. & Stuyck, J. J. J. M. (2002). Effectiveness and efficiency of chlorophacinone poisoning for the control of muskrat (Ondatra zibethicus) populations. *New Zealand Journal of Zoology*, *29*, 33–40.
[36] Greaves, J. H., & Ayres, P. (1969b). Some rodenticidal properties of coumatetralyl. *Journal of Hygiene, Cambridge, 67*, 322- 315.
[37] Hadler M. R.. Redfern, R., & Rowe, F. P. (1975). Laboratory evaluation of difenacoum as a rodenticide. *Journal of Hygiene, Cambridge*, 74, 441-448.
[38] Bull, J. O. (1976). Laboratory and field investigations with difenacoum, a promising new rodenticide. *Proceedings of the Vertebrate Pest Conference, 7*, 72-84.
[39] Redfern, R., Gill, J. E., & Hadler, M. R. (1976). Laboratory evaluation of WBA 8119 as a rodenticide for use against warfarin-resistant and non-resistant rats and mice. *Journal of Hygiene, Cambridge, 77*, 419-426.
[40] Moens, R., & Gigot, J. (1988). Attempts to control muskrats (Ondatra zibethicus l) using a preparation with a difenacoum base. *Revue de l'Agriculture, 41*, 999-1007
[41] Nahas, K., Lorgue, G., & Mazallon, M. (1989). Difethialone (Lm-2219): A new anticoagulant rodenticide for use against warfarin-resistant and -susceptible strains of *Rattus norvegicus* and *Mus musculus*. *Annales de Recherches Veterinaires, 20*,159–164
[42] Huckle, K. R., Hutson, D. H., & Warburton, P. A. (1988). Elimination and accumulation of the rodenticide flocoumafen in rats following repeated oral administration. *Xenobiotica, 18*,1465–1479.
[43] Inglis I. R., Shepherd D. S., Smith P., Haynes P. J., Bull D. S., Cowan D. P., & Whitehead D. (1996). Foraging behaviour of wild rats (*Rattus norvegicus*) towards new foods and bait containers. *Applied Animal Behaviour Science, 47*, 175-190.
[44] Odam, B. E., Pepper, H. W., & Townsend, M.G . (1979). A study of the persistence of warfarin on wheat bait used for the control of grey squirrels (*Sciurus carolinensis*). *Annals of Applied Biology 91*, 81-89.
[45] Chambers, C. M., & Chambers, P. L. (1983). Warfarin and the Grey Squirrel. In C. M. Chambers, P. L. Chambers & S. Gitter (Eds), *Toxicology in the Use, Misuse, and Abuse of Food, Drugs, and Chemicals. Archives of Toxicology (Supplement), vol 6* (pp. 214-221). Springer, Berlin, Heidelberg.
[46] Marshall, E. F. (1984). Cholecalciferol: a unique toxicant for rodent control. *Proceedings of the Vertebrate Pest Conference*, *11*, 95–98.
[47] Tobin, M. E., Matschke G. H., Susihara R. T., McCann, G. R., Koehler, A. E., & Andrews K. J. (1993). Laboratory efficacy of cholecalciferol against field rodents. United States Department of Agriculture. Animal and Plant Health Inspection Service. Denver Wildlife Research Report No. 11-55-002.
[48] Dorman D. C., & Beasley, V. R. (1989). Diagnosis and therapy for cholecalciferol toxicosis. In W.B. Saunders (Ed.), *Current veterinary therapy X. Small animal practice* (pp. 148–152). Philadelphia, USA.
[49] Jolly S. E., Eason, C. T., & Frampton, C. (1993). Serum calcium levels in response to cholecalciferol and calcium carbonate in the Australian brushtail possum. *Pesticide Biochemistry and Physiology, 47*, 159-164.
[50] Eason, C. T., Fagerstone, K. A., Eisemann, J. D., Humphrys, S., O'Hare, J. R., & Lapidge, S. J. (2010b). A review of existing and potential New World and Australasian vertebrate pesticides with a rationale for linking use patterns to registration requirements. *International Journal of Pest Management, 56,* 109-125. DOI: 10.1080/09670870903243463
[51] Gregory, N. G., Milne, L. M., Rhodes. A. T., Litten, K. E., Wickstrom. M., Eason, C. T. (1998). Effects of potassium cyanide on behaviour and time to death in possums. *New Zealand Veterinary Journal, 46*, 60-64.
[52] Fisher, P., Beausoleil N. J, Warburton, B., Mellor, D. J., Campion, M., & Booth, L. (2010). How humane are our pest control tools? P.152 Landcare Research, Lincoln, MAF Biosecurity, Wellington, New Zealand.
[53] Sharp, T. & Saunders, G. (2011). A model for assessing the relative humaneness of pest animal control methods (Second edition). Australian Government Department of Agriculture, Fisheries and Forestry, Canberra, ACT. New Millennium Print, Australia. https://www.agriculture.gov.au/animal/welfare/aaws/humaneness-of-pest-animal-control-methods
[54] Littin, K., Fisher, P., Beausoleil, N., & Sharp, T. (2014). Welfare aspects of vertebrate pest control and culling: Ranking control techniques for humaneness. *Revue scientifique et technique (International Office of Epizootics), 33*, 281–289.
[55] Schmolz, E. (2010) Efficacy of anticoagulant-free alternative bait products against house mice (*Mus musculus*) and brown rats (*Rattus norvegicus*). *Integrative Zoology, 5*,44-52.

[56] Zhelev, G., Lyutskanov, M., Petrov, V., Michaylov, G., Marutsov, P., Koev, K., & Tsvetanov, T. (2013). Efficacy of a cellulose-based rodenticide for control of warfarin-resistant black rats (Rattus rattus). *Bulgarian Journal of Veterinary Medicine, 16*, 134 – 140.
[57] Ineich, I., Bonnet, X., Brischoux, F., Kulbicki, M., Séret, B., & Shine, R. (2007). Anguilliform fishes and sea kraits: neglected predators in coral-reef ecosystems. *Marine Biology, 151*, 793–802.
[58] Witmer G. W., Snow N. P., & Moulton R. S. (2015). Efficacy of potential chemical control compounds for removing invasive American bullfrogs (*Rana catesbeiana*). *SpringerPlus 4*, 1-5. DOI 10.1186/s40064-015-1319-6.
[59] Knight, D. J., & Cooke, M. (2002). Regulatory control of biocides in Europe. In D.J. Knight & M. Cooke (Eds.), *The Biocides Business: Regulation, Safety and Applications*, (pp. 45-74). Weinheim: Wiley-VCH Verlag GmbH.
[60] Buckle, A. P., Sharples, R., & Prescott, C. V. (2005). Europe's biocidal products directive: benefits and costs in urban pest management. In C. Y. Lee & W. Robinson (Eds.), *Proceedings of the Fifth International Conference on Urban Pests* (pp. 343-349). Singapore.
[61] Witmer, G. W. (2018). Perspectives on Existing and Potential New Alternatives to Anticoagulant Rodenticides and the Implications for Integrated Pest Management. In N. van den Brink, J. Elliott, R. Shore, & B. Rattner (Eds.), *Anticoagulant rodenticides and wildlife. Emerging topics in ecotoxicology (principles, approaches and perspectives), Vol 5* (pp. 357-378). Springer, Switzerland.
[62] Bautista, A. C., Woods, L. W., Filigenzi, M. S., & Puschner, B. (2014). Bromethalin poisoning in a raccoon (*Procyon lotor*): diagnostic considerations and relevance to nontarget wildlife. *Journal of Veterinary Diagnostic Investigation, 26,* 154-15.
[63] Peterson, M. E. (2013). Bromethalin. *Topics in Companion Animal Medicine, 28*, 21–23.
[64] Pérez-López, M., Sobhakumari, A., Filigenzi, M. & Poppenga, R. H. (2017). Presente y futuro de un nuevo rodenticida dentro de la Unión Europea La brometalina (Present and future of a new rodenticide within the European Union: Bromethalin. *Anales de Veterinaria de Murcia, 33*, 49-54. https://revistas.um.es/analesvet/article/view/369461/260461
[65] Lund, M. (1988). Detection and monitoring of resistance to anti-coagulant rodenticides in populations of brown rats (Rattus norvegicus) in Denmark. In J.W. Suttie, (Ed.), *Current Advances in Vitamin K Research* (pp. 399-405). Elsevier Science Publishers, New York.
[66] Stone, C. P., Dusek, M., & Aeder, M. (1995). Use of an anticoagulant to control mongooses in Nene breeding habitat. *'Elepaio 54*, 73–78.
[67] Smith D. G., Polhemus, J. T., & VanderWerf, E. A. (2000). Efficacy of fish-flavored diphacinone bait blocks for controlling small Indian mongooses (*Herpestes auropunctatus*) populations in Hawaii. *Elepaio 60*, 47–51.
[68] Mason G. J., & Littin, K. E. (2003). The humaneness of rodent pest control. *Animal Welfare, 12*, 1–37.
[69] Abatan, M. O., Lateefand, I., & Taiwo, V. O. (2006). Toxic effects of non-steroidal anti-inflammatory agents in rats. *African Journal of Biomedical Research, 9*, 219 – 223.
[70] Taiwo, V. O., & Conteh, O. L. (2008). The rodenticidal effect of indomethacin: pathogenesis and pathology. *Veterinarski Arhiv, 78*, 167-178.
[71] Zhelev, G., Koev, K., & Petrov, Vl. (2018). Rodenticidal effectiveness of indomethacin baits in warfarin-resistant roof rats (*Rattus rattus*) and House mice (*Mus musculus*). *Trakia Journal of Sciences, 1*, 22-26.
[72] Roszkowski, A. P., Poos, G. I., & Mohrbacher, R. J. (1964). Selective Rat Toxicant. *Science, 144*, 412–413.
[73] Bova, S., Trevisi, L., Debetto, P., Cima, L., Furnari, M.,Luciani, S., Padrini, R., & Cargnelli, G., (1996). Vasorelaxant properties of norbormide, a selective vasoconstrictor agent for the rat microvasculature. *British Journal of Pharmacology, 177,* 1041–1046.
[74] Zulian, A., Šileikyte˙, J., Petronilli, V., Bova, S., Dabbeni-Sala, F., Cargnelli, G., Rennison, D., Brimble, M.A., Hopkins, B., & Bernardi, P., (2011). The translocator protein (peripheral benzodiazepine receptor) mediates rat-selective activation of the mitochondrial permeability transition by norbormide. *Biochim. Biophys. Acta (BBA) Bioenergetics, 1807*, 1600–1605.
[75] Greaves, J. H. (1966). Some laboratory observations on the toxicity and acceptability of norbormide to wild *Rattus norvegicus* and on feeding behaviour associated with sub-lethal dosing. *Journal of Hygiene, Cambridge, 64,* 275–285.
[76] Campbell, K. J., Beek, J., Eason, C. T., Glen, A. S., Godwin, J., Gould, F., Holmes, N. D., Howald, G. R., Madden, F. M., Ponder, J. B., Threadgill, D. W., Wegmann, A. S., & Baxter, G. S. (2015). The next generation of rodent eradications: innovative technologies and tools to improve species specificity and increase their feasibility on islands. *Biological Conservation, 185*, 47–58.
[77] Eason, C. T., Miller, A., MacMorran, D. B., & Murphy E. C (2014). Toxicology and ecotoxicology of para-aminopropiophenone (PAPP) – a new predator control tool for stoats and feral cats in New Zealand. *New Zealand Journal of Ecology, 38*, 177–188.
[78] Fisher, P., & O'Connor, C. (2007). Oral toxicity of *p*-aminopropiophenone to ferrets. *Wildlife Research 34*, 19–24.
[79] Murphy, E. C., Eason, C. T., Hix, S., MacMorran, D. B. (2007). Developing a new toxin for potential control of feral cats, stoats and wild dogs in New Zealand. In G. W. Witmer, W. C. Pitt, & K. A. Fagerstone, K. A. (Eds.) *Managing vertebrate invasive species: proceedings of an international symposium* (pp. 469–479). USDA/APHIS/WS, National Wildlife Research Centre, Fort Collins,Colorado.

[80] Savarie, P. J., Ping Pan, H., Hayes, D. J., Roberts, J. D., Dasch, G. L., Felton, R. & Schafer, E. W. Jr. (1983). Comparative acute oral toxicity of para-aminopropiophenone. *Bulletin of Environmental Contamination and Toxicology, 30*, 122–126.
[81] Ruell, E. W., Niebuhr, C. N., Sugihara, R. T., & Siers, S. R. (2019). An evaluation of the registration and use prospects for four candidate toxicants for controlling invasive mongooses (*Herpestes javanicus auropunctatus*). *Management of Biological Invasions 10*, 573–596.
[82] Fisher, P. M., O'Connor, C. E. & Murphy, E. C. (2005). Acute oral toxicity of p-aminopropiophenone to stoats. *New Zealand Journal of Zoology 32*, 163–169.
[83] APVMA (2015) Australian Pesticides and Veterinary Medicines Authority (APVMA). Public Release Summary on the Evaluation of the New Active 4-aminopropiophenone (also known as para-aminopropiophenone (PAPP)) in the Products Foxecute Fox Bait & PAPP Wild Dog Bait. APVMA Product Numbers 65095 and 65094. APVMA, Australia. https://apvma.gov.au/sites/default/files/publication/18771-papp_public_release_summary.pdf
[84] Sugihara, R. T., Pitt, W. C., Berentsen, A. R., & Payne., C. G. (2018). Evaluation of the palatability and toxicity of candidate baits and toxicants for mongooses (*Herpestes auropunctatus*). *European Journal of Wildlife Research 64*, 2, https://doi.org/10.1007/s10344-017-1163-9
[85] Bentley, E., & Rowe, M. (1956). Pival, an anti-coagulant rodenticide. *Epidemiology and Infection, 54,* 20–27.
[86] Miller, J. E. (1974). Muskrat control and damage prevention. *Proceedings of the Vertebrate Pest Conference*, 6, 85-89.
[87] Fisher, P., Brown, S., & Arrow, J. (2016). Welfare Impacts of Pindone Poisoning in Rabbits (*Oryctolagus cuniculus*). *Animals, 6*, 19; doi:10.3390/ani6030019 www.
[88] Peters, R. S. (1952). Lethal Synthesis. *Proceedings of the Royal Society of London, 139*, 142-170.
[89] Eason, C. T., Miller, A., Ogilvie, S., & Fairweather, A. (2011). An updated review of the Toxicology and Ecotoxicology of 1080 in relation to its use as a pest control tool in New Zealand. *New Zealand Journal of Ecology, 35*, 1–20.
[90] Sherley, M. (2007). Is sodium fluoroacetate (1080) a humane poison? *Animal Welfare 16*, 449–458.
[91] Twigg L. E, & Parker, R. W. (2010). Is sodium fluoroacetate a humane poison? The influence of mode of action, physiology, effect and target specificity. *Animal Welfare, 19*, 249–263.
[92] Eason, C. T., Shapiro, L., Adams, P., Hix, S., Cunningham, C., MacMorran, D., Statham, M., & Statham, H. (2010c). Advancing a humane alternative to sodium fluoroacetate (1080) for wildlife management- welfare and wallaby control. *Wildlife Research, 37,* 497-503.
[93] Cowled, B. D., Eslworth, P. & Lapidge, S. J. (2008). Additional toxins for feral pig (*Sus scrofa*) control: Identifying and testing Achilles' heels. *Wildlife Research, 35*, 651–662.
[94] Lapidge, S., Wishart, J., Staples, L., Fagerstone, K., Campbell, T. & Eisemann, J. (2012). Development of feral swine toxic bait (Hog-Gone) and bait hopper (Hog-Hopper™) in Australia and the USA. *Proceedings of the Wildlife Damage Management Conference, 14*,19–24.
[95] Campbell, T. A., M. J. Bodenchuk, M. J., Eisemann, J. D., Lapidge, S. J., Staples, L. & Morrow, P. (2012). Preliminary assessment of the HogHopper™ for excluding nontarget wildlife. *Proceedings of the Vertebrate Pest Conference*, 12, 333–336.
[96] Witmer, G. (2013). A preliminary evaluation of sodium nitrite as a rodenticide. Unpublished Internal report, QA-1752. USDA/APHIS/WS/NWRC, 14pp.
[97] Ramey, C. A. , Schafer, E. W. , Fagerstone, K. A. , & Palmateer, S. D. (l994). Active ingredients in APHIS's vertebrate pesticides – use and reregistration status. *Vertebrate Pest Conference 16,* 124–132.
[98] Feare, C. J. (2010). The use of Starlicide in preliminary trials to control invasive common myna *Acridotheres tristis* populations on St Helena and Ascension islands. *Atlantic Ocean Conservation Evidence 7*, 52-61 www.ConservationEvidence.com
[99] Schafer, E. W. Jr. (1984). Potential primary and secondary hazards of avicides. *Proceedings of the Vertebrate Pest Conference*, *11*, 217-222.
[100] Eisemann, J. D., Pipas, P. A., & Cummings, J. L. (2003). Acute and chronic toxicity of compound DRC-1339 (3-chloro-4-methylalanine hydrochloride) to birds. In G.M.Linz (Ed.), *Proceedings of the symposium on management of North American blackbirds* (pp. 49-63). National Wildlife Research Center, Fort Collins, Colorado, USA.
[101] Felsenstein, W. C, Smith, R. P., & Gosselin, R. E. (1974). Toxicological studies on the avicide 3-Chloro-p-Toluidine. *Toxicology and Applied Pharmacology, 28*, 110-125.
[102] Decino, T. J., Cunningham, D. J., & Schafer, E. W. (1966). Toxicity of DRC-1339 to starlings. *Journal of Wildlife Management, 30,* 249-253.
[103] Dhami, M. K. (2009). Review of the biology and ecology of the Common Myna (*Acridotheres tristis*) and some implications for management of this invasive species. Maanaki Whenua Landcare Research, Canterbury, New Zealand. http://www.indianmynaaction.org.au/documents/New%20Zealand%20paper.pdf
[104] Feare, C. J. & Mungroo, Y. (1990). The status and management of the House Crow *Corvus splendens* (Viellot) in Mauritius. *Biological Conservation 51*, 63-70.
[105] Puttoo, M., & Archer, T. (2003). Control and/or eradication of Indian crows (*Corvus splendens*) in Mauritius. National Parks and Conservation Service. Mauritius.
[106] Ryall, C. (2010). Further records and updates of range expansion in house crow *Corvus splendens*. *Bulletin of the British Ornithologists' Club, 130*, 246-254.
[107] Suleiman, A. S. & Taleb. N., (2010). Eradication of the House Crow *Corvus splendens* on Socotra, Yemen. *Sandgrouse 32*, 136–140.
[108] Buck, W. B. (1991). Toxicity of pesticides in livestock. In D. Pimental (Ed.) *CRC Handbook of Pest Management in Agriculture, Vol. II.* (pp. 571-587). CRC Press, Inc., Boca Raton, FL.

[109] Baker, S. E., Ellwood, S. A., Johnson, P. J., Macdonald, D. W. (2016). Moles and Mole Control on British Farms, Amenities and Gardens after Strychnine Withdrawal. *Animals*, 6, 39.
[110] Davies, C. (2006). The death of strychnine. *Pesticide News, June 2006*, 7.
[111] Buckle, A. P. & Eason C. T. (2015). Control methods: Chemical. In A. P Buckle & R. H. Smith (Eds.), *Rodent pests and their control* (2nd Ed., pp. 123–154). Wallingford: CAB International.
[112] Miller, J. E. (2018). Muskrats. Wildlife Damage Management Technical Series. 14. USDA: APHIS. https://digitalcommons.unl.edu/nwrcwdmts/14
[113] Nelson, P. C. (1994). Bird Control in New Zealand using Alpha-chloralose and DRC-1339. *Vertebrate Pest Conference 16,* 259–264.
[114] Parkes, J. & Avarua, R. (2006) Feasibility plan to eradicate Common Mynas *(Acridotheres tristis)* from Mangaia Island, Cook Islands. Landcare Research, Lincoln, New Zealand.
[115] Canning, G. (2011). Eradication of the invasive common myna, *Acridotheres tristis*, from Fregate Island, Seychelles. *Phelsuma 19*, 43-53.
[116] Avery, M. L., & Eisemann, J. D. (2014). Invasive Myna Control in American Samoa. *Proceedings of the Vertebrate Pest Conference, 26,* 140-144.
[117] Parkes, J. (2012). Review of best practice management of common mynas (*Acridotheres tristis*) with case studies of previous attempts at eradication and control: A working document. Contract Report LC986, prepared for Durrell Wildlife Conservation Trust, Landcare Research, Lincoln, New Zealand.
[118] Kuo, P-C., (1982). Solving tree squirrel debarking problems in Taiwan—a review. *Proceedings of the Vertebrate Pest Conference, 10,* 88-89.
[119] Kuo, P-C., & Liao, Y-K. (1988). A five-year evaluation of the silvicultural treatments for the control of squirrel damage in Taiwan. *Proceedings of the Vertebrate Pest Conference, 12,* 205-208.
[120] Pepper, H. W. (1990). Grey squirrel damage control with warfarin. Forestry Commission Research Information Note 180, HMSO, London.
[121] Adriaens, T., Baer, K., Breyne, P., Casaer, J., Devisscher, S., Onkelinx, T. et al., (2015) Successful eradication of a suburban Pallas's squirrel *Callosciurus erythraeus* (Pallas 1779) (Rodentia, Sciuridae) population in Flanders (northern Belgium). *Biolological Invasions. 17*, 2517–2526.
[122] Veron, G., Patou, M-L., Pothet, G., Simberloff, D., & Jennings, A.P. (2006). Systematic status and biogeography of the Javan and small Indian mongooses (Herpestidae, Carnivora). *Zoologica Scripta, 2006*, 1-10.
[123] Woodworth, J. R. & Woodside, D. H. (1953) Mongoose poison experiment. Territory of Hawaii Division of Fish and Game, unpublished report. 18pp.
[124] Pimentel, D. (1955). The control of the mongoose in Puerto Rico. *American Journal of Tropical Medicine and Hygiene 4*, 147-151.
[125] Kridler, E. (1965). *Experimental poisoning of the Hawaiian mongoose with thallium sulfate*. U.S. Fish and Wildlife Service, Honolulu, Hawaii. Unpublished report. 12 pp.
[126] Everard, C. O. & Everard, J. D. (1992). Mongoose rabies in the Caribbean. *Annals of the New York Academy of Sciences, 653,* 356-366.
[127] Roy, S. S., Jones, C. G. & Harris, S. (2002). An ecological basis for control of the mongoose *Herpestes javanicus* in Mauritius: is eradication possible? In: C. R. Veitch, & M. N. Clout (Eds.), *Turning the tide: the eradication of invasive species* (pp. 266-273). IUCN SSC Invasive Species Specialist Group. IUCN, Gland, Switzerland and Cambridge, UK.
[128] Keith, J. O., Hirata, D. N., & Espy, D. L. (1985). Control of mongoose predation on endangered Hawaiian birds. Unpublished progress report. USFWS Denver Wildlife Research Center, 37 pp.
[129] Keith, J. O., Hirata, D. N., & Espy, D. L. (1986). Control of mongoose predation on endangered Hawaiian birds. Unpublished progress report. USDA APHIS Denver Wildlife Research Center. 24pp.
[130] Keith, J. O., & Hirata, D. N. (1988a). Determination of an acute, oral LD50 for diphacinone against mongooses (*Herpestes auropunctatus*). Unpublished final report. U.S. Department of Agriculture, Animal and Plant Health Inspection Service, Denver Wildlife Research Center,USA
[131] Keith, J. O., & Hirata, D. N. (1988b). Laboratory trials to determine mortality of mongooses (*Herpestes auropunctatus*) fed 0.00025% diphacinone bait. Unpublished final report. U.S. Department of Agriculture, Animal and Plant Health Inspection Service, Denver Wildlife Research Center, USA
[132] Keith, J. O., Espy, D. L., & Hirata, D. N. (1988). Small field efficacy trials using diphacinone bait against mongooses (*Herpestes auropunctatus*). Unpublished final report. U.S. Department of Agriculture, Animal and Plant Health Inspection Service, Denver Wildlife Research Center, USA
[133] Keith, J. O., Hirata, D. N., Espy, D. L., Greiner, S., & Griffin, D. (1989). Field Trials to determine efficacy of diphacinone (0.00025%) bait on controlling mongoose predation of endangered Hawaiian birds. Unpublished final report, QA-16. U.S. Department of Agriculture, Animal and Plant Health Inspection Service, Denver Wildlife Research Center, USA.
[134] Keith, J. O., Hirata, D. N., Espy, D. L., Greiner, S., & Griffin, D. (1990). Field evaluation of 0.00025% diphacinone bait for mongoose control in Hawaii. Unpublished Final report USDA/APHIS/Denver Wildlife Research Center, 49pp.
[135] Berentsen, A. R., Pitt, W. C., & Sugihara, R. T. (2018). Ecology of the small Indian mongoose (*Herpestes auropunctatus*) in North America. In W. C. Pitt, J. C. Beasley, & D. W Witmer, (Eds.). *Ecology and Management of terrestrial vertebrate invasive species in the United States* (pp. 251-267. CRC Press, Boca Raton, Florida, US.

[136] Barun, A., Hanson, C. C., Campbell, K. J., & Simberloff, D. (2011). A review of small Indian mongoose management and eradications on islands. In C. R. Veitch, M. N. Clout, & D. R. Towns, (Eds.). *Island invasives: eradication and management* (pp. 17-25). IUCN, Gland, Switzerland.
[137] Creekmore, T. E., Linhart, S. B., Corn, J. L., Whitney, M. D., Snyder, B. D., & Nettles, V. F. (1994). field evaluation of baits and baiting strategies for delivering oral vaccine to mongooses in Antigua, West Indies. *Journal of Wildlife DIseases, 30*, 497-505.
[138] Jesse, D., Blanton, J. D, Meadows, A., Murphy, S. M., Manangan, J., Hanlon, C. A., Faber, M-L., Dietzschold, B., & Rupprecht, C.E. (2006). Vaccination of small Asian mongoose (*Herpestes javanicus*) against rabies. *Journal of Wildlife Diseases, 42*, 663–666.
[139] Scalera, R., & Zaghi, D. (2004). *Alien species and nature conservation in the EU: The role of the LIFE program*. European Commission, Office for Official Publications of the European Communities: 56 pp.
[140] Poole, J. R., Sauey, B. W., Amberg, J. J., & Bajer, P. G. (2018) Assessing the efficacy of corn-based bait containing antimycin-a to control common carp populations using laboratory and pond experiments. *Biological Invasions, 20*, 1809–1920.
[141] Bajer, P. G., Ratna, Ghosal, R., Maselko, M., Smanski, M. J., Lechelt, J. D., Hansen, G., & Kornis, M. S. (2019). Biological control of invasive fish and aquatic invertebrates: a brief review with case studies. *Management of Biological Invasions, 10*, 227–254.
[142] Britton, J. R., Cucherousset, J., Davies, G. D. *et al.* (2010). Non-native fishes and climate change: predicting species responses to warming temperatures in a temperate region. *Freshwater Biology 55*, 1130–1141.
[143] Snow, N., & Witmer, G. (2010). American Bullfrogs as invasive species: a review of the introduction, subsequent problems, management options, and future directions. *Proceedings of the Vertebrate Pest Conference, 24,* 86–89.
[144] Mankad, A., Kennedy, U. & Carter, L. (2019). Biological control of pests and a social model of animal welfare. *Journal of Environmental Management, 247*, 313–322. https://www.sciencedirect.com/science/article/pii/S0301479719308813?via%3Dihub
[145] Moriarty, A. (2004). The liberation, distribution, abundance and management of wild deer in Australia. *Wildlife Research, 31*, 291–299.
[146] Daniel, M. J. (1966). Early trials with sodium monofluoroacetate (compound "1080") for the control of introduced deer in New Zealand. New Zealand Forest Research Institute Technical Paper No. 51, 4.
[147] Fraser, K. W. & Sweetapple, P. J. (2000). A comparison of the effectiveness of two toxic loadings (0.08% and 0.15%) for control of deer during aerial 1080 poisoning using carrot baits. Landcare Research Contract Report LC9900/84 (unpubl.). 22 p.
[148] Batcheler, C. L., & Challies, C. N. (1988). Loss of compound 1080 (sodium monofluoroacetate) from carbopol gel smeared on foliage to poison deer. *New Zealand Journal of Forestry Science, 18*, 109-15.
[149] Burnam, J., & Mengak, M. T. (2007). Managing wildlife damage: Nutria (*Myocaster coypus*). WSFNR Wildlife Management Series No. 12. 6 pages.
[150] Leblanc, D. J. (1994). Nutria. In S . E. Hygnstrom, R. M. Timm, & G. E. Larsen (Eds.), *Prevention and Control of Wildlife Damage*. (pp. B71-B80).Nebraska Cooperative Extension Service, University of Nebraska Lincoln.
[151] Mach, J. J. (2002). Nutria control in Louisiana. *Proceedings of the Vertebrate Pest Conference 20*, 32-39.
[152] Morin, M. F., Merler, N., Naulleau, G., & Dore, M. (1990). Primary toxicity of bromadiolone on the coypu. *Bulletin of Environmental Contamination and Toxicology, 44*,595-601.
[153] Mougenot, C., & Roussel, L. (2005). To poison or to trap? The ecologisation of “pest” control. *Sociologie Ruralis, 45*, 115-129.
[154] Barrat, J., Richomme, C., & Moinet, M. (2010). The accidental release of exotic species from breeding colonies and zoological collections. *Revue Scientifique et Technique-Office International des Epizooties, 29*, 113-122.
[155] Gosling, L. M. & Baker, S. J. (1989). The eradication of muskrats and coypus from Britain. *Biological Journal of the Linnean Society, 38*, 39-51
[156] Bertolino S., & Viterbi, R. (2010). Long-term cost-effectiveness of coypu (*Myocastor coypus*) control in Piedmont (Italy). *Biological Invasions 12*, 2549-2558.
[157] Cliquet, F., Guiot, A. L., Munier, M., Bailly, J., Rupprecht, C. E., & Barrat, J. (2006) Safety and efficacy of the oral rabies vaccine SAG2 in raccoon dogs. *Vaccine, 24*, 4386–4392.
[158] Miller, J. E. (1974). Muskrat control and damage prevention. *Proceedings of the Vertebrate Pest Conference*, *6*, 85-89.
[159] Miller, J. E. (2018). Muskrats. Wildlife Damage Management Technical Series. 14. USDA: APHIS. https://digitalcommons.unl.edu/nwrcwdmts/14
[160] van Melckebeke, J. (1986). Muskusrattenverdelging in België. *Muskusrat and Beheer*, *6*, 19–21.
[161] Barends, F. (2002). The muskrat (*Ondatra zibethicus*): expansion and control in the Netherlands. *Lutra*, *45*, 97–104.
[162] Robertson, P. A., Adriaens, T., Lambin, X., Mill, A., Roy, S., Shuttleworth, C. M., Sutton-Croft, M. (2016) The large-scale removal of mammalian invasive alien species in Northern Europe. *Pest Management Science, 73*, 273–279.
[163] Bos, D. (2017). Information on measures and related costs in relation to species included on the Union list: Ondatra zibethicus. Technical note prepared by IUCN for the European Commission.

[164] Mazaubert, E. (2016). Coypu Controlling populations of harmful aquatic rodents (coypu and muskrats) in the Loire-Atlantique department. GROUPE DE TRAVAIL NATIONAL INVASIONS BIOLOGIQUES EN MILIEUX AQUATIQUES. http://www.gt-ibma.eu/wp-content/uploads/2016/10/Myocastor-coypus2.pdf
[165] Stuyck, J. (2008). Muskusrattenbestrijding in Vlaanderen. Bevalt de nieuwe aanpak. *Zoogdier 19* (3).
[166] Bos, D., Klop, E., Hemert, H. van, LaHaye, M., Hollander, H., Loon, E. van, & Ydenberg, R. (2016). Beheer van Muskusratten in Nederland. Effectiviteit van bestrijding op grond van historie en een grootschalige veldproef. Deel 1 - Samenvatting tussenrapportage en Deel 2 Achtergrond studies (No. A&W rapport 2191). Veenwouden: Altenburg and Wymenga ecologisch onderzoek.
[167] Bos, D., Kentie, R., La Haye M., & Ydenberg, R.C. (2019). Evidence for the effectiveness of controlling muskrat (*Ondatra zibethicus L.)* populations by trapping. *European Journal of Wildlife Research 65*, 45.
[168] VMM. (2010). Ratten op Vlaamse wijze. Erembodegem: Vlaamse Milieumaatschappij-VMM. https://www.vmm.be/publicaties
[169] Scalera, R. & Zaghi, D. (2004). Alien species and nature conservation in the EU. (Ed. B. Julien) European Commission ISBN: 92-894-6022-9
[170] Schreier, T. M., Dawson, V. K. & Larson, W. (2008). Effectiveness of piscicides for controlling round gobies (*Neogobius melanostomus*). *Journal of Great Lakes Research, 34*, 253–264.
[171] Perry, B. D., Garner, N., Jenkins, S. R., McCloskey, K. & Johnston, D. H. (1989). A study of techniques for the distribution of oral rabies vaccine to wild raccoon populations. *Journal of Wildlife Diseases, 25*, 206-217.
[172] UNEP-WCMC (2010). Review of the Grey Squirrel *Sciurus carolinensis*. UNEP-WCMC, Cambridge.
[173] Wood, D. A. & Phillipson, J. (1977). The utilisation of poison hoppers designed for grey squirrel (*Sciurus carolinensis*) control. *Biological Conservation*, *11*, 119– 127.
[174] Pepper, H. W. (1989). Hopper modification for grey squirrel control. Forestry Commission research information note 153. Forestry Commission, Edinburgh, UK.
[175] Pepper, H. W., & D. Stocker. D. (1993). Grey squirrel control using modified hoppers. Forestry Commission research information note 232. Forestry Commission, Edinburgh, UK.
[176] Gurnell, J., & Pepper, H. (1988). Perspectives on the management of red and grey squirrels. In D. C. Jardine (Ed.), *Wildlife management in forests,* (pp. 92-109). ICF, Edinburgh.
[177] Pepper, H., & F. Currie, F. (1998). Controlling grey squirrel damage to woodland. Forestry Commission practice note 4. Edinburgh, UK.
[178] Mayle, B. A., & Broome, A. C. (2013). Changes in the impact and control of an invasive alien: the grey squirrel (*Sciurus carolinensis*) in Great Britain, as determined from regional surveys. *Pest Management Science, 69,* 323–333.
[179] Williams, F., Eschen, R., Harris, A., Djeddour, D., Pratt, C., Shaw, R. H., Varia, S., Lamontagne-Godwin, J., Thomas, S. E., Murphy, S. T. (2010). *The economic cost of invasive non-native species to Great Britain*. CABI Proj No VM10066, pp. 1–99. CABI Publishing, Wallingford.
[180] Barr, J. J. F., Lurz, P. W. W., Shirley, M. D. F., & Rushton, S. P. (2002). Evaluation of immunocontraception as a publicly acceptable form of vertebrate pest species control: the introduced grey squirrel in Britain as an example. *Environmental Management*, *30*, 342–351.
[181] Dunn, M., Marzano, M., Forster, J., & Gill, R. M. A. (2018). Public attitudes towards “pest” management: perceptions on squirrel management strategies in the UK. *Biological Conservation, 222*, 52-63.
[182] Buckle, A. P. & Pelz, H. J. (2015). Rodent control in practice: temperate field crops and forestry. In A. P Buckle & R. H. Smith (Eds.) *Rodent pests and their control* (2nd Ed., pp. 247–268). Wallingford: CAB International.
[183] Gill, R. (2019). *Controlling grey squirrels in forests and woodlands in the UK*. UK Forestry Standard Technical Note ISBN: 978-1-83915-004-3.
[184] Schuchert, P., Shuttleworth, C. M., McInnes C. J., Everest D. J. & Rushton, S. P. (2014). Landscape scale impacts of culling upon a European grey squirrel population: can trapping reduce population size and decrease the threat of squirrelpox virus infection for the native red squirrel? *Biological Invasions, 16*, 2381–2391 (2014).
[185] Shuttleworth, C. M., Schuchert, P., Everest, D. J., McInnes, C. J., Rushton, S. P., Jackson, N. L. *et al.*, (2015). Developing integrated and applied red squirrel conservation programmes: what lessons can Europe learn from a regional grey squirrel eradication programme in North Wales?. C. M. Shuttleworth, P. W. W. Lurz & M. W. Hayward (Eds.), *Red Squirrels: Ecology, Conservation and Management in Europe*, (pp. 233-250). European Squirrel Initiative, Woodbridge, UK.
[186] Bertolino, S., Shuttleworth, C.M., Lurz, P.W.W., Shuttleworth, C.M., Martinoli, A., Wauters, L.A., (2016). The management of grey squirrel populations in Europe: evolving best practice. In, C. Shuttleworth, P. Lurz, P., & Gurnell, J. (Eds.), *The Grey Squirrel: Ecology & Management of an Invasive Species in Europe.* (pp 495-514). European Squirrel Initiative, UK.
[187] Frey, J. K., Iglesias, J., & Herman, K. (2013). Eastern fox squirrel (*Sciurus niger*): new threat to pecan orchards in far west Texas. *Western North American Naturalist Vol. 73*, No. 3, Article 11.
[188] Marsh R. E. (1994). Current (1994) ground squirrel control practices in California. *Proceedings of the Vertebrate Pest Conference*, 16, 95-98.
[189] Proulx, G. & Walsh, K. (2007). Effectiveness of aluminum phosphide, strychnine and chlorophacinone to control Richard-son’s ground squirrels (*Spermophilus richardsonii*) in spring in southern Saskatchewan. Alpha Wildlife Research & Management Ltd. report to Pest Management Regulatory Agency, Ottawa, ON.

[190] Proulx, G., Walsh,K., MacKenzie, N. & MacKenzie, K. (2009). Assessment of the effectiveness of Rozol®, Phostoxin®, Strychnine, RoCon®, and various treatments to control Richardson's ground squirrels (*Spermophilus Richardsonii*) in southern Saskatchewan, in spring and summer 2008. Alpha Wildlife Research & Management Ltd. report to Saskatchewan Agriculture Development Fund, Regina, SK.
[191] Proulx, G. (2011). Field evidence of non-target and secondary poisoning by strychnine and chlorophacinone used to control Richardson's ground squirrels in southwest Saskatchewan. *Proceedings of the Ninth Prairie Conservation of Endangered Species Conference*, 128–134.
[192] Smits R. R., van Horssen, P., van der Winden, J. (2010). A risk analysis of the sacred ibis in The Netherlands Including biology and management options of this invasive species. Bureau Waardenburg bv / Plantenziektenkundige Dienst, Ministerie van LNV.
[193] Lacomba, J. I. (2013). Demonstration strategy and techniques for the eradication of invasive freshwater turtles. LIFE TRACHEMYS (LIFE09 NAT/ES/000529)
[194] Pauli, B. D., Money, S. (2000) Ecotoxicology of pesticides in reptiles. In D.W. Sparling, G. Linder, & CA. Bishop (Eds), *Ecotoxicology of Amphibians and Reptiles.* (Pp 269–289), SETAC Press, Pensacola.
[195] Broom, D. M. (1999). The welfare of vertebrate pests in relation to their management. In D.P. Cowan & C.J. Feare (Eds.), *Advances in Vertebrate Pest Management* (pp. 309-329). Filander Verlag: Furth, Germany.
[196] Littin, K. E., Mellor, D. J., Warburton, B., & Eason, C. T. (2004). Animal welfare and ethical issues relevant to humane control of vertebrate pests. *New Zealand Veterinary Journal, 52*, 1–10.
[197] Meerburg, B. G., Brom, F. W., Kijlstra, A. (2008). The ethics of rodent control. *Pest Management Science, 64*, 1205–1211.
[198] Cowan, P., Warburton, B., & Fisher, P. (2011). Welfare and ethical issues in invasive species management. *European Vertebrate Pest Management Conference, 8*, 44-45.
[199] Dubois, S., Fenwick, N., Ryan, E. A., Baker, L., Baker, S. E., Beausoleil, N. J., et al. (2017). International consensus principles for ethical wildlife control. *Conservation Biology 31*, 753–760. doi: 10.1111/cobi.12896.
[200] Beausoleil N. J., Fisher P., Mellor D. J., & Warburton B. (2012). Ranking the negative impacts of wildlife control methods may help to advance the Three Rs. *ALTEX Proc., 1 (WC8)*, 481–485.
[201] EPA (Environment Protection Agency of the United States). (1995) R.E.D. (Reregistration Eligibility Decision) facts. Starlicide (3-chlorop-toluidine hydrochloride). www.epa.gov/oppsrrd1/REDs/factsheets/2610f act.pdf
[202] Fajt, J.R (1997). *Method of fish management by poison fish bait method of making the bait, and formulation of bait.* US Patent 5,674,518,1997
[203] PSD (Pesticide Safety Directorate) 1997 *Assessment of Humaneness of Vertebrate Control Agents -Evaluation of Fully Approved or Provisionally Approved Products, No.* 171 *(December* 1997). Pesticides Safety Directorate: York, UK.
[204] Cowan, P., & Warburton, B. (2011). Animal welfare and ethical issues in island pest eradication. In C.R. Veitch, M. N. Clout, D. R. Towns, (Eds.), *Island Invasives: Eradication and Management* (pp. 418-421). IUCN: Gland, Switzerland.
[205] Fisher, P., Campbell, K. J., Howald, G. R., & Warburton B. (2019). Anticoagulant rodenticides, islands, and animal welfare accountancy. *Animals, 9*, 919; doi:10.3390/ani911091.
[206] Sandøe, P. & Gamborg, C. (2017) Animal welfare impact assessments: a good way of giving the affected animals a voice when trying to tackle wild animal controversies? *Journal of Agricultural and Environmental Ethics*, 30, 571–578.
[207] Gibson, T. J., & Jackson, E. L. (2017). The economics of animal welfare. *Revue scientifique et technique (International Office of Epizootics), 36*, 125-135.
[208] European Commission (1998) Council Directive 98/8/EC of the European Parliament and of the Commission of 16 February 1998 concerning the placing of biocidal products on the market. Official Journal of the European Communities L 123(1):63.
[209] Paparella, M. (2006). Rodenticides-an animal welfare paradox? *ALTEX Alternatives to Animal Experimentation, 23*, 51–52.

Appendix 15. Shooting

1. Measure name			
1.1. English:	Shooting		
1.2. Lethal or non-lethal:	Lethal		
1.3. Other languages (if available):			
Bulgarian	Отстрел при лов	Italian	Abbattimento
Croatian	Pucanje	Latvian	Nošaušana
Czech	Lov střelnou zbraní	Lithuanian	Šaudymas
Danish	Skydning	Maltese	
Dutch	Afschot	Polish	Odstrzał
Estonian	Laskmine	Portuguese	Tiro
Finnish	Ampuminen	Romanian	Vânătoare cu arme de foc
French	Tir	Slovak	Lov strelnou zbraňou - poľovníctvo
German	Schießen	Slovenian	Streljanje - lov
Greek	Πυροβολισμός - Κυνήγι	Spanish	Disparo
Hungarian	Lelövés	Swedish	Skytte
Irish			

2. Technical details of measure
2.1.a. Measure description
Shooting as a wildlife management tool involves the use of various designs of firearms as a lethal method of control, and can be undertaken by trained professional staff, and in some cases also by sport/recreational hunters. The types of weapon used for wildlife control typically include rifles and shotguns, although air powered weapons and pistols are also used in some circumstances. Firearms are available in a wide variety of calibres and gauges, single or multiple shot designs, and can be used with a range of types of ammunition. Firearms are also used by personnel actively searching for or pursuing an animal, by others using a static location such as a high seat, or used from a vehicle, with further examples of their application from trucks, boats, and helicopters [1]. The nature of the species being controlled also influences the choice of weapon, ammunition or mode of shooting. More powerful weapons are used on larger species and are more likely to ensure a humane kill [2], small or more mobile species such as birds may favour the use of shotguns which produce a spread of small pellets rather than a single bullet. Shooting is widely used as a control method, particularly for alien birds and mammals, but also reptiles and amphibians. It provides a selective method that can kill animals at a distance, a unique characteristic of this method. This feature makes it particularly useful for species that may not be readily trapped or captured, or those animals within a population that develop trap-shyness. Shooting has provided the main method used in a number of successful eradications and is a key component of many widescale control programmes.

The ownership and use of firearms are strongly controlled in most Member States, with various limits on the type and size of weapon, ammunition and number of shots that the weapon may carry without reloading. Most Member States require firearms to be registered, securely kept, and may require formal training before licenses are issued. Given the safety issues and public concern associated with the use of firearms, many Member States also place restrictions on the sites where they can be used, often including limits to their use near roads or buildings.

As well as the use of firearms as a control method, their use in hunting for sport is a widespread activity. This brings a range of traditions, societies, legislation and ownership issues associated with land access, the numbers of animals that may be killed or the seasons when this can take place. While both control and sports hunting involve the lethal use of firearms, their objectives are different and those measures designed to conserve species from over-hunting, may differ from the approaches used for control, for example the control of species during what sport hunters would consider the close, or protected season. For example, hunting includes restrictions, traditions and measures to ensure the level of removal should not compromise numbers in future years, while for eradication the objective is to reduce the population to zero as quickly as possible. The ongoing programme to remove the ruddy duck from Europe is an example of this, where control has included shooting throughout the year [3] which is in contrast to the close season and winter only hunting for other wildfowl species. In some cases alien species may also be hunted, or were even introduced for the purpose of hunting, so balancing the concerns of hunters with the objectives of control can be an issue. This does not apply to all AIS, which include many species which would not normally be hunted. To reduce the potential conflicts between hunting and the objectives of eradication, some alien species eradication programmes employ dedicated control operatives, but hunters also provide a large and experienced pool of people with skills in the use of firearms and of the animals, and can form part of control programmes.

Firearm ammunition has traditionally been based on lead projectiles and weapons have been designed around the mass and ductile nature of this material. However, there are increasing concerns regarding the toxicological effects of lead from firearms, both as an environmental contaminant, particularly over wetlands, and the consequences of lead inclusion within the carcass of shot animals, for human health [4] and natural food webs [5]. A variety of approaches have been taken to reduce these risks, including the replacement of lead with other metals for use in particular circumstances. However, this is an ongoing issue and risks remain.

Against this background, many Member States produce lists of weapons, ammunition and modes of shooting considered appropriate or approved for use on different species. This includes descriptions of the type of weapon (e.g. rifles, pistols, shotguns, air weapons), their power (e.g. calibre or bore), appropriate ammunition (e.g. solid shot, bullets, shotgun cartridges), mode of shooting (e.g. from a high seat, from a vehicle), time (e.g. day or night using night vision/thermal imaging, close seasons) and location (e.g. away from roads or buildings) and that may be licensed to shoot particular species. The use of shooting as a control method needs to be viewed against this background and the limits will vary by Member State and by species. These need to be reviewed on an individual basis and licenses may be required to use firearms for the control of IAS.

Following the approach adopted internationally for the welfare testing of kill-traps, Hampton et al propose a four-step testing process for new shooting (ballistic) technologies to ensure their effectiveness and humaneness.

2.1.b. Integration with other measures

It is also regularly used in combination with other methods such as traps [6,7,8] given the restrictions on the use of shooting on some sites, and the changing cost-effectiveness of different methods as animal density changes. Shooting can also be used in association with Judas animals and in association with dogs to help track and locate the animals [9]. For programmes where trapping is the principle control method, shooting may also be needed for the control of that proportion of the population that become trap shy. Given its flexible nature, shooting can be used alongside many other control methods, for example pond-draining to control bullfrogs or turtles [8,10,11].

2.2.a. Availability - species and objectives										
Objective	Unknown objective		Rapid Eradication		Management					
					Eradication		Control		Containment	
Species	Avail.	Ref(s).	Avail.	Ref(s).	Avail.	Ref(s).	Avail.	Ref(s).	Avail.	Ref(s).
Acridotheres tristis			A	12	P	12	A	47	P	
Alopochen aegyptiaca			A		A		A	8, 13, 27, 28	A	8, 13
Callosciurus erythraeus			A		A		A		A	
Corvus splendens			A	8	A	8	P		P	
Herpestes javanicus							A		A	
Lepomis gibbosus										
Lithobates catesbeianus			P		A	8	A	8,51,52	A	8
Muntiacus reevesi			A	8	A	8	A	8	A	8
Myocastor coypus			A	7	A	7	A	7, 29, 30, 31	A	7
Nasua nasua			A		A		P		P	
Nyctereutes procyonoides			A	9	A	9	A	9, 29, 32	A	9
Ondatra zibethicus			A	26	A	26	A	26, 29	A	26
Oxyura jamaicensis			A	3	A	3	A	3, 27	A	3
Perccottus glenii										
Plotosus lineatus										
Procyon lotor			A		A		A	6, 29, 33, 34	A	6
Pseudorasbora parva										
Sciurus carolinensis			A		A		A	14	A	14
Sciurus niger			P		P		A		A	
Tamias sibiricus			P		P		P		P	
Threskiornis aethiopicus			A	13	A	13	A	13	A	13
Trachemys scripta			P		P		A	8,10,11,35	A	8,10,11

2.2.b. Application – EU Member States and objectives										
Objective	Unknown objective		Rapid Eradication		Management					
					Eradication		Control		Containment	
Country	Avail.	Ref(s).	Avail.	Ref(s).	Avail.	Ref(s).	Avail.	Ref(s).	Avail.	Ref(s).
Austria										
Belgium			X	8	X	8	X	8	X	8
Bulgaria										
Croatia										

Cyprus										
Czech Republic										
Denmark							X	36,37,38, 39,40	X	36,37,38,39, 40
Estonia										
Finland										
France			X	13	X	13				
Germany							X	41,42,43, 44	X	41,42,43,44
Greece										
Hungary										
Ireland					X	15				
Italy			X	7	X	7	X	7	X	7
Latvia										
Lithuania										
Luxembourg							X	45,46	X	45,46
Malta										
Netherlands										
Poland										
Portugal										
Romania										
Slovakia										
Slovenia										
Spain					X	15	X	47		
Sweden			X	26	X	9	X	9	X	9
United Kingdom*					X	15				

* Not an EU Member State

3. Humaneness of the measure			
3.1. Welfare for all measures			
Measure type (if applicable):	**Humaneness impact categories**		
Domain:	**No impact**	**Mild-Moderate**	**Severe - Extreme**
1: Water deprivation, food deprivation, malnutrition	Shooting does not involve the capture or restraint of animal and its use should not be associated with these concerns.		
2: Environmental challenge	Shooting does not involve the capture or restraint of animal and its use should not be associated with these concerns.		
3: Injury, disease, functional impairment	If used appropriately, shooting should result in a rapid instantaneous death for the majority of animals [49, 50], with no consequences for their welfare.		Shooting carries the risk of wounding with potentially severe consequences for the welfare of the animal concerned. Studies [16] have assessed rates of potential wounding by hunters shooting at targets. They conclude that rifles 'killed' better than shotguns and 'wounded' less. There was no regime that had no probability of 'wounding'; however, the latter varied dramatically across the trials with different types of gun, ammunition and shooters' skill level. Other studies [17] document the reported outcomes of 2281 shots fired at deer by hunters in the UK. Overall, 96% of deer were hit, of which 93% were killed outright by the first shot. Similar studies of

			rabbit shooting found a first-shot wounding rate of 12% [18]. This risk can be reduced by the selection of appropriate firearms and ammunition, the training of the shooters, and measures to ensure wounded animals are followed and despatched with a second shot.
4: Behavioural, interactive restriction	Shooting does not involve the capture or restraint of animal and its use should not be associated with these concerns.		
5: Anxiety, fear, pain, distress, thirst, hunger etc.	If used appropriately, shooting should result in a rapid instantaneous death for the majority of animals [49, 50], with no consequences for their welfare.	If the animal is chased as part of the process, or other animals are disturbed by this activity, they may experience mild to moderate stress.	If other animals observe the shooting of another member of their own species, they may suffer stress which, in some circumstances, could be considered severe, for example if a highly sentient species sees its offspring or parent killed. However, more evidence is needed to assess animal's perception of death and how this varies between species [19].

3.2. Mode of death (if relevant)

Measure type (if applicable):	**Immediate death (i.e. no suffering)**	**Not immediate death (mild - moderate suffering)**	**Not immediate death (severe - extreme suffering)**
Rationale:	If used appropriately, shooting should result in a rapid instantaneous death for the majority of animals [49, 50], with no consequences for their welfare.		Shooting carries the risk of wounding with potentially severe consequences for the welfare of the animal concerned. This risk can be reduced by the selection of appropriate firearms and

			ammunition, the training of the shooters, and measures to ensure wounded animals are followed and quickly despatched with a second shot.

3.3. Humaneness summary	If used appropriately, shooting should result in a rapid instantaneous death for the majority of animals, with no consequences for their welfare. However, shooting carries the risk of wounding with potentially severe consequences for the welfare of the animal concerned. This risk can be reduced by the selection of appropriate firearms and ammunition, the training of the shooters, and measures to ensure wounded animals are followed and quickly despatched with a second shot.

4. Costs and effectiveness of the measure	
General effectiveness of the measure	Shooting is widely used as a wildlife management tool. This reflects its selective nature, its ability to control animals at a distance, and the flexibility offered by the range of weapons and applications available. Shooting has provided the main method for the control of the Ruddy Duck [3], Sacred Ibis ([13], and is widely used for the control of large alien mammals such as deer and coypu [7,8] It also contributes to the control of other smaller IAS such as bullfrogs and turtles [8,10,11].

4.1. Case studies	
CASE STUDY #1	
Measure type (if relevant):	Shooting
Species:	*Oxyura jamaicensis* - Ruddy Duck
Objective:	Eradication of a well-established and widespread population in the UK as part of a wider international strategy to remove this species from Europe [15].
Use of measure	Control was primarily through the use of firearms, principally .223 rifles and five-cartridge semi-automatic 12-guage shotguns. Shooting was conducted both from the shore and from boats, with small teams operating independently through most of the breeding season but with all of the control officers working together on the larger wintering sites. Published papers [3] provide a detailed description of the control strategy.
Combined with other measure(s):	Shooting was the main method used for the control of this species. A number of other methods were also used, including live trapping and egg-oiling, but these were considered much less effective than shooting and only small numbers of animals were controlled in this way [3].
Country(ies) of application:	This case study relates to the UK, but the larger programme of eradication from Europe has also included control in Spain, Ireland, France, Belgium and the Netherlands [15].

Geographic scale (km^2) and/or population size measure applied to:	Control was undertaken throughout the UK, an area of 242,000 km2. Considering activities across all six European states with established populations, these covered a combined area of 1.5m km2.
Time period:	Initial control trials in the UK began in 1993. Nationwide control was concentrated over the period 2005-2013. A low level of control has subsequently been maintained to prevent this species re-establishing a breeding population in the UK given the presence of birds in neighboring countries. Control in Spain, France, Belgium and the Netherlands is ongoing, but the total European population has been reduced by over 93% between 2000 and 2013 [15].
Effort:	Details of effort and costs are given in a review published by IUCN (2018). Between 2005 and 2011, the main period of control, the project employed eight full-time staff.
Costs:	Overall costs:
	Details of effort and costs are given in a review [20]. Between 2005 and 2011, the main period of control, the project cost € 3,770,771 (2018 prices). Between 2011 and 2018, the UK invested a further total of € 814,000 in ongoing control.
	Personnel costs:
	52.6% of the costs between 2005 and 2011 [20].
	Equipment and infrastructure:
	4.3% of the costs between 2005 and 2011 [20].
	Other, including overheads:
	36.5% including overheads, travel and subsistence costs, consumables e.g. running costs for vehicles, and bonus payments for staff [20].
Effectiveness:	Shooting is a highly effective method of controlling this species. The UK population was reduced from over 6,000 individuals in 2000 to around 20 by 2017 [20].

CASE STUDY #2	
Measure type (if relevant):	Shooting
Species:	*Threskiornis aethiopicus* - Sacred Ibis
Objective:	Rapid Eradication, Eradication
Use of measure	Control used a combination of shotguns, .22 long rifles and .222 Remingtons equipped with scopes and moderators. Wooden decoys were used at times to attract the birds ([13,21,22]. Shooting was undertaken by government agency officials (ONCFS) occasionally assisted by other authorised persons. Shooting took place at feeding sites, near to their breeding colonies and on the birds regular travel routes [13].
Combined with other measure(s):	Shooting was combined with the hand capture and trapping of adult birds, chicks and the removal of eggs at their nesting colonies and in parks, together with the use of a stupefying bait (Alpha-chloralose) [13].
Country(ies) of application:	France

Geographic scale (km²) and/or population size measure applied to:	Control measures applied in two regions of France, the Atlantic coast (Loire-Atlantique, Morbihan and Vendée departments) and the southern coast around the Mediterranean Basin (Departments of Aude, Hérault, Gard, Bouches-du-Rhône and Alpes-Maritimes). In 2006/7, prior to control, the Atlantic region contained 1,700 breeding pairs and a total population of 5,000 birds; the Mediterranean region included a minimum of 360 birds [13].
Time period:	The main period of control in the Atlantic region was between 2007-2013 [13].
Effort:	In the Mediterranean Basin, control included the assistance of at least 20 people [13].
Costs:	Overall costs:
	No overall costs for these programmes are available. Reports of costs per bird removed vary widely depending on the stage of the programme. For the Mediterranean Basin, during the early stages when the largest number of birds were removed, the reported costs were less than EUR 40 per bird. In the latter stages, when small number of birds were widely spread, costs per bird removed rose to EUR 700-1300 [13].
	Personnel costs:
	Not available
	Equipment and infrastructure:
	Not available
	Other, including overheads:
	Not available
Effectiveness:	On the Atlantic coast, over the period 2006-2013, 6626 birds were shot and 2720 nests controlled which reduced the population from 1700 pairs to 280-300, a reduction of 82%. In the Mediterranean Basin, 395 adults and 90 chicks were removed between 2007 and 2013, after which only three animals were thought to remain in the area. Control in both areas has continued, but the current status needs to be confirmed [13].

4.2. Costs effectiveness summary	Shooting with a combination of shotguns and rifles has provided the main method for the eradication of both ruddy duck and sacred ibis. The UK ruddy duck population was reduced from over 6,000 individuals in 2000 to around 20 by 2017 (IUCN 2018). Shooting was used to control Sacred Ibis numbers in two regions of France. Both populations were substantially reduced over a seven-year period and eradication appears a feasible objective. It is unlikely that these results could have been achieved without using shooting as the main control method. Shooting also provides the main method of control for large mammalian IAS such as deer and coypu [7,8], it has also been used to supplement the control smaller IAS such as squirrels, amphibians and reptiles [8,10,11].

5. Side effects	
Non-target native species, their habitats and the broader environment:	Positive:
	The use of firearms is highly selective and carries only a small risk to non-target species. Reports [3] document 29 non-target birds being killed during the programme to remove over 6,200 ruddy ducks, a non-target rate of less than 0.5%, mainly through having been hit by the back-pattern from shotguns (i.e. pellets that missed their intended target), although there were also two cases of mis-identification.
	Negative:
	Shooting, and associated activities such as the use of boats for the ruddy duck work, can cause disturbance to other bird species using a wetland. Case studies of the effects of ruddy duck shooting on Gadwalls and Shovelers found that these species left the disturbed site and flew to alternative sites within about 3 km. On the majority of occasions, most birds returned within one day of the cull, and sometimes before the end of the same day. The culls were not considered to have had a lasting negative impact upon either species [23]. The impact on disturbance on other bird species is thought to be more related to the frequency of disturbance rather than the intensity of a particular occasion. By managing the frequency of visits to particular sites and avoiding particularly sensitive periods such as the breeding season, the effects of disturbance can be minimised. Efforts to minimise disturbance associated with control was also a feature of the Sacred Ibis control operations in France [13]. The use of lead-based ammunition is a potential source of environmental contamination. The repeated use of lead shot in an area, particularly over water, can lead to environmental contamination, with significant consequences for other wildlife including waterbirds [24]. Alternative non-toxic ammunition is available [25], for example the ruddy duck programme used tungsten-matrix shot [3].
Other invasive alien species:	Positive:
	Negative:
Public health and well-being:	Positive:
	Negative:
	The use of firearms brings potential risks to the general public which need to be carefully managed. Due to these risks, the use of firearms may not be always be possible, for example in built up areas. Training in the safe use of firearms is essential, and for more powerful weapons can be a requirement for their use. The use of experienced shooters can greatly reduce the risks, for example the ruddy duck programme employed professional, trained staff [3] given the use of firearms in public places. The risks associated with their use can be reduced by the selection of appropriate weapons, for example shotguns carry lower risks of shot carrying over long distances compared to rifles. The calibre of the weapon should also be chosen to be appropriate for the species, balancing the need for a clean kill with the greater risks associated with larger, more powerful

	weapons. Knowledge of the area where shooting will take place will reduce the risks, for example, identifying safe fire zones and the presence of back-stops prior to undertaking control.
Economic:	Positive:
	In many cases shooting provides a cost-effective method of control. The skills needed are widely available, the capital costs are low and its flexible nature allows the level of effort to be closely controlled, unlike trapping where the need for trap checking brings on-going man-power needs.
	Negative:

6. Conclusion

Overall assessment of the measure (qualitative)

Shooting is widely used as a wildlife management tool and can be highly cost-effective. This reflects its selective nature, its ability to control animals at a distance, and the flexibility offered by the range of weapons and applications available. Shooting has provided the main method for the control of the Ruddy Duck [3]), Sacred Ibis [13] and is widely used for the control of large alien mammals such as deer and coypu [7,8]. It has also been used to supplement other methods for the control of reptiles and amphibians [8,10,11].

If used appropriately, shooting should result in a rapid death for the majority of animals, with only mild to moderate consequences for their welfare. Shooting carries the risk of wounding with potentially severe consequences for the welfare of the animal concerned. This risk can be reduced by the selection of appropriate firearms and ammunition, the training of the shooters, and measures to ensure wounded animals are followed and quickly despatched with a second shot.

Assessor:	Peter Robertson
Reviewer 1:	Riccardo Scalera
Reviewer 2:	Sandro Bertolino

7. References

1 Bayne, P., Harden, B., Pines, K. and Taylor, U., 2000. Controlling feral goats by shooting from a helicopter with and without the assistance of ground-based spotters. *Wildlife Research*, *27*(5), pp.517-523.

2 Hampton, J.O., Adams, P.J., Forsyth, D.M., Cowled, B.D., Stuart, I.G., Hyndman, T.H. and Collins, T., 2016. Improving animal welfare in wildlife shooting: the importance of projectile energy. *Wildlife Society Bulletin*, *40*(4), pp.678-686.

3 Henderson, I., 2009. Progress of the UK Ruddy Duck eradication programme. *British Birds*, *102*(12), p.680.

4 Pain, D.J., Cromie, R.L., Newth, J., Brown, M.J., Crutcher, E., Hardman, P., Hurst, L., Mateo, R., Meharg, A.A., Moran, A.C. and Raab, A., 2010. Potential hazard to human health from exposure to fragments of lead bullets and shot in the tissues of game animals. *PloS one*, *5*(4), p.e10315.

5 Pauli, J.N. and Buskirk, S.W., 2007. Recreational shooting of prairie dogs: A portal for lead entering wildlife food chains. *The Journal of Wildlife Management*, *71*(1), pp.103-108.

6 Fischer, M.L., Sullivan, M.J., Greiser, G., Guerrero-Casado, J., Heddergott, M., Hohmann, U., Keuling, O., Lang, J., Martin, I., Michler, F.U. and Winter, A., 2016. Assessing and predicting the spread of non-native raccoons in Germany using hunting bag data and dispersal weighted models. *Biological Invasions*, *18*(1), pp.57-71.

7 Panzacchi, M., Cocchi, R., Genovesi, P. and Bertolino, S., 2007. Population control of coypu *Myocastor coypus* in Italy compared to eradication in UK: a cost-benefit analysis. *Wildlife Biology*, *13*(2), pp.159-171.
8 Adriaens, T., Branquart, E., Gosse, D., Reniers, J., Vanderhoeven, S. 2019. Feasibility of eradication and spread limitation for species of Union concern sensu the EU IAS Regulation (EU 1143/2014) in Belgium. Report prepared in support of implementing the IAS Regulation in Belgium. Institute for Nature and Forest Research, Service Public de Wallonie, National Scientific Secretariat on Invasive Alien Species, Belgian Biodiversity Platform. DOI: https://doi.org/10.21436/17033333.
9 Dahl, F. & Åhlén, P.A., 2017. Information on measures and related costs in relation to species included on the Union list: *Nyctereutes procyonoides*. Technical note prepared by IUCN for the European Commission.
10 Fowler, J.F. & Avery, J.L., 1994. Turtles. Prevention and control of wildlife damage (Eds SE Hygnstrom and RM Timm), pp. F-27–F31. University of Nebraska, Lincoln, Nebraska.
11 Davis, J.T., 1976. Turtle control in farm ponds. Texas Agricultural Extension Service, Texas A & M University. College Station, Texas.
12 Feare, C.J., van der Woude, J., Greenwell, P., Edwards, H.A., Taylor, J.A., Larose, C.S., Ahlen, P.A., West, J., Chadwick, W., Pandey, S. and Raines, K., 2017. Eradication of common mynas *Acridotheres tristis* from Denis Island, Seychelles. *Pest Management Science*, *73*(2), pp.295-304.
13 Sarat, E., Mazaubert, E., Dutartre, A., Poulet, N. and Soubeyran, Y., 2015. Invasive alien species in aquatic environments. Practical information and management insights. Volume 2. Management insights. Onema. Knowledge for action series, 252 pp.
14 Central Science Laboratory, 2009. Review of methods of humane destruction of grey squirrels (*Sciurus carolinensis*). Scottish Natural Heritage Commissioned Report No. 317.
15 Robertson, P.A., Adriaens, T., Caizergues, A., Cranswick, P.A., Devos, K., Gutiérrez-Expósito, C., Henderson, I., Hughes, B., Mill, A.C. and Smith, G.C., 2015. Towards the European eradication of the North American ruddy duck. *Biological Invasions*, *17*(1), pp.9-12.
16 Fox, N.C., Blay, N., Greenwood, A.G., Wise, D. and Potapov, E., 2005. Wounding rates in shooting foxes (*Vulpes vulpes*). *Animal Welfare*, *14*(2), pp.93-102.
17 Aebischer, N.J., Wheatley, C.J. and Rose, H.R., 2014. Factors associated with shooting accuracy and wounding rate of four managed wild deer species in the UK, based on anonymous field records from deer stalkers. *PloS one*, *9*(10), p.e109698.
18 Hampton, J.O., Forsyth, D.M., Mackenzie, D. and Stuart, I., 2015. A simple quantitative method for assessing animal welfare outcomes in terrestrial wildlife shooting: the European rabbit as a case study. *Animal Welfare*, *24*(3), pp.307-17.
19 Monsó, S., 2019. How to tell if animals can understand death. *Erkenntnis*, pp.1-20.
20 IUCN, 2018. Compilation of costs of prevention and management of invasive alien species in the EU. Technical note prepared by IUCN for the European Commission.
21 Yésou, P. & Clergeau, P., 2005. Sacred Ibis: a new invasive species in Europe. *Birding World* 18 (12): 517-526.
22 Yésou, P., Clergeau, P., Bastian, S., Reeber, S. and Maillard, J.F., 2017. The Sacred Ibis in Europe: ecology and management. *British Birds*, *110*, pp.197-212.
23 Briggs, B., 2007.The use of waterbodies in South-West London by Gadwall and Shoveler: implications for nature conservation. Unpublished D. Phil thesis, University of Oxford.
24 Scheuhammer, A.M. and Norris, S.L., 1996. The ecotoxicology of lead shot and lead fishing weights. *Ecotoxicology*, *5*(5), pp.279-295.
25 Caudell, J.N., Stopak, S.R. and Wolf, P.C., 2012. Lead-free, high-powered rifle bullets and their applicability in wildlife management. *Human-Wildlife Interactions*, *6*(1), pp.105-111.
26. Annual report 2019 - Swedish raccoon dog project, including additional tasks on raccoon (NV-03794-15), muskrat (NV-01089-18), water turtles (Trachemys scripta S.p) and Egyptian goose (NV08788-18), and Sibirian chipmunk (NV-02057-19). https://jagareforbundet.se/contentassets/f110ce2f2e8643d083259c1d1d24d7f2/arsrapport-svenska-mardhundsprojektet-2019.pdf
27 Miljøstyrelsen. (2017). Handlingsplan mod invasive arter. https://mst.dk/media/143350/handlingsplan_invasive-arter_juni17.pdf
28 De Sousa, T. (2019). Plan d'action pour espèces exotiques envahissantes au Grand-Duché de Luxembourg: l'Ouette d'Égypte, Alopochen aegyptiacus (Linnaeus, 1766). In L. Administration de la nature et des forêts (Ed.), (13/12/2019 ed.).
29 Forvaltningsplan for mink, mårhund og vaskebjørn i Danmark (2020). https://mst.dk/media/191343/netversion-miljoestyrelsen_forvaltningsplan_2020-mink-maarhund-og-vaskebjoern.pdf
30 De Sousa, T. (2019). Plan d'action pour espèces exotiques envahissantes au Grand-Duché de Luxembourg: le Ragondin, Myocastor coypus (Molina, 1792). In L. Administration de la nature et des forêts (Ed.), (Vol. 20 pp).
31 Nutria – Management- und Maßnahmenblatt zu VO (EU) Nr. 1143/2014 (2018). https://neobiota.bfn.de/unionsliste/art-19-management.html
32 Marderhund – Management- und Maßnahmenblatt zu VO (EU) Nr. 1143/2014 (2019). https://neobiota.bfn.de/unionsliste/art-19-management.html
33 De Sousa, T. (2019). Plan d'action pour espèces exotiques envahissantes au Grand-Duché de Luxembourg: le Raton laveur, Procyon lotor (Linnaeus, 1758). In L. Administration de la nature et des forêts (Ed.), (13/12/2019 ed.).

34 Waschbär – Management- und Maßnahmenblatt zu VO (EU) Nr. 1143/2014 (2018). https://neobiota.bfn.de/unionsliste/art-19-management.html
35 De Sousa, T. (2019). Plan d'action pour espèces exotiques envahissantes au Grand-Duché de Luxembourg: la Tortue de Floride, Trachemys scripta ssp. (Schoepff, 1792). In L. Administration de la nature et des forêts (Ed.), (13/12/2019 ed.).
36 Bekendtgørelse om eftersøgning og aflivning af nødstedt vildt - BEK nr. 827 (2016). https://www.retsinformation.dk/eli/lta/2016/827
37 Miljø- og, F. (2019). Bekendtgørelse af lov om jagt og vildtforvaltning - LBK nr. 265.
38 Bekendtgørelse om våben og ammunition der må anvendes til jagt m.v. - BEK nr.1397 (2020).
39 Bekendtgørelse om vildtskader - BEK. 1006 (2020). https://www.retsinformation.dk/eli/lta/2020/1006
40 Forvaltningsplan for mink, mårhund og vaskebjørn i Danmark (2020). https://mst.dk/media/191343/netversion-miljoestyrelsen_forvaltningsplan_2020-mink-maarhund-og-vaskebjoern.pdf
41 Bundesnaturschutzgesetz vom 29. Juli 2009 (BGBl. I S. 2542), das zuletzt durch Artikel 290 der Verordnung vom 19. Juni 2020 (BGBl. I S. 1328) geändert worden ist (2009). https://www.gesetze-im-internet.de/bnatschg_2009/BJNR254210009.html
42 Tierschutzgesetz in der Fassung der Bekanntmachung vom 18. Mai 2006 (BGBl. I S. 1206, 1313), das zuletzt durch Artikel 280 der Verordnung vom 19. Juni 2020 (BGBl. I S. 1328) geändert worden ist (2006). https://www.gesetze-im-internet.de/tierschg/BJNR012770972.html
43 Richtlinie 92/43/EWG des Rates vom 21. Mai 1992 zur Erhaltung der natürlichen Lebensräume sowie der wild lebenden Tiere und Pflanzen (FFH-Richtlinie) (1992).
44 Bundesjagdgesetz in der Fassung der Bekanntmachung vom 29. September 1976 (BGBl. I S. 2849), das zuletzt durch Artikel 291 der Verordnung vom 19. Juni 2020 (BGBl. I S. 1328) geändert worden is (1976). https://www.gesetze-im-internet.de/bjagdg/BJNR007800952.html
45 Loi du 18 juillet 2018 concernant la protection de la nature et des ressources naturelles et modifiant (2018). http://data.legilux.public.lu/eli/etat/leg/loi/2018/07/18/a771/jo
46 Règlement grand-ducal du 16 décembre 2011 concernant l'emploi des armes et munitions de chasse, les moyens autorisés pour l'exercice de la chasse ainsi que l'emploi du chien de chasse.
47 Cruz, S. and Reynolds, S.J. (2019). Eradication and control programmes for invasive mynas (*Acridotheres* spp.) and bulbuls (*Pycnonotus* spp.): defining best practice in managing invasive bird populations on oceanic islands. In: C.R. Veitch, M.N. Clout, A.R. Martin, J.C. Russell and C.J. West (eds.) (2019). Island invasives: scaling up to meet the challenge, pp. 302–308. Occasional Paper SSC no. 62. Gland, Switzerland: IUCN.
48 Hampton, J.O., Arnemoc, J.M., Barnsley, R., Cattet, M., Daoust, P-Y, DeNicola, A.J., Eccles, G., Fletcher, D., Hinds, L.A., Hunt, R., Portas, T., Stokke, S., Warburton, B. & Wimpenny, C. 2021. Animal welfare testing for shooting and darting free-ranging wildlife: a review and recommendations. *Wildlife Research*, https://doi.org/10.1071/WR20107
49 Hampton, J.O., Forsyth, D.M., Mackenzie, D.I. and Stuart, I.G. 2015. A simple quantitative method for assessing animal welfare outcomes in terrestrial wildlife shooting: the European rabbit as a case study. Animal Welfare, 24: 307-317 doi: 10.7120/09627286.24.3.307
50 Hampton, J.O. & Forsyth, D.M. 2016. An assessment of animal welfare for the culling of peri-urban kangaroos. *Wildlife Research*, 43, 261–266. http://dx.doi.org/10.1071/WR16023
51 Berroneau, M., Detaint, M. and Coïc, C. (2008). Bilan du programme de mise en place d'une stratégie d'éradication de la grenouille taureau Lithobates catesbeianus (Shaw 1802) en Aquitaine (2003-2007) et perspectives. *Bull Soc Herpétol France*, 127:35–45.
52 Kamoroff, C., Daniele, N., Grasso, R.L., Rising, R., Espinoza, T. and Goldberg, C.S. (2020) Effective removal of the American bullfrog (*Lithobates catesbeianus*) on a landscape level: long term monitoring and removal efforts in Yosemite Valley, Yosemite National Park. *Biological Invasions,* 22:617–626. https://doi.org/10.1007/s10530-019-02116-4(0123456789().,-volV)(01234567

Appendix 16. Drowning traps

1. Measure name			
1.1. English:	Drowning traps		
1.2. Lethal or non-lethal:	Lethal		
1.3. Other languages (if available):			
Bulgarian	Капани за удавяне	Italian	Trappole da annegamento
Croatian	Potopne zamke	Latvian	Slīcinoši slazdi
Czech	„Topící“ pasti	Lithuanian	Skandinamieji spąstai
Danish	Drukne fælder	Maltese	
Dutch	Verdrinkingsvallen	Polish	Płapki topiące
Estonian	Uppumislõksud	Portuguese	Armadilhas de afogamento
Finnish	Hukuttavat ansat/raudat	Romanian	Capcane pentru înec
French	Pièges par noyade	Slovak	Pasce na topenie – “topiace” pasce
German	Ertränkungsfalle	Slovenian	Pasti za utopitev
Greek	Παγίδες πνιγμού	Spanish	Trampas de ahogamiento
Hungarian	Vízbefullasztó csapdák	Swedish	Dränkande fångstredskap
Irish			

2. Technical details of measure
2.1.a. Measure description
Drowning traps are traps set either underwater to restrain the animal, or so that the animal will be pulled underwater once caught. Both are intended to cause the hypoxia-induced death of the animals. Body-gripping, bait traps and conibear traps set to drown the animal are then classified as drowning traps and are covered by this assessment. Drowning traps are used to kill semiaquatic mammal species. Regarding IAS of Union Concern, drowning traps are used to control muskrats [8,9]. The method is potentially applicable with coypu but there are no experiences about it. In areas where the European Otter is present, the width of the entrances is limited to 9 cm to prevent trapping of this protected species. In the EU, it is only permitted to use used bait traps featured with a protection against pecking by waterfowl and only with the application of a protective cover up to the water level [1]. In some Member States, the use of drowning traps is illegal (e.g. in Italy). Being a semiaquatic species, muskrats are physiologically adapted to life in water. They could remain underwater in drowning traps for a period longer than 300-second, the time limit specified in the AIHTS for the loss of the corneal and palpebral reflexes. While it is still controversial if the times to irreversible unconsciousness (TIU) should be calculated from the moment the animal starts to show distress behaviour, the use of drowning traps seems contrary to Art.7b of the AIHTS, which require member states to ensure that the trapping methods applied in their territory comply with these

standards. A Technical Workshop on International Trapping Standards agreed that there is a need to develop alternative multi-capture muskrat traps that can meet the requirements of the higher AIHTS welfare categories [4].

2.1.b. Integration with other measures

In some cases, shooting and clubbing are applicable as additional means [1], or the drowning traps are used in combination with body-gripping, bait traps and conibear traps set on a raft [2], see for example Case Study 1.

2.2.a. Availability - species and objectives

Objective	Unknown objective		Rapid Eradication		Management					
					Eradication		Control		Containment	
Species	Avail.	Ref(s).	Avail.	Ref(s).	Avail.	Ref(s).	Avail.	Ref(s).	Avail.	Ref(s).
Acridotheres tristis										
Alopochen aegyptiaca										
Callosciurus erythraeus										
Corvus splendens										
Herpestes javanicus										
Lepomis gibbosus										
Lithobates catesbeianus										
Muntiacus reevesi										
Myocastor coypus	A	[14]					A	[14]	A	[14]
Nasua nasua										
Nyctereutes procyonoides										
Ondatra zibethicus	A	[10]					A	[8, 9]	A	[10]
Oxyura jamaicensis										
Perccottus glenii										
Plotosus lineatus										
Procyon lotor										
Pseudorasbora parva										
Sciurus carolinensis										
Sciurus niger										
Tamias sibiricus										
Threskiornis aethiopicus										
Trachemys scripta										

2.2.b. Application – EU Member States and objectives												
Objective	Unknown objective		Rapid Eradication		Management							
					Eradication		Control		Containment			
Country	Avail.	Ref(s).	Avail.	Ref(s).	Avail.	Ref(s).	Avail.	Ref(s).	Avail.	Ref(s).		
Austria												
Belgium							X	[9]				
Bulgaria												
Croatia												
Cyprus												
Czech Republic												
Denmark												
Estonia												
Finland												
France												
Germany							X	[9]				
Greece												
Hungary												
Ireland												
Italy												
Latvia												
Lithuania												
Luxembourg												
Malta												
Netherlands							X	[7, 9]				
Poland												
Portugal												
Romania												
Slovakia												
Slovenia												
Spain												
Sweden												
United Kingdom*			X	[10]								

* Not an EU Member State

3. Humaneness of the measure			
3.1. Welfare for all measures			
Measure type (if applicable):	**Humaneness impact categories**		
Domain	**No impact**	**Mild-Moderate**	**Severe - Extreme**
1: Water deprivation, food deprivation, malnutrition	No impact, as the animal is restrained in the trap for a few minutes before dying.		
2: Environmental challenge	No impact, as the animals is exposed to ambient conditions that are within its thermoneutral range.		
3: Injury, disease, functional impairment		No data are available on the injuries to which muskrats are exposed when captured in drowning traps. If the animals are captured with body-gripping traps, they could presumably get injured as species of terrestrial animals captured by this kind of traps are. Teeth breakage and skin abrasions easily occur in metal box traps [3]. However, as the death occurs relatively quickly (see 3.2), the impact of injuries is mid.	
4: Behavioural, interactive restriction			Before dying by suffocation, trapped muskrats experience agitation from not being able to perform a natural behaviour that the animal is highly motivated to perform (breathing) [3, 4, 5]. This highly stressful condition is prolonged until the moment of death, happening after several minutes [3, 5].
5: Anxiety, fear, pain, distress, thirst, hunger etc.			Distress is unlikely to occur immediately after entry into the drowning trap because semi-aquatic mammals like the muskrats routinely spend some time underwater without

			experiencing distress or pain [4]. For an animal in a drowning trap, distress and possibly pain, likely starts when it first attempts to, and thereby finds that it is unable to, come to the surface to breathe. In experimental conditions, after means of 61 - 76 seconds muskrats started biting the wire mesh of the cage, then showing clear signs of distress [4]. This condition is prolonged until the death of the animal, occurring after around additional 300sec. Serum corticosterone concentration (indicating stress level) in post mortem blood samples taken from the heart of the drowned muskrats was found to be eight times higher than the basal levels [4].

3.2. Mode of death (if relevant)			
Measure type (if applicable):	**Immediate death (i.e. no suffering)**	**Not immediate death (mild - moderate suffering)**	**Not immediate death (severe - extreme suffering)**
Rationale:			Death by drowning-induced hypoxia is a slow process for semi-aquatic species and even after struggling, which consumes oxygen more quickly, electroencephalogram loss occurs for muskrats after an average of 6 minutes [4]. Death by drowning induced hypoxia is not considered an acceptable method of euthanasia by veterinary and laboratory researchers [6, 7].

3.3. Humaneness summary	Before dying by suffocation, trapped muskrats experience agitation from not being able to perform natural behaviour that the animal is highly motivated to perform (breathing) [3, 4, 5]. Death by drowning induced hypoxia is not considered an acceptable method of euthanasia by veterinary and laboratory researchers [6, 7]. Even if the period between onset of distress and irreversible unconsciousness for muskrats in a drowning trap is within the 300 seconds limit specified in the AIHTS and Welfare Category C of the Improved Standards [4], experts have consistently recommended to develop new muskrat traps that can meet better criteria of animal welfare [3, 4].

4. Costs and effectiveness of the measure	
General effectiveness of the measure	Drowning traps are more efficient if used in situations with small ranging water levels [1]. The extensive analysis of data collected on the use muskrat trapping in the Netherlands (presented in Case Study 1), indicates that trapping affects muskrat population density only if the levels of investment are in adequate proportion to population size. However, the study doesn't specify if only drowning traps have been used, and no more specific assessments of the drowning traps' effectiveness have been published.

4.1. Case studies	
CASE STUDY #1	
Measure type (if relevant):	Drowning trap/unspecified lethal traps
Species:	Muskrat (*Ondatra zibethicus*)
Objective:	Control
Combined with other measure(s):	The study does not specify which kind of traps have been used. Control effort was made using lethal traps only, no poison. As drowning traps are the most commonly used traps for muskrats [1], it seems reasonable to conclude that most of the captures have been made using this kind of trap. However, the use of other lethal traps can't be excluded. In fact, in some cases, shooting and clubbing are applicable as additional means [1], or the drowning traps are used in combination with body-gripping, bait traps and conibear traps set on a raft [2].
Country(ies) of application:	The Netherlands
Geographic scale (km^2) and/or population size measure applied to:	Whole Netherlands (12 provinces): 41543km2. This equates to an average of 10.35 animals removed per km2 per year.
Time period:	1941 - 2013
Effort:	On average, populations were observed to decline when the annual effort exceeded 1.4 h/km/y. Prior to the peak year, all the 12 provinces covered by the study showed increasing catches, in some cases lasting decades, in spite of generally increasing effort. Probably, the effort was not intensive enough to cause a

	decline. After the peak, catch was limited by muskrat population size, and extra effort further depressed the population, reducing the catch in the following year.
Costs:	Overall costs:
	Cost is not specified. It seems progressively cheaper to maintain control at lower population density. This is corroborated by the study's finding showing that lower investments were made as each new phase of control was attained.
	Personnel costs:
	Equipment and infrastructure:
	Other, including overheads:
Effectiveness:	The study results indicate that trapping affects muskrat population density, provided that the levels of investment are in adequate proportion to population size. However, control measures are expensive, large numbers of animals are killed and non-target species are killed as well, directly or indirectly, as side-effects of the control measures. Further, existing alternatives to trapping could mitigate or prevent damage.
Reference(s):	[8]

4.2. Costs effectiveness summary	Available literature rarely specifies the type of lethal trap used to control/eradicate muskrat populations. As drowning traps are the most commonly used traps for muskrats [1], one could argue that most of the captures have been made using this kind of trap and the following considerations are based on this assumption. However, the use of other lethal traps cannot be excluded. Several authors have doubted the efficacy of muskrat control programmes, arguing that annual catch rates were too low relative to recruitment to result in a significant population decrease [9]. In the UK, the effectiveness of muskrat control was demonstrated in five well-documented cases that resulted in complete eradication—but all were small, recently established and isolated populations [10]. In Germany, a study concluded that the populations are more affected by habitat and weather conditions than by trapping efforts, and more work is needed on the real results of the trapping programme [11]. In the Netherlands, ongoing muskrat control limits muskrat population size, but it has been recommended to carefully study the feasibility of alternative methods to prevent damage and to undertake a cost-benefit analysis [8, 9].

5. Side effects		
Non-target native species, their habitats and the broader environment:	Positive:	
	Negative:	
	Important negative side effects of muskrats control programmes using drowning traps include bycatch of fish, birds and mammals, disturbance of nature areas and blocking of ecological corridors by muskrat traps [11].	
Other invasive alien species:	Positive:	
	Where both species are present, drowning traps could trap simultaneously muskrats and coypus [1].	
	Negative:	
Public health and well-being:	Positive:	
	Negative:	
Economic:	Positive:	
	Negative:	
	The economic benefit of using drowning traps to control muskrat populations compared to preventing damages has not been demonstrated and seems questionable [8, 9].	

6. Conclusion	
Overall assessment of the measure (qualitative)	
The use of drowning traps causes severe suffering in target and non-target animals. Death by drowning induced hypoxia is not considered an acceptable method of euthanasia by veterinary and laboratory researchers which have consistently recommended to develop new muskrat traps that can meet better criteria of animal welfare. Live trapping and the humane dispatch of the animals is a more humane lethal method already applied for muskrats or other semi-aquatic mammals, like the coypus in Italy [13]. In addition, large numbers of non-target animals are inhumanely killed as well, directly or indirectly, as side-effects of the muskrats' trapping by drowning traps. The evaluation of the cost-effectiveness of controlling muskrat populations compared to the implementation of measures to prevent damages deserves further consideration.	
Assessor:	Ilaria Di Silvestre
Reviewer 1:	Riccardo Scalera
Reviewer 2:	Sandro Bertolino

7. References

[1] FACE, UETA, IFF (2014). Best Practice Guidelines for Trapping of Mammals in Europe 2013-2014 - *Ondatra zibethicus*.

[2] https://forestry.ca.uky.edu/muskrat_lethal#:~:text=A%20muskrat%20float%20used%20to,quick%20drown%22%20device%20is%20used.

[3] Iossa, G., Soulsbury C.D. and Harris, S. (2007). Mammal trapping: a review of animal welfare standards of killing and restraining traps. Animal Welfare 2007, 16: 335-352 ISSN 0962-7286

[4] Talling J.C. & Inglis I.R. (2009) Improvements to trapping standards. DG ENV.

[5] Gilbert FF, Gofton N. ¡1982). Terminal dives in mink, muskrat and beaver. Physiol Behav. 1982;28(5):835-840. doi:10.1016/0031-9384(82)90200-1

[6] Close B, Banister K, Baumans V, Bernoth E-M, Bromage N, Bunyan J, Erhardt W, Flecknell P, Gregory N, Hackbarth NGH, Morton D and Warwick C (1996). Recommendations for euthanasia of experimental animals: Part 1. Laboratory Animals (London) 30: 293-316

[7] Beaver BV, Reed W, Leary S, McKiernan B, Bain F, Schultz R, Bennett BT, Pascoe P, Shull E, Cork LC, Francis-Floyd R, Amass KD, Johnson R, Schmidt RH, Underwood W, Thornton GW and Kohn B (2001). Report of the AVMA Panel on Euthanasia. Journal of the American Veterinary Medical Association 218: 669-696.

[8] van Loon, E., Bos, D., van Hellenberg Hubar, C., and Ydenberg, R. (2016). A historical perspective on the effects of trapping and controlling the muskrat (*Ondatra zibethicus*) in the Netherlands. Pest Management Science, (October 2015). http://doi.org/10.1002/ps.4270

[9] Bos, D., Kentie, R., La Haye, M. et al. (2019). Evidence for the effectiveness of controlling muskrat (*Ondatra zibethicus* L.) populations by trapping. Eur J Wildl Res 65, 45 (2019). https://doi.org/10.1007/s10344-019-1279-1

[10] Baker, S.J., 2010. Control and eradication of invasive mammals in Great Britain. Revue scientifique et technique, 29(2), p.311.

[11] Pelz HJ. (1984). Spread of the Musk-Rat in the Federal Republic of Germany. Bull. OEPP 14: 153–157 14 (1984).

[12] Bos D, Hollander H, Klop E, Ydenberg RC (2017) Bijvangsten bij de muskusrattenbestrijding. Ontwikkeling tussen 2007 en 2016. Zoogdier 28:23–25

[13] Bertolino, S., Cocchi, R. (2018). Piano di gestione nazionale della Nutria *Myocastor coypus*. https://www.minambiente.it/sites/default/files/archivio/allegati/biodiversita/Piano_gestione_nutria_12032018.pdf

[14] Kendrot, S.R. (2011). Restoration through eradication: protecting Chesapeake Bay marshlands from invasive nutria (*Myocastor coypus*). Pages 313-319 In: Veitch, C. R.; Clout, M. N. and Towns, D. R. (eds.). 2011. Island invasives: eradication and management. IUCN, Gland, Switzerland.

Appendix 17. Goodnature® self-resetting (M026c)

1. Measure name			
1.1. English:	Goodnature® self-resetting (M026c)		
1.2. Lethal or non-lethal:	Lethal		
1.3. Other languages (if available):			
Bulgarian	Самозареждащи се капани тип Goodnature®	Italian	Goodnature®: trappole autoripristinanti
Croatian	Samopostavljajuće zamke	Latvian	Goodnature® pašuzlādes atsperu slazdi
Czech	Samo-nástražné, smrtící pasti (Goodnature®)	Lithuanian	Goodnature® savigaudžiai spąstai
Danish	Goodnature® selv nulstillende fælde	Maltese	
Dutch	Goodnature® vallen	Polish	Automatyczne pułapki Goodnature
Estonian	Goodnature® ise-vinnastavad püünised	Portuguese	Armadilhas automáticas (Goodnature®)
Finnish	Goodnature® itsestään virittyvä	Romanian	Capcane Goodnature® cu revenire automată
French	Pièges à réinitialisation automatique Goodnature®	Slovak	Samo -nástražné pasce
German	Goodnature® Auto-Reset-Falle	Slovenian	Samonastavljive pasti
Greek	Goodnature® Παγίδες αυτόματης επαναφοράς	Spanish	Trampas automáticas (Goodnature®)
Hungarian	„Goodnature" önfelhúzó csapdák	Swedish	Goodnature®
Irish			

2. Technical details of measure
2.1.a. Measure description
Goodnature® traps are designed to kill an animal entering the trap with a blow to the head. The trap is powered by a CO_2 cylinder and is self-resetting. It is capable of killing multiple animals on a single CO_2 cylinder but the cylinder otherwise needs to be replaced on a six-monthly basis, along with the species-specific lure used with the trap. These traps are used for the removal of a variety of mammal species and are commonly attached vertically to a tree trunk or post. As a target animal investigates the trap, it triggers the trap, causing gas from the canister to propel a piston to strike the animal on the skull, with the aim of killing it instantly. The carcass then drops to the ground and the trap resets for the next capture [1]. This type of trap is a relatively new innovation and there is little scientific evidence yet relating to its effectiveness, or its effects on non-target species [2]. Different models are available for mongoose (A18 Mongoose Trap), mink (A18 Mink Trap), grey squirrels and rats (A18 Grey Squirrel Trap), rats, stoats, mice and weasels (A24 Rat and Stoat Trap), rats and mice (A24 Pro) and possum (A12). The model number reflects the number of times the trap can reset itself on a single gas cylinder. The A18 Grey Squirrel Trap, A18 Mink Trap, A24 Rat and Stoat Trap and A24 Pro are welfare approved in the UK to AIHTS (Agreement on International Humane Trapping Standards), meaning they meet EU standards. The A24 is certified under the NAWAC (New Zealand National Animal Welfare Advisory Committee) humaneness guideline [3,4]. The A18 is targeted at American mink in Scandinavia and mongoose in Hawaii [5], and is currently undergoing humaneness testing with mongoose, according to the manufacturer. There are reports that the

Goodnature® Grey Squirrel Trap is used in the UK [2] but no published studies seem to exist. Under the various Spring Trap Approval Orders (STAOs) for the different UK countries there are varying requirements for the traps to be used with a specific type of entrance tunnel, or with a tunnel and at a height of >30cm above ground to avoid non-target capture [6,7].

2.1.b. Integration with other measures

Sometimes Goodnature® self-resetting traps have been trialed in combination with other methods [8]. Some animals may be trap-shy and alternative methods may be needed if eradication is the aim.

2.2.a. Availability - species and objectives

Objective	Unknown objective		Rapid Eradication		Management					
					Eradication		Control		Containment	
Species	Avail.	Ref(s).	Avail.	Ref(s).	Avail.	Ref(s).	Avail.	Ref(s).	Avail.	Ref(s).
Acridotheres tristis										
Alopochen aegyptiaca										
Callosciurus erythraeus	P		P		P		P		P	
Corvus splendens										
Herpestes javanicus	P		P		P		A	5	P	
Lepomis gibbosus										
Lithobates catesbeianus										
Muntiacus reevesi										
Myocastor coypus										
Nasua nasua										
Nyctereutes procyonoides										
Ondatra zibethicus										
Oxyura jamaicensis										
Perccottus glenii										
Plotosus lineatus										
Procyon lotor										
Pseudorasbora parva										
Sciurus carolinensis	A	2,6,7	P		P		P		P	
Sciurus niger	P	9	P		P		P		P	
Tamias sibiricus	P		P		P		P		P	
Threskiornis aethiopicus										
Trachemys scripta										

2.2.b. Application – EU Member States and objectives										
Objective	**Unknown objective**		**Rapid Eradication**		**Management**					
					Eradication		**Control**		**Containment**	
Country	Avail.	Ref(s).	Avail.	Ref(s).	Avail.	Ref(s).	Avail.	Ref(s).	Avail.	Ref(s).
Austria										
Belgium										
Bulgaria										
Croatia										
Cyprus										
Czech Republic										
Denmark										
Estonia										
Finland										
France										
Germany										
Greece										
Hungary										
Ireland										
Italy										
Latvia										
Lithuania										
Luxembourg										
Malta										
Netherlands										
Poland										
Portugal										
Romania										
Slovakia										
Slovenia										
Spain										
Sweden										
United Kingdom*	X	2								

* Not an EU Member State

3. Humaneness of the measure			
3.1. Welfare for all measures			
Measure type (if applicable):	**Humaneness impact categories**		
Domain	**No impact**	**Mild-Moderate**	**Severe - Extreme**
1: Water deprivation, food deprivation, malnutrition	No impact occurs under any domain before the killing method (trap triggering) is applied.		
2: Environmental challenge	No impact occurs under any domain before the killing method (trap triggering) is applied.		
3: Injury, disease, functional impairment	No impact occurs under any domain before the killing method (trap triggering) is applied.		
4: Behavioural, interactive restriction	No impact occurs under any domain before the killing method (trap triggering) is applied.		
5: Anxiety, fear, pain, distress, thirst, hunger etc.	No impact occurs under any domain before the killing method (trap triggering) is applied.		

3.2. Mode of death (if relevant)			
Measure type (if applicable):	**Immediate death (i.e. no suffering)**	**Not immediate death (mild - moderate suffering)**	**Not immediate death (severe - extreme suffering)**
Rationale:	Manufacturer's own reports state that Goodnature® traps kill mongoose 'instantly' [5]. A18 Grey Squirrel Trap (for grey squirrels and rats), A18 Mink Trap (for mink but a similar model is available for mongoose) and models for other species have been approved under AIHTS standards in the UK [6,7]. This means that the traps exceeded the standard of causing irreversible unconsciousness to ≥80% of 12 animals		NAWAC testing was not able to show categorically that unconsciousness occurred instantly because of the practicalities involved. Improvements may have been made since these data were recorded (in 2010 and 2011), but until more evidence is available, the time to death and associated

	within 5 minutes [10]. Goodnature® traps used with ship rats (*Rattus rattus*), brushtail possums (*Trichosurus Vulpecula*) and stoats *(Mustela erminea)* rendered them irreversibly unconscious in 15-29s [3], 21-156s [11] and 22-38s [4] respectively and therefore met the humaneness criteria under the NAWAC 3 minute guideline. Note, these times included the time for assessors to reach trapped animals, so the times to irreversible consciousness may have been less than those reported. However, a recent study (in which the only Norway rat triggering an A24 rat trap received a non-lethal injury) suggests that more research may be required on their humaneness and efficacy with Norway rats [21]. The manufacturer reports that the A18 Mongoose trap is currently undergoing welfare testing [12].		variation with these traps remains uncertain, although they have passed the legal thresholds of AIHTS and NAWAC testing. However, cases where immediate death may not occur (possibly leading to severe-extreme suffering) cannot be ruled out.

3.3. Humaneness summary	A18 trap models have been approved in the UK (under AIHTS) for grey squirrels and American mink, while the A18 mongoose trap is currently undergoing humaneness testing. However, it is possible at worst that, for most squirrels and mink tested, traps meeting this standard take up to 5 minutes to become unconscious and that a small proportion take longer. Goodnature® claim that their A18 mongoose traps cause instant death [5], but the only data found on time to irreversible unconsciousness are unable to support such claims for ship rats, brushtail possums and stoats because of the practicalities of testing unconsciousness instantly after trap triggering. These data were collected in 2010 and 2011 and improvements may have occurred in the interim. However, until more evidence is available, the time to death and associated variation with these traps remains uncertain, although they have passed the legal thresholds of AIHTS and NAWAC testing. Therefore, cases where immediate death may not occur (possibly leading to severe-extreme suffering) cannot be ruled out.

4. Costs and effectiveness of the measure	
General effectiveness of the measure	Because the Goodnature® self-resetting traps have only been available for a few years, there is little scientific evidence yet relating to their effectiveness, or their effects on non-target species [2]. Goodnature® have updated their traps and species-specific lures over time and so earlier trials may not be representative of currently available models. For example, older versions of the A24 trap and the A12 trap were concluded to be ineffective for rat and possum control respectively, due in part to mechanical failure related to the leaking of gas from the cylinder and failings of the long-life auto-lures [13]. Mechanical failures could also potentially result in injuries to, rather than death of, target animals [14]. However, the failures reported predate the approval of any Goodnature® traps in the UK and, according to Carter *et al*. [15], the mechanical issues have been resolved. Goodnature® conducted a trial of the A18 Mongoose Trap in collaboration with the USFWS in Hawaii and claimed that mongoose numbers declined by 60% within the first few days and that thereafter the mongoosenumbers and the capture of re-invading individuals continued to decline, but the trial may have been only <1 month long [5]. In preliminary trials of A24 Rat and Stoat Traps and A12 Possum Traps in New Zealand, possum Residual Trap Catch Index (RTCI) declined from 15% to 5.7% 17 months later after 15 months of trapping (a similar level of control to that achieved previously with cyanide baits). Possum (*Trichosurus vulpecula*) control was comparably effective to using best-practice 'kill traps' (this probably means spring traps) available at the time and more effective than poison baits. The minimum number of rats killed per trap per night reduced from 0.15 to 0.01 over 2 months and remained below 0.03 over the 4 month trapping period [16]. Trapping rats (*Rattus norvegicus* and *Rattus rattus*) and possum in New Zealand using A24 Rat and Stoat Traps and A12 Possum Traps, respectively, reduced activity of all species at the study site to ≤10% within 9 months [15]. Franklin [14] reported that a grid of A24 Rat Traps at one site killed more rats per hectare than a large-scale snap trap grid at another site, despite using 65% less labour.

4.1. Case studies	
CASE STUDY #1	
Measure type (if relevant):	Kill traps – Goodnature® self-resetting (M026c - CS004)
Species:	*Herpestes javanicus*
Objective:	Control
Use of measure	Field-test of 20 x Goodnature® A18 Mongoose Traps over 20 ha, with the aim of demonstrating that the A18 trap is an effective way of controlling mongoose. The trial was conducted by the manufacturer in collaboration with the USFWS (US Fish and Wildlife Service) [5].

Combined with other measure(s):	Not applicable.
Country(ies) of application:	US (Hawaii)
Geographic scale (km^2) and/or population size measure applied to:	20 ha
Time period:	The time period was not specified directly but the trial was described as taking place 'in November', so probably no more than a month.
Effort:	20 traps over a time period of < one month.
Costs:	Overall costs:
	No costs are mentioned.
	Personnel costs:
	Equipment and infrastructure:
	Other, including overheads:
Effectiveness:	The Manufacturer's own brief outline report says: "*The results were extremely positive, with mongoose numbers dropping by 60 percent within the first few days of the trial. Ongoing results continued to see the numbers and the capture of re-invading individuals decline. The success of the project provides good evidence for setting up trap networks across larger areas*."

4.2. Costs effectiveness summary	The only case study identified was reported by the manufacturer so no independent information is available on the effectiveness or costs of deployment for the IAS under consideration. The capital outlay of buying self-resetting traps is greater than for traditional traps [17], but since traps do not need to be reset regularly there should be a reduction in field effort and overall costs over time compared to more labour-intensive methods [18,19]. Goodnature® traps may also be useful in remote/inaccessible places [19,20]. In a multi-species trial involving the use of A24 Rat and Stoat Traps on rats and A12 Possum Traps on possum, the cost of installing self-resetting traps for long-term suppression of rats within a 64 ha area was higher than for other methods (Victor snap traps and anticoagulants), but not for DOC-200 traps. However, the cumulative maintenance costs of self-resetting traps were lower than those estimated for 1st generation anticoagulants (after 5 years) and Victor snap traps (after 7 years), when field work was carried out by contractors [15].

5. Side effects	
Non-target native species, their habitats and the broader environment:	Positive:
	Self-resetting traps may have reduced welfare and conservation impacts on non-target native species compared to poison baiting [18]. However, it is important to use them according to manufacturer's instructions or in the way for which they have been approved (e.g. with the appropriate tunnel or a certain distance above ground). Predators can safely scavenge target animal carcasses from beneath the trap without risk of non-target effects such as being poisoned.
	Negative:
	Self-resetting traps are relatively new and there is little evidence yet of their impacts on non-target species [2] but their risks to non-target species of similar size and habits are potentially great as it may be difficult to exclude them. In the UK, it is recommended that the A18 Grey Squirrel Trap should be placed on a tree above ground level to minimise risks to hedgehogs *(Erinaceus europaeus)* (some of the UK STAOs require that Goodnature® traps are used ≥30cm above ground-level and all UK STAOSs require them to be used with a particular type of tunnel to reduce non-target access). However, this will not exclude red squirrels *(Sciurus vulgaris)* or pine marten *(Martes martes)* and the traps cannot be used where these occur [2]. Weka or woodhen *(Gallirallus australis)* have been accidentally killed by Goodnature® traps set for other species in New Zealand because the traps were set too close to the ground [15]. Self-resetting traps are likely to pose a risk to non-target native species in at least some parts of the EU too.
Other invasive alien species:	Positive:
	Self-resetting traps may potentially kill non-target alien species of similar size and habit to the target species. For example, the A18 mongoose trap could potentially kill American mink where these are present.
	Negative:
	There is a risk of injury or suffering if non-target alien species, of similar size and habits to target species, have access to a trap. If particular welfare risks are identified, then efforts should be made to exclude the species concerned.
Public health and well-being:	Positive:
	There is no risk of ingesting toxins and probably reduced risk of injury from interference with the trap compared to a traditional spring trap. Operators do not need to handle poisons or have a qualification/certificate.
	Negative:
	There could potentially be a risk of injury if members of the public deliberately interfered with a self-resetting trap but there are no data on this.
Economic:	Positive:
	While the capital outlay of buying self-resetting traps is greater than for traditional traps, self-resetting traps do not need to be reset regularly and so there should be a reduction in field effort and overall costs over time compared to more labour-intensive methods [18].
	Negative:
	Goodnature® self-resetting traps are more expensive to buy per unit than traditional traps, so require a bigger capital outlay upfront [17].

6. Conclusion

Overall assessment of the measure (qualitative)

A18 Goodnature® traps are available for killing grey squirrels and small Indian mongoose among the IAS species. It has been suggested that they may potentially also be suitable for use with fox squirrels *(Sciurus niger)* [9], although individuals of this species can be much heavier than grey squirrels and mongoose, and traps would need to undergo welfare testing for this species. Also, subject to appropriate testing, the A18 trap might be considered for Pallas squirrels *(Callosciurus erythraeus)* and the A24 for Siberian chipmunks *(Tamias sibiricus),* based on bodyweights. It is difficult to make firm conclusions about Goodnature® self-resetting traps at this stage as they have only been available for a few years and problems were reported with mechanical and other failures among some trap models in 2012 and 2013. These problems may have been resolved now but there have been few formal studies in the interim to produce data on effectiveness and non-target impacts for traps targeting any species, let alone the IAS species under focus here. However, the few more recent reports (mainly on trapping rats and possums) indicate that the traps are considered to be effective at significantly reducing target species numbers and maintaining them at lower levels. The only study identified for one of the IAS species *(Herpestes javanicus)*, was conducted jointly between Goodnature® themselves and the USFWS, and only superficial results are reported. No results are available yet for humaneness testing of the A18 trap for this species although, according to the manufacturer, this is underway [12] and the comparable A18 models for grey squirrels *(Sciurus carolinensis)* and American mink *(Mustela vison)* have passed humaneness testing (to AIHTS standards) in the UK. Until more evidence is available, the time to death and associated variation with these traps remains uncertain, although they have passed the legal thresholds of AIHTS and NAWAC testing. However, cases where immediate death may not occur (possibly leading to severe-extreme suffering) cannot be ruled out. Injury or capture of some non-target species may be minimised by using exclusion measures, such as appropriate tunnels or setting traps above certain heights. Upfront costs for buying and installing these traps are multiple times that for traditional traps but using them is far less labour-intensive because of their self-resetting ability, and overall costs have been reported to be recouped compared to anti-coagulant rodenticides and snap traps over a few years.

Assessor:	Sandra Baker
Reviewer 1:	Peter Robertson
Reviewer 2:	Riccardo Scalera

7. References

1. Goodnature® (current) *Join the trapping revolution*. https://goodnature.co.nz
2. Gill, R. (2019) *Controlling grey squirrels in forests and woodlands in the UK*. UK Forestry Standard Technical Note. https://www.forestresearch.gov.uk/research/controlling-grey-squirrels-forests-and-woodlands-uk/
3. Jansen, P. (2011) *The Goodnature® A24 automatic rat and stoat kill trap evaluation of humaneness [ship rats]: 2011*. Ministry for Primary Industries, NAWAC, Wellington.
4. Jansen, P. (2011) *The Goodnature® A24 automatic rat and stoat kill trap evaluation of humaneness [stoats]*: 2011. Ministry for Primary Industries, NAWAC, Wellington.
5. Goodnature® (2018) *Goodnature trap tackles most voracious invasive species*. https://goodnature.co.nz/blogs/news/goodnature-trap-tackles-most-voracious-invasive-species.
6. UK Government (2018a) *The Spring Traps Approval (England) Order 2018*. http://www.legislation.gov.uk/uksi/2018/1190/made
7. UK Government (2018b) *The Spring Traps Approval (Scotland) Order 2018*. http://www.legislation.gov.uk/ssi/2018/389/article/4/made
8. Vanderwerf, E.A. and Young, L.C. (2014) Breeding biology of red-tailed tropicbirds Phaethon rubricauda and response to predator control on O'ahu, Hawai'i. *Marine Ornithology*, 42, 73-76.

9. Frey, J.K., Iglesias, J., Herman, K. (2013) Eastern Fox Squirrel (*Sciurus Niger*): New Threat to Pecan Orchards in Far West Texas. *Western North American Naturalist*, 73(3), 382-385.
10. FACE (current) AIHTS – *Agreement on International Humane Trapping Standards*. https://www.face.eu/international-agreements/aihts/
11. Jansen, P. (2010) *The Goodnature™ self-resetting possum kill trap evaluation of humaneness: 2010*. Ministry for Primary Industries, NAWAC, Wellington.
12. Penberthy, A. (2020) *Goodnature personal communication*. hello@goodnature.co.nz
13. Gillies, C., Gorman, N., Crossan, I., Harawira, R., Hawaikirangi, R., Long, J., and McCool, E. (2012) A second progress report of DOC S&C Investigation 4276, *Operational scale trials of self-resetting traps for ground-based pest control for conservation in NZ forests*. Department of Conservation Science and Capability Group Report, Hamilton, New Zealand. 24 pp.
14. Franklin, K. (2013) *Informational report on the use of Goodnature®A24 rat traps in Hawaii*. Unpublished report, Pacific Cooperative Studies Unit, Research Corp. of the University of Hawaii, Oahu Army Natural Resources Program.
15. Carter, A., Barr, S., Bond, C., Paske, G., Peters, D. and van Dam, R. (2016) Controlling sympatric pest mammal populations in New Zealand with self-resetting, toxicant-free traps: a promising tool for invasive species management. *Biological Invasions*, 18, 1723-1736.
16. Peters, D., Schumacher, K., Schumaker, R., and Baigent, D. (2014) Goodnature automatic traps for vertebrate pest control: Field trials using new kill traps targeting animal pests in New Zealand. In R. Timm and J. O'Brien. (Eds.), *Proceedings of the 26th Vertebrate Pest Conference*. (pp. 405–410), Davis, California: University of California.
17. Witmer, G.W. and Shiels, A.B. (2018) Ecology, impacts, and management of invasive rodents in the United States. pgs. 193-219. In: W.C. Pitt, J.C. Beasley, and G.W Witmer, editors. *Ecology and Management of terrestrial vertebrate invasive species in the United States*. CRC Press, Boca Raton, FL. 403 pp.
18. Campbell, K.J., Beek, J., Eason, C.T., Glen, A.S., Godwin, J., Gould, F., Holmes, N.D., Howald, G.R., Madden, F.M., Ponder, J.B., Threadgill, D.W., Wegmann, A.S., Baxter, G.S. (2015) The next generation of rodent eradications: Innovative technologies and tools to improve species specificity and increase their feasibility on islands. *Biological Conservation*, 185, 47-58.
19. Shiels, A.B., Bogardus, T., Rohrer, J., Kawelo, K. (2019) Effectiveness of snap and A24-automated traps and broadcast anticoagulant bait in suppressing commensal rodents in Hawaii. *Human–Wildlife Interactions* 13, 226–237.
20. Pejchar, L., Lepczyk, C.A., Fantle-Lepczyk, J.E., Hess, S.C., Johnson, M.T., Leopold, C.R., Marchetti, M., McClure, K.M. and Shiels, A.B. (2020) Hawaii as a Microcosm: Advancing the Science and Practice of Managing Introduced and Invasive Species. *Bioscience*, 70(2), 184-193.
21. Ryan EA 2021 Non-target interactions and humane evaluation of a captive bolt trap on commensal rodents. MSc Thesis, University of British Columbia, Canada

Appendix 18. Spring traps

1. Measure name	
1.1. English:	Spring traps
1.2. Lethal or non-lethal:	Lethal
1.3. Other languages (if available):	

Bulgarian	Пружинни капани	Italian	Trappole letali – a molla
Croatian	Zamke s oprugom	Latvian	Atsperu slazdi
Czech	Pružinové pasti	Lithuanian	Spyruokliniai spastai
Danish	Ben hold fælde / klapfælde	Maltese	
Dutch	Veerklemmen	Polish	Pułapki sprężynowe
Estonian	Vedruga töötavad püünised	Portuguese	Armadilhas operadas por mola
Finnish	Raudat	Romanian	Capcane cu arc
French	Pièges à ressort	Slovak	Pružinové pasce
German	Schlagfallen	Slovenian	Pasti na vzmet
Greek	Παγίδες με ελατήριο	Spanish	Trampas operadas con resorte
Hungarian	Rugós csapdák	Swedish	Slagfällor
Irish			

2. Technical details of measure
2.1.a. Measure description
Spring trapping involves the use of a lethal trap that is powered by a spring. There are two types of spring trap: one has spring-powered bars that kill an animal by crushing a vital region of the body, generally the neck; the other has rotating jaws which have two hinged metal frames that allow a torsion spring to rotate the frames in a scissor-like action [1]. Spring traps are used for the detection or removal (by killing) of a variety of mammal species. They are used with or without a bait or lure. Some spring traps meet AIHTS (Agreement on International Humane Trapping Standards) approval, meaning (for the species concerned here) that they cause irreversible unconsciousness within 5 minutes in ≥80% of 12 individuals of the species concerned [2]. Lists of AIHTS approved traps for different species in the UK are available in the Schedules of the Spring Traps Approval Orders (STAOs) for England, Wales, Scotland and Northern Ireland (e.g., https://www.legislation.gov.uk/uksi/2018/1190/made). These approved traps must be used as approved (e.g. inside a specific tunnel where stipulated to minimize non-target capture or injury). The EU is a signatory of the AIHTS [1], so spring traps used in EU Member States need to meet AIHTS approval, so traps approved for a particular species in the UK should be acceptable throughout the EU for trapping the same species. Several types of spring traps are approved to AIHTS standards for grey squirrels (*Sciurus carolinensis*) (e.g., in the UK these include the BMI Magnum, DOC, Fenn, Kania, KORO, Solway and Springer traps [e.g., 3,4]. Squirrel spring traps are often attached directly onto a wall, tree trunk or wooden post and cannot be used in areas where non-target protected species (e.g. red squirrels (*Sciurus vulgaris*) or pine martens (*Martes martes*) are present [5]).

However, previously in the UK, grey squirrels were generally managed with poison where red squirrels were absent and with live cage trapping or shooting where reds were present [6,7,8], although sporting estates and woodland owners probably use both live and spring traps to control greys in order to protect trees. Toxins for squirrel control are no longer available for use in the UK, so traps and shooting now form the main control methods.

Spring traps are also available for muskrats (*Ondatra zibethicus*), raccoon dogs *(Nyctereutes procyonoides),* raccoons *(Procyon lotor)* and small Indian mongoose (*Herpestes auropunctatus*). Muskrats, raccoons and raccoon dogs are listed on the AIHTS list of fur-bearers; these species may be spring trapped in several EU Member States [9, Table 2.2] and spring traps must meet AIHTS standards. Spring trapping is generally not allowed in Italy. Muskrats can be 'active trapped' where drowning traps are set underwater at burrow entrances (generally Conibear traps or Ground Clamps), or 'passive trapped' using live-traps, drowning traps, baited body-gripping Conibears mounted on floating islands, or cages containing body-gripping traps [10]. The two latter trap types are covered for muskrats in this assessment but spring traps for muskrats are mainly used as drowning traps [11].

Live-traps, leghold traps, neckhold traps, snares, drowning traps and Goodnature® self-resetting traps are covered in other assessments.Traps that have been used with small Indian mongoose include the DOC 250 trap, the KORO large double spring trap and the Victor 160 Conibear trap [12,13]. Fenn traps have also been suggested [14]. The Conibear® trap (no. 220-2), is the most commonly used body-gripping trap for controlling coypu (nutria) *(Myocastor coypus)*. Nos. 160-2 and 330-2 Conibear® traps can also be used [15]. Leg-hold trapping is the method most often used by pecan growers to control fox squirrels, but a comparative study of leg-hold, cage and lethal spring traps identified spring traps as best overall on the basis of efficiency, cost, selectivity and humaneness [16] and kill-trapping has been suggested as the preferred method for controlling fox squirrels on pecan farms in the U.S. [17].

2.1.b. Integration with other measures

Spring trapping may be supplemented with other measures. For example, the effectiveness of muskrat spring trapping programmes is sometimes improved by killing of animals observed during other activities, for example shooting which is possible at dawn and dusk when muskrats are most active or during high water conditions, when muskrat are forced to sit and wait in trees [18], or by habitat modification [19]. Muskrat spring traps are probably also used in combination with drowning traps (kill traps set underwater). Kill-trapping may also be used with exclusion measures, such as fencing [20].

2.2.a. Availability - species and objectives

Objective	**Unknown objective**		**Rapid Eradication**		**Management**					
					Eradication		**Control**		**Containment**	
Species	Avail.	Ref(s).	Avail.	Ref(s).	Avail.	Ref(s).	Avail.	Ref(s).	Avail.	Ref(s).
Acridotheres tristis										
Alopochen aegyptiaca										
Callosciurus erythraeus	P		P		P		P		P	
Corvus splendens										
Herpestes javanicus	A	12, 13, 14			P		P		P	
Lepomis gibbosus										
Lithobates catesbeianus										
Muntiacus reevesi										
Myocastor coypus	A	15								

Nasua nasua	P				P		P		P	
Nyctereutes procyonoides	A	9			P		P		P	
Ondatra zibethicus	A	18			A	21, 22	A	23, 24, 25, 26	A	24
Oxyura jamaicensis										
Perccottus glenii										
Plotosus lineatus										
Procyon lotor	A	9	P		P		A		P	
Pseudorasbora parva										
Sciurus carolinensis	A	27	P		P	P	A		P	
Sciurus niger	P		P		P		P		P	
Tamias sibiricus	P		P		P		P		P	
Threskiornis aethiopicus										
Trachemys scripta										

2.2.b. Application – EU Member States and objectives

Objective	Unknown objective		Rapid Eradication		Management					
					Eradication		Control		Containment	
Country	Avail.	Ref(s).	Avail.	Ref(s).	Avail.	Ref(s).	Avail.	Ref(s).	Avail.	Ref(s).
Austria										
Belgium					X	23	X	18, 23, 24, 25		
Bulgaria										
Croatia										
Cyprus										
Czech Republic										
Denmark										
Estonia										
Finland										
France							X	18		
Germany							X	26	X	24
Greece										
Hungary										
Ireland										
Italy										
Latvia										
Lithuania										
Luxembourg										

Malta										
Netherlands					X	18, 24	X	18, 24, 25	X	18
Poland										
Portugal										
Romania										
Slovakia										
Slovenia										
Spain										
Sweden										
United Kingdom*	X	27			X	28				

* Not an EU Member State

3. Humaneness of the measure			
3.1. Welfare for all measures			
Measure type (if applicable):	**Humaneness impact categories**		
Domain	**No impact**	**Mild-Moderate**	**Severe - Extreme**
1: Water deprivation, food deprivation, malnutrition	No impact occurs under any domain before the killing method (trap triggering) is applied.		
2: Environmental challenge	No impact occurs under any domain before the killing method (trap triggering) is applied.		
3: Injury, disease, functional impairment	No impact occurs under any domain before the killing method (trap triggering) is applied.		
4: Behavioural, interactive restriction	No impact occurs under any domain before the killing method (trap triggering) is applied.		
5: Anxiety, fear, pain, distress, thirst, hunger etc.	No impact occurs under any domain before the killing method (trap triggering) is applied.		

3.2. Mode of death (if relevant)			
Measure type (if applicable):	**Immediate death (i.e. no suffering)**	**Not immediate death (mild - moderate suffering)**	**Not immediate death (severe - extreme suffering)**
Rationale:	Some spring traps are reported to cause immediate irreversible unconsciousness in which case they should cause no suffering (for example with two small Indian mongoose in DOC 250 traps). Cause of death was multiple skull fractures [12].	Some traps meet various trap welfare standards, e.g., the NAWAC or AIHTS. While these generally require irreversible unconsciousness within 3 or 5 minutes, some such traps may convincingly surpass these thresholds, e.g., Conibear 110™ traps for muskrats [2]. DOC traps have the potential to be one of the most humane traps for grey squirrels as it causes extensive destruction of the brain [28]. Parrott et al [28] concluded, that only DOC traps and the Kania 200 and Kania 250 traps should be used with grey squirrels.	Concerns have been raised over the time taken to cause irreversible unconsciousness in certain species by a number of spring traps including Fenn and BMI traps [28 and references cited therein, 29]. There are reports of raccoons being alive after 5 hours trapped in Conibear traps [Gilbert Proulx, pers. comm.]. Some spring traps set for grey squirrels have caught individuals (found alive) by extremities, including head and leg [28,30]. When Conibear traps, set on runways, were checked the next morning, all trapped muskrats were dead [31], but this is not an acceptable measure of humaneness.

3.3. Humaneness summary	
	The welfare impact of spring traps is likely to vary widely among species, trap model and the way the trap is used. While traps that have passed AIHTS or NAWAC standards for a species can generally be expected to cause irreversible unconsciousness within 5 or 3 minutes respectively, other traps have not been tested in this way. And, anyway, more information is needed on how quickly traps cause irreversible unconsciousness, rather than whether this occurs in fewer than 5 or 3 minutes as these are arguably quite long time thresholds. Conibear110TM and Leprich spring traps each caused irreversible unconsciousness in 12 muskrats within 31.5 +/- 16.3 s and 184.0 +/- 31.7 s respectively [1] indicating that they would meet AIHTS approval but not the more stringent standards suggested by Proulx and Barrett [32] or Talling and Inglis [9]. Fenn traps have received mixed reviews regarding inconsistent strike locations and laboratory testing (admittedly, >10 years ago) consistently failed to render ≥70% of (non-squirrel) target species irreversibly unconscious within 3 minutes (with 95% confidence) [see references cited in 28]. Concerns have been raised over how quickly the BMI trap kills fox squirrels [29]. DOC traps have passed NAWAC standards (consistently causing irreversible unconsciousness in less than three minutes in all species they were tested on) [28].

	In order to minimize welfare impact on target animals, traps need to strike the head or neck with sufficient force to cause cranial or upper vertebra destruction [28]. Achieving a quick and clean kill also depends on traps being set properly, e.g., body-gripping traps should be set so that the rotating jaws close on the top and bottom of the target animal's neck [33]. Two of 19 (10.5%) of grey squirrels caught in Magnum 116 bodygrip traps were caught by extremities [30], while 52% of grey squirrels caught in Fenn IV traps were found alive [34]. Zelin et al. [35] found muskrats were 'relatively easy to kill' with a thorax strike (as by a body-gripping trap), potentially because they have a flat (more readily crushed) thorax, but did not say how quickly animals died. Traps must be checked regularly so that injured animals can be killed quickly and trapped non-target species dealt with appropriately. In some jurisdictions it is considered unnecessary to check lethal traps regularly on the assumption that trapped animals will be dead. For example, passive muskrat traps are sometimes not checked for as long as a month [10] but when animals are struck by a trap at a sub-optimal strike location they may suffer a protracted and painful death if not found quickly [28]. Efficient kill trapping could have lower welfare impacts than live trapping (followed by killing), because live trapped animals are held under stress and sometimes sustain injury before being killed, for example, as suggested for fox squirrels [16]. Until more evidence is available, it is difficult to assess the humaneness of many spring traps but potentially severe-extreme suffering cannot be ruled out in many cases currently.

4. Costs and effectiveness of the measure	
General effectiveness of the measure	Live-trapping is considered the best approach for controlling grey squirrel numbers, particularly where reds are present [36], because grey squirrels are easy to live trap [Seymour 1961, cited in 37]. Kill trapping grey squirrels is generally less efficient than live trapping, but live-trapping is not efficient when natural tree seeds are available, because squirrels are not attracted to the bait at the traps and it is labour-intensive and costly [38]. The main measure to manage muskrat populations is trapping. Muskrat trapping methods are well described and there is a lot of experience of trapping in muskrat population management and control [18]. Unfortunately, most information sources report live-capture methods rather than the use of spring-traps or they simply test different measures rather than being part of an eradication or control effort [e.g., 31]. Four muskrat populations were successfully eradicated from large areas (up to 2,800km2) in the UK in the 1930s (using leghold traps, which are outside of this assessment) and the success of the campaign was attributed to: the need for control was clear and the campaigns were well supported; action was taken at a relatively early stage and was well organised; there was no immigration from outside the control areas; and trapping continued well after the last muskrat had been caught [21].

	Muskrat trapping is most effective over autumn/winter and effectiveness is mainly influenced by trapping intensity, but scale of operation, coordination and motivation of staff are also factors [18] as well as trapper skill, knowledge of muskrat behavior, persistence and the trap type used [19]. Maintaining a high trapping intensity (percentage of population removed) requires less effort at lower population sizes. Body-gripping traps (such as the Conibear) are extremely effective when set in runs [19]. Barun et al [39] report that kill traps have been used successfully in an eradication campaign to capture small Indian mongoose on Okinawa and Amami-Oshima. The effectiveness of spring traps for mongoose control depends primarily on the type of bait used, spacing between traps, the area over which they are placed, the skill and experience of the trapper in trap placement and setting. Trapping is labour and time intensive, potentially taking decades of effort (depending on population size and available resources) with no guarantee of success. Numerous strategies for the mechanical removal of mongoose are in use with no standardized measure to evaluate efficacy [40]. For eradication campaigns, multiple trap and bait/ scent types should be considered, as wariness or aversion to one combination may not be transferable to others [39]. DOC 250 spring traps set in best practice boxes were more effective than Tomahawk live cage traps at catching mongoose in Hawaii [12]. There was no difference in ability to trap mongoose among the DOC 250 trap, the Koro large double spring trap or the Victor 160 Conibear trap in the U.S. Virgin Islands [13]. In general, however, live box traps (outside the scope of this assessment) have been the primary method used to control and eradicate mongoose, e.g., from six islands by live box trapping and/or secondary poisoning, while mongoose were prevented from establishing on mainland North America when the first few immigrants were caught using an unspecified type of trapping, on Dodge Island, Florida in 1976. Two island mongoose eradication attempts have failed, one after ten years of using an unspecified type of trapping [39]. Most information sources found regarding the effectiveness of grey squirrel trapping refer to live-trapping. However, in a comparison of Magnum 116 spring traps and live capture traps, grey squirrels were equally likely to be caught in both [30] but evidence from spring trapping suggests no reduction in the number of squirrels caught [E. Brun cited in Huxley, 2003, cited in 41].

4.1. Case studies	
CASE STUDY #1	
Measure type (if relevant):	Kill traps – spring operated
Species:	*Herpestes javanicus*
Objective:	Eradication
Use of measure	Following live-trapping between 2000 and 2004, a spring-trapping programme on Amami-Oshimi island, Japan, was begun from 2005 [39]. In 2013, mongoose numbers were reported to have declined to a few

	individuals and a new 10-year eradication plan was started supplemented with sniffer dogs, camera traps and hair traps [42].
Combined with other measure(s):	No.
Country(ies) of application:	Japan
Geographic scale (km^2) and/or population size measure applied to:	Not specified.
Time period:	7 years.
Effort:	By 2009, 44-48 full-time trappers were employed.
Costs:	Overall costs:
	Personnel costs:
	Equipment and infrastructure:
	Other, including overheads:
Effectiveness:	>7,500 mongoose were killed over 5 years but the project was ongoing after 2009.

CASE STUDY #2	
Measure type (if relevant):	Kill traps – spring operated
Species:	*Sciurus carolinensis*
Objective:	Containment
Use of measure	Kill-trapping using Fenn Mk IV spring traps was added to existing shooting efforts by the RSPP (Red Squirrel Protection Partnership) in an effort to eradicate grey squirrels from Northumberland and parts of Durham [28]. Spring traps are set inside wooden boxes. Each box, which may be fixed to the trunk of a tree or set on the ground, contains one kill-trap and is usually baited. Control is instigated in response to public sightings, systematic survey work or as part of an ongoing programme. The aim is to completely eradicate grey squirrels from Northumberland. The first kill was made on 10th February 2007. A Fenn Vermin Trap Mark IV (Heavy Duty) is currently approved for grey squirrels under the UK STAOs but it is not known whether this is the same as used here (or the Fenn traps criticised by Parrott et al [28]).
Combined with other measure(s):	Live-trapping followed by shooting, and shooting in the drey or on the ground.
Country(ies) of application:	UK

Geographic scale (km^2) and/or population size measure applied to:	By July 2008, an area of 810 km^2 was signed up to the project which operates throughout Northumberland and in parts of Durham.
Time period:	The original project was funded for 3 years (2007-2010) with the first squirrel killed in February 2007. The work may have continued beyond the 3 years as more information, dated 2009 and updated in 2013, reveals that the eradication effort failed and grey squirrel numbers were increasing [43].
Effort:	One full-time trapper, two full-time shooters and 1,000 volunteers year-round. No data are available on trapping or shooting effort.
Costs:	Overall costs:
	£166,000 (50,000 GBP p.a.)
	Personnel costs:
	1,000 volunteers
	Equipment and infrastructure:
	Other, including overheads:
Effectiveness:	No before, during or after surveys were conducted of grey squirrel numbers to evaluate success, although it was considered that eradication (at least locally) had been achieved. 20,157 squirrels were killed between 10th February 2007 and 30th September 2008. Ultimately, the eradication effort failed and grey squirrel numbers were increasing [43].

4.2. Costs effectiveness summary	Spring trapping is very labour-intensive (though less so than cage trapping) and can be difficult in some terrains. A cost-benefit analysis indicated that DOC 250 traps were more cost-effective than live traps for mongoose in Fiji [44]. In a comparison of trap types for fox squirrels, spring traps (110 body traps) were identified as better than live cage traps and baited and unbaited leghold traps because spring traps had moderate efficiency, low relative cost, high selectivity and were relatively humane [16].

5. Side effects	
Non-target native species, their habitats and the broader environment:	Positive:
	Negative:
	Trapping of any kind may lead to non-target capture [21,24, 45]. For example, in muskrat trapping, brown rats (*Rattus norvegicus*), water voles (*Arvicola amphibius/terrestris*), fish and water birds are the most common non-target captures and there is a strong positive correlation between the number of muskrat traps deployed and the number of non-target individuals caught [18]. The most important factor in selective

	trapping is location [10] and non-target capture may be minimized by proper placement and design of traps. Kill-traps may kill or injure non-target animals whereas this may be less likely with live traps. Trapping fox squirrels could risk the capture of the native red squirrels (*S. vulgaris*) where present and spring-trapping may not be appropriate [46]. Spring traps set for grey squirrels may catch and kill birds [30]. A trial of excluders for the prevention of native species from entering grey squirrel spring traps in tunnels revealed that this reduced the utility of grey squirrel trapping [47].
Other invasive alien species:	Positive:
	Negative:
	Capture of non-target alien species is possible (although this may be desirable). Examples might include *Sciurus carolinensis, Tamias sibiricus, Callosciurus erythraeus*, which all have populations in different European countries, where trapping for one species could also trap the others. See notes on non-target native species above.
Public health and well-being:	Positive:
	Since many mammals are predominantly nocturnal, or are present around buildings or settlements, trapping is often the safest method for restraint [48].
	Negative:
	In muskrat trapping, there are risks of human injury or death from accidents with vehicles or flooding due to the often difficult terrain. Trapping muskrats at high population densities is physically demanding work and leads to physical strain on trappers, and especially joint complaints [18]. In case the trap springs while setting, each muskrat trapper must have knowledge how to free himself [10] and use of setting tongs and a safety gripper is recommended to minimize risk of trapper injury [33]. Muskrats are associated with transmission of several diseases to humans so this should be mitigated for when trapping/handling muskrats [49,50] and this could add to costs.
Economic:	Positive:
	Negative:
	Spring trapping is very labour-intensive (though less so than cage trapping) and therefore expensive. People opposed to trapping may damage/remove trapping equipment, especially in urban areas, resulting in repair or replacement costs.

6. Conclusion
Overall assessment of the measure (qualitative)
Lethal spring traps are available for use with muskrats, raccoons, raccoon dogs, mongoose and grey squirrel and may be suitable for ring-tailed coati, fox squirrels, Siberian chipmunks or Pallas' squirrels, subject to appropriate testing. Spring traps are likely to be of variable welfare impact but this could range between no suffering and severe to extreme suffering. Some spring traps are reported to cause irreversible unconsciousness instantly. While some traps may not have not been formally approved, others are approved under the AIHTS or NAWAC standards but this could still mean that

animals remain conscious for minutes and more detail is needed about time to irreversible unconsciousness. Until more evidence is available, it is difficult to assess the humaneness of many spring traps but potentially severe-extreme suffering cannot be ruled out in many cases currently.

Spring traps do not seem to be the primary measure used with grey squirrels, muskrats or mongoose, because they may be less effective than other measures, they are more labour intensive and pose a threat to non-target native species where present. Spring traps may be cost-effective in the long-term and provided that eradication work is started early.

Assessor:	Sandra Baker
Reviewer 1:	Riccardo Scalera
Reviewer 2:	Sandro Bertolino

7. References

1. Iossa, G., Soulsbury, C.D. and Harris, S. (2007) Mammal trapping: a review of animal welfare standards of killing and restraining traps. *Animal Welfare*, 16: 335-352.
2. FACE (current) AIHTS – Agreement on International Humane Trapping Standards. https://www.face.eu/international-agreements/aihts/
3. UK Government (2018a) The Spring Traps Approval (England) Order 2018. http://www.legislation.gov.uk/uksi/2018/1190/made
4. UK Government (2018b) The Spring Traps Approval (Scotland) Order 2018. http://www.legislation.gov.uk/ssi/2018/389/article/4/made
5. Gill, R. (2019) *Controlling grey squirrels in forests and woodlands in the UK*. UK Forestry Standard Technical Note. https://www.forestresearch.gov.uk/research/controlling-grey-squirrels-forests-and-woodlands-uk/
6. Genovesi, P. and Bertolino, S. (2001) Human dimension aspects in invasive alien species issues: the case of the failure of the grey squirrel eradication project in Italy. In, *The great reshuffling: human dimensions of invasive alien species* (J.A. McNeely, ed.). International Union for Conservation of Nature, Gland, Switzerland, and Cambridge, 113-119.
7. Mayle, B., Pepper, H., Ferryman, M. (2004). Controlling grey squirrel damage to woodlands. Forestry Commission Practice Note 4 (Revised). Forestry Commission, Edinburgh.
8. Lawton, C., Cowan, P., Bertolino, S., Lurz, P.W.W. and Peters, A.R. (2010) The consequences of introducing non-indigenous species: two case studies, the grey squirrel in Europe and the brushtail possum in New Zealand. *Revue Scientifique et Technique (International Office of Epizooties),* 29(2): 287-298.
9. Talling, J.C.; Inglis, I.R. (2009) Improvements to trapping standards; DG ENV, p 361. https://ec.europa.eu/environment/biodiversity/animal_welfare/hts/pdf/Final_report.pdf
10. FACE, UETA and IFF (2013/14) *Best Practice Guidelines for Trapping of Mammals in Europe*. FACE, UETA, IFF, http://www.face.eu/sites/default/files/attachments/trapping_guidelines_-_ondatra_zibethicus.pdf
11. Shuler, J. (2000) A history of muskrat problems in northeastern California. *Proceedings of the Vertebrate Pest Conference*, 19(19): 146-153.
12. Peters, D., Wilson, L., Mosher, S., Rohrer, J., Hanley, J., Nadig, A., Silbernagle, M., Nishimoto, M. and Jeffrey, J. (2011) Small Indian mongoose – management and eradication using DOC 250 kill traps, first lessons from Hawaii. In, Veitch, C. R.; Clout, M. N. and Towns, D. R. (eds.). *Island invasives: eradication and management*. IUCN, Gland, Switzerland. Pp 225-227.
13. Pollock, C.G. and Hairston, J. (2013) Mongoose Trap Preference at Sandy Point National Wildlife Refuge, US Virgin Islands. *Marine Turtle Newsletter*, 139: 3-6.
14. Roy, S.S., Jones, C.G. and Harris, S. (2002) An ecological basis for control of mongoose *Herpestes javanicus* in Mauritius: is eradication possible? In, C.R. Veitch and M.N. Clout (eds). *Turning the tide: the eradication of invasive species, pp* 266-273. IUCN SSC Invasive Species Specialist Group. IUCN. Gland. Switzerland and Cambridge. UK.
15. Wildlife Damage Management (2019) Nutria damage management. https://wildlife-damage-management.extension.org/nutria-damage-management/#Trapping
16. Huggins, J.G. (1996) Economic effectiveness, efficiency and selectivity of fox squirrel trapping in pecan groves. In R.M. Timm & A.C. Crabb, (Eds.) Proceedings of the 17th Vertebrate Pest Conference. University of California, Davis, U.S. Pp 123-126.

17. Frey, J.K., Iglesias, J. and Herman, K. (2013) Eastern fox squirrel (Sciurus niger): new threat to pecan orchards in far west Texas. *Western North American Naturalist,* 73(3): pp. 382–385.
18. Bos, D. 2017. *Information on measures and related costs in relation to species included on the Union list: Ondatra zibethicus*. Technical note prepared by IUCN for the European Commission. https://circabc.europa.eu/sd/a/7cf3a0bb-8ac3-48be-8e12-12c7783caa3e/TSSR-2016-003%20Ondatra%20zibethicus.pdf
19. Miller, J.E. (2018) *Muskrats*. Wildlife Damage Management Technical Series, U.S. Department of Agriculture: Animal and Plant Health Inspection Service. https://www.aphis.usda.gov/wildlife_damage/reports/Wildlife%20Damage%20Management%20Technical%20Series/Muskrat-WDM-Technical-Series.pdf
20. Jackson, J.J. (1994) Tree Squirrels. *The Handbook: Prevention and Control of Wildlife Damage,* 10. https://digitalcommons.unl.edu/icwdmhandbook/10?utm_source=digitalcommons.unl.edu%2Ficwdmhandbook%2F10&utm_medium=PDF&utm_campaign=PDFCoverPages
21. Gosling, L. M., and Baker, S.J. (1989). The Eradication of muskrats and coypus from Britain. *Biological Journal of the Linnean Society*, 38(1), 39–51.
22. Robertson, P.A., Adriaens, T., Lambin, X., Mill, A., Roy, S., Shuttleworth, C.M. and Sutton-Croft, M. (2016). The Large-Scale Removal of Mammalian Invasive Alien Species in Northern Europe. Pest Management Science, 17(2): 273-279.
23. VMM. (2010). Ratten op Vlaamse wijze. Erembodegem: Vlaamse Milieumaatschappij-VMM. https://www.vmm.be/publicaties
24. Bos, D., E. Klop, Hemert, H. van, LaHaye, M., Hollander, H., Loon, E. van, and Ydenberg, R. (2016). Beheer van Muskusratten in Nederland. Effectiviteit van bestrijding op grond van historie en een grootschalige veldproef. Deel 1 - Samenvatting tussenrapportage en Deel 2 Achtergrond studies (No. A&W rapport 2191). Veenwouden: Altenburg and Wymenga ecologisch onderzoek.
25. van Loon, E., Bos, D., van Hellenberg Hubar, C., and Ydenberg, R. (2016). A historical perspective on the effects of trapping and controlling the muskrat *(Ondatra zibethicus)* in the Netherlands. *Pest Management Science*, October 2015. http://doi.org/10.1002/ps.4270
26. Halle & Pelz (1990) On the efficiency of muskrat *(Ondatra zibethica)* control from trapping data ascertained in Bremen. *Zeitschrift fuer angewandte Zoologie*, 77(2): 205-218.
27. Scalera, R., Cozzi, A., Caccamo, C. and Rossi, I. (2017) *A catalogue of LIFE projects contributing to the management of alien species in the European Union*. Platform Meeting on Invasive Alien Species (IAS) 29-30 November 2017, Milan (Italy). LIFE14 IP/IT/000018 Nature Integrated Management to 2020 (GESTIRE 2020). Pp. 140.
28. Parrott, D., Quy, R., Van Driel, K., Lurz, P., Rushton, S., Gurnell, J., Aebischer, N. and Reynolds, J. (2009) *Review of red squirrel conservation activity in northern England*. Natural England Commissioned Report NECR019. Natural England, Worcester, UK. http://publications.naturalengland.org.uk/publication/46008
29. Huggins, J.G. (1999). Gray and fox squirrel trapping: A review. In, Proulx G, editor. *Mammal trapping*. Sherwood Park, Alpha Wildlife Research and Management Ltd., pp. 117–129.
30. Shuttleworth, C.M., Mill, A. and Van Der Waal, Z. (2017) A preliminary comparison of Magnum 116 bodygrip traps with a live capture trap design during the control of grey squirrels *(Sciurus carolinensis)*. *International Journal of Pest Management*, 63(4): 316-321.
31. Parker, G.R. (1983) An evaluation of trap types for harvesting muskrats in New Brunswick. *Wildlife Society Bulletin*, Winter, 11(4): 339-343.
32. Proulx, G. and M. W. Barrett (1994). Ethical considerations in the selection of traps to harvest American martens and fishers. In, *Martens, sables, and fishers; Biology and Conservation*. Buskirk, S.W., Harestad, A.S., Raphael, M.G. and Powell, R. Cornell University Press, Ithaca, New York, USA: 192-196.
33. AFWA (Association of Fish and Wildlife Agencies) (2014) Best Management Practices for Trapping Muskrats in the United States. https://www.dec.ny.gov/docs/wildlife_pdf/trapbmpsmuskrat.pdf
34. Shuttleworth CM, Knott H, Bailey M. 2002. Red squirrel conservation on Anglesey, North Wales. A report on the first five years of the Anglesey red squirrel project. Llangefni, Anglesey: Menter Mon.
35. Zelin, S., Jofriet, J.C., Percival, K. and Abdinoor, D.J. (1983) Evaluation of humane traps: momentum thresholds for furbearers. *Journal of Wildlife Management*, 47(3):863–868.
36. Bertolino, S. and Genovesi, P. (2003) Spread and attempted eradication of the grey squirrel *(Sciurus carolinensis)* in Italy, and consequences for the red squirrel *(Sciurus vulgaris)* in Eurasia. *Biological Conservation*, 109: 351-358.
37. Lawton, C. and Rochford, J. (2007) The recovery of grey squirrels *(Sciurus carolinensis)* populations after intensive control programmes. *Biology and Environment: Proceedings of the Royal Irish Academy*, 107B (1): pp. 19-29.
38. Rushton, S.P., Gurnell, J., Lurz, P.W.W. and Fuller, R.M. (2002) Modeling impacts and costs of gray squirrel control regimes on the viability of red squirrel populations. The Journal of Wildlife Management, 66(3): 683-697.
39. Barun, A., Hanson, C.C., Campbell, K.J. and Simberloff, D. (2011) A review of small Indian mongoose management and eradications on islands. In, Veitch, C. R.; Clout, M. N. and Towns, D. R. (eds.). 2011. *Island invasives: eradication and management*. IUCN, Gland, Switzerland. Pp 17-25.

40. Berentsen, A.R., Pitt, W.C. and Sugihara, R.T. (2017) Ecology of the small Indian mongoose *(Herpestes auropunctatus)* in North America. In W.C. Pitt, J. Beasley and G.W. Witmer (Eds.), *Ecology and management of terrestrial vertebrate invasive species in the United States*. Pp 251-267.
41. Harris, S., Soulsbury, C.D. and Iossa, G. (2006) Is culling of grey squirrels a viable tactic to conserve red squirrel populations? Unknown report. https://www.onekind.scot/wp-content/uploads/0811_grey_squirrel_populations.pdf
42. Abe, S. (2013, November). Eradication project of invasive alien mongooses on Amami-oshima Island, Japan. In *First Asia Park Congress, Sendei City*.5656.
43. Henderson, T. (2009/2013) Culling fails to stop march of grey squirrels, The Journal, 26 Sep 2009/26 June 2013
44. Brown, P. and Daigneault, A. (2015) Managing the Invasive Small Indian Mongoose in Fiji. *Agricultural and Resource Economics Review*, 44(3): 275–290.
45. Klop, E., van der Heide, J. E., and Schoppers, E. (2011). Bijvangsten muskusrattenbestrijding Trends, oorzaken en maatregelen (No. 9W2767). Groningen: Royal Haskoning.
46. Baiwy, E., Schockert, V. and Branquart, E. (2015) *Risk analysis of the Fox squirrel,* Sciurus niger*, Risk analysis report of non-native organisms in Belgium*. Cellule interdépartementale sur les Espèces invasives (CiEi), DGO3, SPW / Editions, updated version, 34 pp.
47. Short, M.J. and Reynolds, J.C. (2001) Physical exclusion of non-target species in tunnel-trapping of mammalian pests. *Biological Conservation*, 98: 139-147.
48. FACE (2013) *Annual report*.
49. Triplet, P. (2019) (update –original text 2009). *Ondatra zibethicus* CABI. Invasive Species Compendium. Alien Species Factsheet. http://www.cabi.org/isc/datasheet/71816
50. Wobeser, G., Campbell, G. D., Dallaire, A., and McBurney, S. (2009). Tularemia, plague, yersiniosis, and Tyzzer's disease in wild rodents and lagomorphs in Canada: A review. *Canadian Veterinary Journal-Revue Veterinaire Canadienne*, 50(12), 1251–1256.

Appendix 19. Cage Traps

1. Measure name	
1.1. English:	Cage Traps
1.2. Lethal or non-lethal:	Non-Lethal

1.3. Other languages (if available):

Bulgarian	Капани тип клетка	Italian	Trappole a gabbia
Croatian	Kavezne zamke	Latvian	Būru slazdi
Czech	Sklopce	Lithuanian	Gyvagaudžiai spąstai
Danish	Levende fælde / burfælde / trådfælde	Maltese	
Dutch	Kooivallen	Polish	Pułapki klatkowe
Estonian	Kastlõksud	Portuguese	Armadilhas de gaiola/caixa
Finnish	Loukut	Romanian	Capcane tip cușcă
French	Pièges à cages	Slovak	Sklopce
German	Käfigfallen, Lebendfangfallen	Slovenian	Pasti v obliki kletke
Greek	Παγίδες κλωβοί	Spanish	Cajas trampa
Hungarian	Ketreccsapdák	Swedish	Burfällor, levandefångstfällor
Irish			

2. Technical details of measure

2.1.a. Measure description

Cage traps can be used for the live capture of animals in terrestrial environments. A variety of related devices, including various forms of fish traps, are placed within aquatic environments, but these are considered in a separate assessment dealing with purely aquatic environments.

Cage traps typically have two features, a 'cage' to hold the captured animal, and some form of entrance which allows the animal to enter, but not to leave. The cage element comes in a variety of sizes, allowing cage traps to hold animals of different sizes, or multiple animals of the same species. Traps vary from small, single capture traps for small mammals [1], to large, semi-permanent, multi-capture corral traps for large ungulates [2]. These are typically made of wire, but the size of the mesh and its strength varies depending on the species. For some birds, netting stretched over a wooden frame is used to reduce weight and minimise the risk of injury [3]. Solid metal boxes are used in some cases, such as Longworth traps for small mammals [1], as they provide some degree of shelter from the elements and predation.

The entrance can include a variety of designs, such as spring or gravity operated doors, funnel and drop-in types. These designs are typically used to capture single animals. Funnel traps include a loose wire tube which allows the animal to enter, but the narrow end leading into the trap makes it difficult for the animal to exit. These traps often use a soft wire funnel which can be trampled by any animal in the trap, further complicating their exit. Drop-in traps include an entrance in the roof which allows the animal to 'drop' down into the cage, but which complicates exit by a jumping or flying animal.

Cage traps are used to capture a wide range of different species and their siting, setting and operation need to be tailored to the species if they are to be used humanely and effectively. Traps need to be of an appropriate size and a wide variety of commercial designs are available to cater to this from companies such as Havahart and Tomahawk, although there are many different suppliers. The choice of entrance design is also species specific, traps with spring operated doors are most frequently used to capture mammals, funnel and drop-in traps are more widely used for capturing birds. Various traps have also been used to capture reptiles and amphibians [4]. The behaviours of the target species, and possible non-target species in the same area can also be an important consideration when choosing or setting traps. Traps have the potential to catch a range of non-target species. Care in the choice, setting and baiting of the traps can minimise this risk, although it cannot be completely removed. Where traps are placed can add an element of selectivity to the species they catch. Traps for inquisitive predatory species such as American mink are typically set in tunnels [5] or on floating rafts [6]. Coypu traps may be set on floating platforms on water bodies [7]. Cage traps for corvids are typically set away from vegetation on open ground [3]. The size and design of the doors fitted to traps, their placement, or the baits used, can also be used to provide an element of species specificity. For example, the door and tunnel size used for mink traps may help prevent the unintended capture of otters in the same areas [5]. Although cage traps have been used in very limited cases for the capture of ruddy ducks (*Oxyura jamaicensis*), they are unlikely to provide a cost-effective method for the control of this species and their usefulness is limited to very specific circumstances [44]. This is because ruddy ducks spend the majority of their time on open water and are only rarely seen on land, which limits the attractiveness of traps for the control of this species. Various methods are used to attract the target species to enter, these include the use of suitable food as a bait or various odour-based lures [8,9]. Live animals can also be used as decoys in cage traps [10,11], but this approach raises particular concerns given the welfare of the decoy, and these designs are considered in a separate assessment of decoy traps.

Muskrats, raccoons and raccoon dogs are among the species listed under the Agreement on International Humane Trapping Standards AIHTS [12]. Cage traps are a type of restraining trap under the AIHTS. Under the AIHTS the humaneness of a cage trap is judged on the basis of a list of certain behavioural and injury indicators. For a cage trap to be considered humane, ≥80% of ≥20 trapped animals must show none of these indicators. This approval system limits the designs of trap that may be used to capture these particular species.

As cage traps hold live animals, they need to be designed and operated in such a way as to minimise risks to the welfare of the animal. These choices are heavily dependent on the nature of the species being trapped and the area of use. Captive animals can struggle in the cage, and the appropriate choice of cage dimensions, mesh size, wire gauge and treatment can be important to minimise the risk of injury. Burrowing species may attempt to dig their way out of the trap, so choice of flooring can be important to minimise risks to claws. Cages made of netting stretched over a wooden frame can be safer for some birds, which may fly in the trap and risk injury from metal mesh. The location in which cage traps are set can carry risks and trap setting needs to consider the risks associated with flooding, exposure to inclement weather, or vulnerability to potential predators. The size of the species and local climate also carry risks, for example small animals may be at particular risk of chilling or hunger while in the trap, while in hot climates animals may risk over-heating and dehydration. When trapping small mammals in Longworth traps, it is standard practice to include straw or paper as bedding material and sufficient food to maintain the animal overnight. In hot weather, the provision of shade and water can be equally important.

A review of injury rates associated with cage traps for mammals [13], compared them with other widely used devices such as snares or leg-hold traps. They conclude that animals captured in cage traps appear to undergo fewer traumas than those captured in snares and leg-hold traps [14]. Significantly, if checked regularly and used correctly, mortality rates associated with cage traps approach zero. Wounds appear to be less severe, with most injuries confined to skin abrasions and broken teeth, and injuries are often reduced by improved trap design and reduced mesh size [14,15]. Cage traps can capture a range of species, but unlike other trap methods, non-target species are typically released physically unharmed, the only distress experienced generally being that of restraint. Longworth traps set in areas where shrews may be accidentally captured should have a small hole to facilitate their escape since they have a high metabolic rate and often die in traps.

One of the largest studies of welfare impacts associated with cage traps [16], describes the injuries associated with capture for 5964 Badgers *Meles meles*. Traps were set overnight by trained, professional trappers. 88% of badgers received no detectable injuries as a result of being confined in the trap. Of those that were injured, 72% received only minor skin abrasions. A minority (1.8% of the total) acquired damage to the teeth or jaws that may have caused serious pain. Although trap rounds were commenced in the early morning, badgers were no more likely to sustain injuries when they remained in traps until later in the day. Coatings intended to give the wire mesh a smoother surface, were associated with a reduction in the incidence of minor skin abrasions, although it may have slightly increased the frequency of less common but more serious abrasions. A modification of the door design was associated with reduced tooth damage.

In addition to physical trapping injuries, welfare impacts on animals confined in cage traps may also include stress. Cage traps caused an increase in cortisol compared to untrapped individuals [17], but cortisol levels were in cage-trapped animals were lower than for individuals caught in leg-hold traps [17,18,19,20]. Significantly this was not related to injuries and therefore pain [20]. Both cage traps and leg-hold traps caused an increase in body temperature, heart rate and some blood metabolites, associated with increased activity, but animals caught in cage traps showed lower values than those caught in leg-hold traps, indicating lower physical activity when trapped [17,20].

The length of time an animal is held in the trap before being removed brings increasing risks to its welfare, and reduces the trap's ability to catch further animals. Legal requirements vary between Member States, but traps typically require at least daily checking to remove captured animals. More frequent checking is recommended to reduce these risks, but requires a choice to be made between the risks to animal welfare, the logistics of frequent checking and the disturbance it can bring to the local environment. A knowledge of the biology of the species can help with these choices. For species particularly active at first light, a check in the hours just after dawn will reduce the risk of animals being held for long periods. Similarly, for species at risk of predation when in the trap, a check just before dusk may reduce the risks from nocturnal predators. New technologies offer alternative approaches, such as electronic devices attached to the trap to send a message when the trap is triggered [21]. While commercial designs are available, their utility can be limited by access to a communication network, their cost, battery life and the frequency of false alerts. Even where these approaches are used, visual checking may also be needed to ensure animals are not held for long periods should the devices fail.

When removing animals from traps, care is needed to prevent stress and injury, to protect the health and safety of the operator and also to reduce the risk of other animals associating the trap with humans. Some species can become stressed when approached or handled. Covering the trap, minimising the number of people involved, or checking at night may all be useful in particular circumstances. Some captured animals may need to be given an anaesthetic while in the trap before they can be safely handled [22], others may best be moved into a dark container or sack to reduce stress. Minimising animal handling time is important to reduce stress. Wild animals present physical and health risks for the operator, particularly when handling larger or aggressive animals or those that may carry pathogens. The use of appropriate clothing such as gloves or possibly masks and goggles may be required. Operators may need vaccinations against high risk pathogens, or regular health surveillance. Some species, particularly those that live in social groups, may learn to associate traps with humans if they see captured animals being removed. This may lead to trap-shyness. Checking traps and removing animals when other animals are unlikely to be present, for example at night for diurnal species, may help reduce this risk.

As cage traps capture animals alive, they can be used in circumstances where there are risks of capturing non-target species which can be released unharmed. For example, live cage traps were chosen for capturing American mink in the Hebrides in Scotland, as lethal spring traps were considered to pose risks to otter populations in the area (Roy et al 2015). Similarly, cage traps are used to control grey squirrels in areas where native red squirrels are also present, as this species can then be released [23].

Cage traps suffer a number of shortcomings. Some species or individuals may not enter traps and will remain untrapped requiring other methods to remove these individuals. The frequency and implications of individual variation in trapability, including both trap-happy and trap-shy individuals, have been widely studied [24,25]. Large traps may also be bulky and difficult to transport, increasing their cost. The requirement for daily checking carries significant implications for man-power, often requiring dedicated trappers to check traps on a routine basis for as long as they are in operation. This may make cage traps less cost-effective as target animal densities are reduced, and other methods such as shooting or active searching using dogs [5] may become more cost-effective in the latter stages of a management programme. There are also site-specific limitations to the use of traps, for example, there will be some areas where human or animal disturbance, or legal restrictions, will mitigate against their use.

2.1.b. Integration with other measures

Cage traps are a widely used method for rapid eradication, eradication, control and containment for many different species. However, given the limitations associated with trap shy individuals, their low cost-effectiveness when capture rates are low, and limits to the sites where they can be set, cage traps are often used in association with other methods. For many programmes, they are used in association with shooting where the strengths and weaknesses of the two methods often complement each other.

Live capture in cage traps allows animals to be treated in a variety of ways post-capture. Thus cage traps are often used in association with bringing animals into captivity [26], to allow sterilisation [27] or the application of immunocontraceptive vaccines by injection ([28]. Captured animals may also be humanely despatched using a variety of different methods which are considered in other assessments.

2.2.a. Availability - species and objectives

Objective	Unknown objective		Rapid Eradication		Management					
					Eradication		Control		Containment	
Species	Avail.	Ref(s).	Avail.	Ref(s).	Avail.	Ref(s).	Avail.	Ref(s).	Avail.	Ref(s).
Acridotheres tristis	A		A	10	A	10	A		A	
Alopochen aegyptiaca	A		A		A		A	29	A	29
Callosciurus erythraeus	P		P		A	29	A	29	A	29
Corvus splendens	P		A							
Herpestes javanicus	P		A		A	30	A	30	A	30
Lepomis gibbosus										
Lithobates catesbeianus										
Muntiacus reevesi	A				A	29	A	29	A	29
Myocastor coypus	A		A		A	7	A	4, 46	A	4, 46
Nasua nasua	P		A	31	A	31	A	31	A	
Nyctereutes procyonoides	A		A	32	A	32	A	32, 47	A	32, 47
Ondatra zibethicus					A	29	A	29, 48, 49	A	4, 48, 49
Oxyura jamaicensis			A		A		A		A	
Perccottus glenii										

Plotosus lineatus										
Procyon lotor	A		A	26	A	33	A	47, 50, 51	A	47, 50, 51
Pseudorasbora parva										
Sciurus carolinensis	A		A		A	23	A	23	A	23
Sciurus niger	A		A		A	29	A	29	A	29
Tamias sibiricus	A		A		A	29	A	29, 52	A	29, 52
Threskiornis aethiopicus	P		P		P		P		P	
Trachemys scripta	A		P		A	4	A	4, 53, 54	A	4, 53, 54

2.2.b. Application – EU Member States and objectives										
Objective	**Unknown objective**		**Rapid Eradication**		**Management**					
					Eradication		**Control**		**Containment**	
Country	Avail.	Ref(s).	Avail.	Ref(s).	Avail.	Ref(s).	Avail.	Ref(s).	Avail.	Ref(s).
Austria										
Belgium			A	29	A	29	A	29	A	29
Bulgaria										
Croatia										
Cyprus										
Czech Republic										
Denmark			A	32	A	32	A	32	A	32
Estonia										
Finland			A	32	A	32	A	32	A	32
France										
Germany										
Greece										
Hungary										
Ireland										
Italy			A	33	A	33	A	34	A	34
Latvia										
Lithuania										
Luxembourg										
Malta										
Netherlands										
Poland										
Portugal										
Romania										
Slovakia										
Slovenia										

Spain										
Sweden			A	32	A	32	A	32	A	32
United Kingdom*			A	26	A	5	A	7	A	7

* Not an EU Member State

3. Humaneness of the measure			
3.1. Welfare for all measures			
Measure type (if applicable):	**Humaneness impact categories**		
Domain	**No impact**	**Mild-Moderate**	**Severe - Extreme**
1: Water deprivation, food deprivation, malnutrition		Provided trap checking frequency is appropriate to the species and local environmental conditions, trapping should not exceed short-term water or food restrictions that are within usual tolerance levels for the species.	
2: Environmental challenge		Provided trap checking frequency is appropriate to the species and local environmental conditions trapping should not cause more than short term exposure to environmental conditions which are outside the normal range encountered by the animal but remain within their physiological adaptive capacity.	
3: Injury, disease, functional impairment		Provided trap checking frequency is appropriate to the species and local environmental conditions, the impacts should not exceed disease/injury/functional impairment that results in	

		moderately severe debility or incapacity but from which recovery would normally occur spontaneously.	
4: Behavioural, interactive restriction		Provided trap checking frequency is appropriate to the species and local environmental conditions, trapping should not exceed mild interference with the behavioural needs of an animal.	Trapping carries a risk that captured animals may be approached or attacked by predators when confined in a trap. This carries the risk of severe physical consequences or stress but can be mitigated against by frequent trap checking robust trap construction and appropriate trap placement.
5: Anxiety, fear, pain, distress, thirst, hunger etc.		Provided trap checking frequency is appropriate to the species and local environmental conditions, trapping should not result in more than moderate anxiety, fear, pain, sickness, breathlessness, nausea, lethargy/ weakness, dizziness, unsatisfied thirst and/or hunger or other negative affective experiences causing distress.	

3.2. Mode of death (if relevant)			
Measure type (if applicable):	**Immediate death (i.e. no suffering)**	**Not immediate death (mild - moderate suffering)**	**Not immediate death (severe - extreme suffering)**
Rationale:	N/A		

3.3. Humaneness summary	If used appropriately by trained personnel, cage traps offer a flexible and relatively humane method of animal capture and restraint. If traps are checked at intervals appropriate for the species and are used correctly (e.g. avoiding extreme conditions and unsuitable locations), mortality rates associated with cage traps approach zero. Wounds appear to be less severe compared to other methods of animal capture or restraint, with most injuries confined to skin abrasions and broken teeth. The risks of these injuries can often be reduced by improved trap

	design and reduced mesh size as experience of their use on the species is gained [13]. Use of trap alarms are recommended to minimize time in the trap. As cage traps capture animals alive, they are often used in association with other methods for the treatment or despatch of the animal. The implications of these approaches are discussed elsewhere.

4. Costs and effectiveness of the measure	
General effectiveness of the measure	Cage trapping provides a widely used, flexible and effective method to catch and restrain a wide variety of different species. It is a frequently used and successful method to support the rapid eradication, eradication, control and containment of many invasive alien species and has been the method of choice for many large scale control programmes [5,23,29,34,35]. Cage traps also have a number of shortcomings. The requirement for regular checking brings significant implications for cost, and their cost-effectiveness can be low in situations where the rate of animal capture is poor. For most species, a proportion of animals may be trap shy and reluctant to enter traps. There are also likely to be areas where cage traps cannot be used, for example where there are significant levels of disturbance, which can limit their ability to achieve complete geographic coverage of an area. For eradication programmes these can be significant issues and alternative methods may need to be used alongside trapping.

4.1. Case studies	
CASE STUDY #1	
Measure type (if relevant):	Live Cage Trapping
Species:	American Mink *Neovison vison*
Objective:	Eradication of an established mink population on a series of islands, UK
Use of measure	Studies [5] describe how cage traps were chosen as the use of lethal spring traps was considered to be a risk for native otter (*Lutra lutra*) populations which were also present in the area. A total of 2545 live capture cage traps were dug into the ground and set in tunnels during the first 3 months of the project. At any time, around 10 % were set to catch animals, with the remainder left locked shut to prevent captures. This approach reduced the manpower needed to repeatedly set, lift and relocate traps, relying instead on large numbers of pre-located traps being used in rotation. The location of each trap was recorded using GPS. Traps were set and baited and were in use for a 1 or 2 week period, when they were checked daily. As the work progressed, lures based on secretions from the anal glands of captured animals were also placed in the traps to improve trapping success [9]. On average, each trap was in use during four or five separate periods per year.
Combined with other measure(s):	While the majority of animals were caught in cage traps set throughout suitable habitats in the area, when mink densities were low animals were also located using dogs and then a high density of cage traps were set in the immediate area to catch these remaining individuals (Roy et al 2015).

	Captured animals were humanely dispatched with a shot to the brain stem using .22 calibre air pistols.
Country(ies) of application:	This example of the use of cage traps is from the UK, in particular the chain of islands off the west coast of Scotland known as the Uists. American mink are also widely controlled in Europe, with accounts from Finland [36], Estonia [37], England [38], Scotland [39], as well as Iceland [40]. However, these programmes not have been based solely on cage traps and have used a wide variety of methods including lethal and leg-hold trap designs.
Geographic scale (km^2) and/or population size measure applied to:	Mink were established throughout the island chain known as the Outer Hebrides. The project [5] aimed to start control in the southern islands where mink had only recently established, and to then move north as experience was gained. This first phase of the programme involved the eradication of mink from the island groups of North Uist, Benbecula and South Uist, collectively known as The Uists, but also aimed to reduce mink density from neighbouring South Harris to minimise the risk of recolonisation from islands further to the North. The control site in the Uists comprised approximately 356 islands and skerries totalling 850 km^2. The area contained a complex mixture of freshwater and saltwater habitats, with 1116 km of coastline, 2416 km of loch shore and 189 km of rivers and streams.
Time period:	Control in the Uists was concentrated over the period 2001-2005. After completion of this phase, control was extended north to eradicate mink from the rest of the island chain of the Outer Hebrides, including the large islands of Harris and Lewis [41]. This latter phase of control was based on the use of lethal spring traps as experience in the Uists suggested the risk to native otters was small, obviating the need to use cage traps.
Effort:	Traps were in operation throughout the year for 4-5 years. Over this period effort comprised 100,824 trap nights (one trap in operation for one night=one trap night) on the Uists, [5]. This involved 23.5 man years of trapper effort [42]. An additional 41,674 trap nights were deployed on South Harris.
Costs:	Overall costs:
	£1.175m at 2018 prices
	Personnel costs:
	No breakdown available, but an estimate could be made based on the known number of man-years of effort.
	Equipment and infrastructure:
	No breakdown available
	Other, including overheads:
	No breakdown available
Effectiveness:	Successful. Overall a total of 228 mink was caught in The Uists, with the last capture in March 2005. After this date, despite a further seven months of intensive trapping and searching effort, no further signs of mink were found and they were considered likely to have been removed from this region.

CASE STUDY #2	
Measure type (if relevant):	Cage trap
Species:	Raccoon *Procyon lotor*

Objective:	Rapid Eradication
Use of measure	Cage traps have been used to capture individual non-native species with the objective of rapid eradication, capturing escaped animals to reduce the risk of them forming a self-sustaining breeding population. Accounts [26] provide an example of this approach, describing the capture of an individual raccoon from a town in the UK. The presence of the animal was identified through reports from the public, with a cluster of sightings in a local area over a period of months, suggesting one or more animals surviving in the wild for some time. The location of the animal was confirmed by the use of field signs and camera traps. A cage trap was then set to capture the animal. This was a large metal mesh cage trap with a gravity powered door designed for use on badgers. The trap was left unset, but baited for a few days for the animal to familiarise itself and begin to visit the trap on a regular basis, with this being confirmed through the use of cameras. Once the bait was being regularly taken, the trap was set. The door was triggered to lock when the animal disturbed the bait placed at the back of the cage. The trap was fitted with an electronic device to send a phone message to the operator when the trap was sprung. In this case, the operator was able to visit the trap within an hour of the animal being caught. The captured animal was then coaxed from the trap into a smaller holding cage for transport. This holding cage was fitted with separate handles to allow it to be carried by the operator without the risk of them being bitten or scratched, and covered with a blanket for transport to calm the animal. The animal was then brought to a central holding facility and anaesthetised before being removed from the holding cage.
Combined with other measure(s):	The captured animal was brought into captivity, surgically sterilised by a vet, and then rehomed in a local zoo.
Country(ies) of application:	This example is from the UK, but similar approaches are widely used to capture individual animals in many other countries.
Geographic scale (km^2) and/or population size measure applied to:	The original sightings were spread over a distance of 2km over a period of a few months. The number of animals involved was unknown when trapping started, but further sightings ceased after one animal was captured, suggesting that only a single animal was involved.
Time period:	The period from the first sighting of a free-living raccoon, to its successful capture was around two months. Once the focus of the animal's activity was found, and a suitable trapping site identified, the process of pre-baiting, setting the trap and capturing the animal took a few days.
Effort:	One person working part-time over one month.
Costs:	Overall costs:
	Not recorded
	Personnel costs:
	Not recorded, but less than one month's costs for one person.
	Equipment and infrastructure:
	Not recorded, but this used an existing trap and equipment designed for the capture and restraint of native badgers.

	Other, including overheads:
	Not recorded.
Effectiveness:	Fully effective. The animal was quickly captured and removed from the wild.

CASE STUDY #3	
Measure type (if relevant):	Cage Trap
Species:	*Myocastor coypus* Coypu
Objective:	Local control and damage reduction
Use of measure	Since the 1960s, released coypu *Myocastor coypus* have established self-sustaining populations across large areas of Italy. Under Italian law, coypu can be harvested year-round for damage prevention, but control operations require official authorisation and can only be undertaken by authorised operators. The only two coypu control techniques allowed in Italy are live-trapping by means of cage-traps, and direct shooting. Animals caught in cage traps are despatched by shooting or euthanised 34]. Given the widespread nature of the Coypu population, there is no realistic prospect of eradication, so the most common management policies are permanent control campaigns carried out locally in response to social pressures and perceptions of damage. Studies [34] present a detailed cost-benefit analysis of the large scale control of this species in Italy between 1995 and 2000. This details both the use of cage traps and shooting as part of a combined approach to reduce economic damage and slow or prevent the spread of this species. Data for this account was obtained by questionnaires sent to regional control groups, and focused on the costs of control and associated damage. A total of 220,688 coypus were removed during 1995-2000; 54% through trapping and 46% through direct shooting. A longer term view of coypu control in the region of Piedmont i[35] documents an initial increase in coypu control and a decline in reported damage, but only after control had been in place for a number of years.
Combined with other measure(s):	This programme used a combination of cage trapping and shooting to control local populations. Trapped coypus were either shot or, according to Directive 93/119/CE on animal welfare or euthanised at the capture site [34].
Country(ies) of application:	This example is based on experience in Italy, although the control of this species is widespread throughout its introduced range in Europe (4,29]. Similar methods were used to eradicate this species from the UK [7].

Geographic scale (km²) and/or population size measure applied to:	Over the period 1995-2000, the Coypu range in Italy was estimated as 68,600km^2, although the species was still spreading and its future range was predicted to be 2.5-3.5 times as large [34].	
Time period:	This example relates to the period 1995-2000. However, this approach to damage management would need to be continued indefinitely to maintain any benefits.	
Effort:	Over the period 1995-2000 the number of operators officially annually involved in control work increased from 241 to 1,479, and the number of traps purchased annually rose from 1,260 to 7,155. Over this period the average control intensity was estimated as 1.2 coypu removed/year/km^2 at total cost of EUR 2,614,408. between 1995 and 2000.	
Costs:	Overall costs:	
	During 1995-2000, EUR 2,614,408 were spent on direct coypu control. It was estimated that 60% of the total cost of control was devoted to cage trapping. The estimate of cost per animal removed was € 13.25/coypu trapped and € 8.21/coypu shot.	
	Personnel costs:	
	Not directly recorded, but coypu control using both cage trapping and shooting involved an average of 789 personnel per year.	
	Equipment and infrastructure:	
	Not recorded, but over the period 1995-2000 a total of 20,430 coypu cage traps were purchased.	
	Other, including overheads:	
	Not recorded	
Effectiveness:	At a national level, the applied control effort neither effectively contained the ongoing rapid population expansion nor the increase in economic losses. Despite the removal of 220,688 animals between 1995-2000, the coypu removal rate did not exceed the population rate of increase [34]. At a more local scale, some well-planned control programmes did achieve significant results in terms of population containment [35], reduction of economic losses [43] and the preservation of biodiversity [35]. The case study [34] relates to 1995-2000 which was the period when the coypu was rapidly spreading within Italy. A longer-term view (1997-2006) in the region of Piedmont has been published [35]. These authors document an initial increase in coypu numbers and control efforts, but that reported damage only began to decline after a number of years of control. In Piedmont a continuing trapping and shooting control program to limit crop-damage by coypu proved successful and cost-effective.	

CASE STUDY #4	
Measure type (if relevant):	Cage Trap

Species:	*Callosciurus erythraeus* Pallas's squirrel
Objective:	Eradication
Use of measure	Using wire live capture cage traps a total of 249 Pallas' squirrels have been captured during 3 years (winter and spring months only), neutered and temporarily rehomed in a rescue centre in Opglabbeek, Belgium [45]. The animals have then been relocated in various zoos and sanctuaries. In captivity a Pallas' squirrel can live up to 15 years.
Combined with other measure(s):	The method was combined with keeping in captivity (not evaluated here).
Country(ies) of application:	Netherlands
Geographic scale (km^2) and/or population size measure applied to:	50 Km^2 near Weert, Ell and in the adjacent area
Time period:	Project started in 2008 and ended in 2014 – trapping between 2011-2013
Effort:	During the winter months of 2011-2012 300 traps were deployed, this was repeated in the winter of 2012-2013.
Costs:	Overall costs:
	The overall cost of the capture phase is about EUR 330,000 (assigned to labour costs). This information is unavailable for keeping the animals in captivity.
	Personnel costs:
	EUR 330,000
	Equipment and infrastructure:
	Other, including overheads:
Effectiveness:	249 squirrels have been captured in 3 year. One more Pallas' squirrel has been detected in 2015 and captured. The population has been successfully eradicated. To our knowledge no escapes have occurred from captivity.

4.2. Costs effectiveness summary	Cage traps have provided a cost-effective method to support a number of large scale control and eradication programmes [5,23,35,45]. Where the objective is eradication, success is measured by the removal of the entire population. In such cases, cage traps are best used in combination with another method to remove any trap shy members of the population, and to locate animals when capture rates are low and the continued man-power needs for regular trap checking can have low cost-effectiveness. Where cage trapping is used to reduce spread and damage as part of a long-term control programme, care is needed to ensure that effort is sufficient to achieve the stated objectives [34,35], this is a general statement for all control methods and not specific to cage traps.

	The flexibility and humaneness of cage traps also makes them an effective method for the removal of animals during rapid eradication exercises [26].

5. Side effects	
Non-target native species, their habitats and the broader environment:	Positive:
	Live capture traps have the advantage that non-target captures can be released unharmed. This can enable their use in areas where other species are also at risk of capture. For example, this was the motivation for selecting live capture traps for mink control in the Uists, as juvenile otters were originally considered a potential bycatch and a method that allowed their release unharmed was required [5]. Similarly, live capture cage traps are recommended for the control of grey squirrels in areas that also contain the native red squirrel, which can be released if captured [23]. In addition technology is currently being developed on 'smart life traps' which are using image recognition to identifiy if the correct species (coypu or muskrat) have been captured (see Life MICA project – Management of invasive coypu and muskrat in Europe https://lifemica.eu/).
	Negative:
	Cage traps can catch non-target species although these can be released after capture.
Other invasive alien species:	Positive:
	Traps set for one species may also capture other alien species. For example, traps set for mink on the Uists also caught introduced brown rats and ferrets which were also present on the islands [5].
	Negative:
Public health and well-being:	Positive:
	Negative:
Economic:	Positive:
	Negative:

6. Conclusion

Overall assessment of the measure (qualitative)

Cage trapping provides a widely used, flexible and effective method to catch and restrain a wide variety of different species. It is a frequently used and successful method to support the rapid eradication, eradication, control and containment of many invasive alien species and has been the method of choice for many large scale control programmes [5,23,29,34,35].

If used appropriately by trained personnel, cage traps offer a flexible and relatively humane method of animal capture and restraint. If checked regularly and used correctly, mortality rates associated with cage traps approach zero. Wounds appear to be less severe, than with other methods of animal capture or restraint, with most injuries confined to skin abrasions and broken teeth. The risks of these injuries can often be reduced by improved trap design and reduced mesh size as experience of their use on the species is gained [13]. Risks to non-target species are less with cage traps than with lethal traps and other control methods.

Cage traps also have a number of shortcomings. The requirement for regular checking brings significant implications for cost and their cost-effectiveness can be low in situations where the rate of animal capture is poor. For most species, a proportion of animals may also be trap shy and reluctant to enter traps. There are also likely to be areas where cage traps cannot be used, for example where there are significant levels of disturbance, which can limit their ability to achieve complete geographic coverage of an area. For eradication programmes these can be significant considerations and alternative methods are typically needed alongside trapping to overcome these issues.

Assessor:	Peter Robertson
Reviewer 1:	Sandra Baker
Reviewer 2:	Riccardo Scalera

7. References

1 Anthony, N.M., Ribic, C.A., Bautz, R. and Garland Jr, T., 2005. Comparative effectiveness of Longworth and Sherman live traps. *Wildlife Society Bulletin*, *33*(3), pp.1018-1026.
2 Williams, B.L., Holtfreter, R.W., Ditchkoff, S.S. and Grand, J.B., 2011. Trap style influences wild pig behavior and trapping success. *The Journal of Wildlife Management*, *75*(2), pp.432-436.
3 Hartley, F.G., Campbell, S.T. & Jamieson, S. 2016. Assessing the nature and use of corvid cage traps in Scotland: Part 2 of 4 – Field survey of trap use in Scotland 2014-15. *Scottish Natural Heritage Commissioned Report No. 932.*
4 Sarat, E., Mazaubert, E., Dutartre, A., Poulet, N. and Soubeyran, Y., 2015. Invasive alien species in aquatic environments. Practical information and management insights. Volume 2. Management insights. Onema. Knowledge for action series, 252 pp.
5 Roy, S.S., Chauvenet, A.L.M. & Robertson, P.A. Removal of American mink (Neovison vison) from the Uists, Outer Hebrides, Scotland. *Biol Invasions* 17, 2811–2820 (2015). https://doi.org/10.1007/s10530-015-0927-y
6 Reynolds, J.C.; Richardson, S.M.; Rodgers, B.J.E.; Rodgers, O.R.K. Effective control of non-native American mink by strategic trapping in a river catchment in mainland Britain. J. Wildl. Manag. 2013, 77, 545-554, doi:10.1002/jwmg.500.
7 Gosling, L.M. & Baker, S.J. 1989: The eradication of muskrats and coypus from Britain. - Biological Journal of Linnaean Society 38: 39-51.
8 Andelt, W.F. and Woolley, T.P., 1996. Responses of urban mammals to odor attractants and a bait-dispensing device. *Wildlife Society Bulletin*, pp.111-118.
9 Roy, S.S., Macleod, I. and Moore, N.P., 2006. The use of scent glands to improve the efficiency of mink (*Mustela vison*) captures in the Outer Hebrides. *New Zealand Journal of Zoology*, *33*(4), pp.267-271.
10 Saavedra S (2010) Eradication of invasive Mynas from islands. Is it possible? Aliens 29

11 Huysentruyt, F, Adriaens, T., De Bus, K., Van Moer, K., Standaert, S., and Casaer, J.. "Testing the efficacy of a floating multicapture trap for invasive Egyptian geese (*Alopochen aegyptiacus*)." In *IUGB (International Union Of Game Biologists) Congress 2013*. 2013.
12 EU (1998) https://ec.europa.eu/world/agreements/downloadFile.do?fullText=yes&treatyTransId=1428
13 Iossa, G., Soulsbury, C.D. and Harris, S., 2007. Mammal trapping: a review of animal welfare standards of killing and restraining traps. *Animal Welfare*, *16*(3), p.335.
14 Powell RA and Proulx G 2003 Trapping and marking terrestrial mammals for research: integrating ethics, performance criteria, techniques, and common sense. *ILAR Journal 44:* 259-276
15 Short J, Turner B and Risbey D 2002 Control of feral cats for nature conservation. III. Trapping. *Wildlife Research 29:* 475-487
16 Woodroffe, R., Bourne, F.J., Cox, D.R., Donnelly, C.A., Gettinby, G., McInerney, J.P. and Morrison, W.I., 2005. Welfare of badgers (*Meles meles*) subjected to culling: patterns of trap-related injury. *Animal Welfare*, *14*(1), pp.11-17.
17 White PJ, Kreeger TJ, Seal US and Tester JR 1991 Pathological responses of red foxes to capture in box traps. *Journal of Wildlife Management 55:* 75-80
18 Kreeger TJ, White PJ, Seal US and Tester JR 1990 Pathological responses of red foxes to foot hold traps. *Journal of Wildlife Management 54:* 147-160
19 Cross ML, Swale E, Young G and Mackintosh C 1999 Effect of field capture on the measurement of cellular immune responses in wild ferrets (*Mustela furo*), vectors of bovine tuberculosis in New Zealand. *Veterinary Research 30:* 401-410
20 Warburton B, Gregory NG and Bunce M 1999 Stress response of Australian brushtail possums captured in foot-hold and cage traps. In: Proulx G (ed) *Mammal Trapping* pp 53-66. Alpha Wildlife Research & Management Ltd: Alberta, Canada
21 Larkin, R.P., VanDeelen, T.R., Sabick, R.M., Gosselink, T.E. and Warner, R.E., 2003. Electronic signaling for prompt removal of an animal from a trap. *Wildlife Society Bulletin*, pp.392-398.
22 Fahlman, Å., 2008. *Advances in wildlife immobilisation and anaesthesia* (Vol. 2008, No. 84).
23 Schuchert, P., Shuttleworth, C.M., McInnes, C.J., Everest, D.J. and Rushton, S.P., 2014. Landscape scale impacts of culling upon a European grey squirrel population: can trapping reduce population size and decrease the threat of squirrelpox virus infection for the native red squirrel?. *Biological invasions*, *16*(11), pp.2381-2391.
24 Nichols, J.D., Hines, J.E. and Pollock, K.H., 1984. Effects of permanent trap response in capture probability on Jolly-Seber capture-recapture model estimates. *The Journal of Wildlife Management*, *48*(1), pp.289-294.
25 King, C.M., Davis, S.A., Purdey, D. and Lawrence, B., 2003. Capture probability and heterogeneity of trap response in stoats (*Mustela erminea*). *Wildlife Research*, *30*(6), pp.611-619.
26 APHA (2016) Rebel Raccoon Rehomed. https://aphascience.blog.gov.uk/2016/08/01/rebel-raccoon-rehomed/
27 Scapin, P., Ulbano, M., Ruggiero, C., Balduzzi, A., Marsan, A., Ferrari, N. and Bertolino, S., 2019. Surgical sterilization of male and female grey squirrels (*Sciurus carolinensis*) of an urban population introduced in Italy. *Journal of Veterinary Medical Science*, pp.18-0319.
28 Massei, G., Cowan, D.P., Coats, J., Bellamy, F., Quy, R., Pietravalle, S., Brash, M. and Miller, L.A., 2012. Long-term effects of immunocontraception on wild boar fertility, physiology and behaviour. *Wildlife Research*, *39*(5), pp.378-385.
29 Adriaens, T., Branquart, E., Gosse, D., Reniers, J. and Vanderhoeven, S., 2019. Feasibility of eradication and spread limitation for species of Union concern sensu the EU IAS Regulation (EU 1143/2014) in Belgium. Report prepared in support of implementing the IAS Regulation in Belgium.
30 Yamada, F., 2002. Impacts and control of introduced small Indian mongoose on Amami Island, Japan. *Turning the tide: the eradication of invasive species. IUCN SSC Invasive Species Specialist Group, IUCN, Gland*, pp.389-392.
31 Mayol, J., Álvarez, C. and Manzano, X., 2009. Presence and control of the coati, *Nasua nasua* L, and other carnivores introduced in recent times in Mallorca. *Bolletí de la Societat d'Història Natural de les Balears*, *52*, pp.183-191.
32 Dahl, F., Åhlén, P-A, Swartström, J., Lindström, M. Simmelsgaard Platz, ML. (2013) Management of the invasive Raccoon Dog (Nyctereutes procyonoides) in the north-European countries. LIFE09 NAT/SE/000344 Swedish Association for Hunting and Wildlife Management, Öster-Malma, 611 91 Nyköping, Sweden. https://ec.europa.eu/environment/life/project/Projects/index.cfm?fuseaction=home.showFile&rep=file&fil=LIFE09_NAT_SE_000344_LAYMAN.pdf
33 Mazzamuto, M.V., Panzeri, M., Bisi, F., Wauters, L.A., Preatoni, D. and Martinoli, A., 2020. When management meets science: adaptive analysis for the optimization of the eradication of the Northern raccoon (Procyon lotor). *Biological Invasions*, pp.1-12.
34 Panzacchi, M., Cocchi, R., Genovesi, P. and Bertolino, S., 2007. Population control of coypu Myocastor coypus in Italy compared to eradication in UK: a cost-benefit analysis. *Wildlife Biology*, *13*(2), pp.159-171.
35 Bertolino, S. and Viterbi, R., 2010. Long-term cost-effectiveness of coypu (Myocastor coypus) control in Piedmont (Italy). *Biological invasions*, *12*(8), pp.2549-2558.
36 Nummelin, J. and Högmander, J., 1998. Uusi menetelmä minkin poistamiseksi ulkosaaristossa on tuottanut hyviä tuloksia. *Metsästäjä*, *47*(1), pp.16-18.
37 Genovesi P., 2000. Guidelines for eradication of terrestrial vertebrates: a European contribution to the invasive alien species issue. Council of Europe document T-PVS, vol. 65, Strasbourg, 30 pp

38 Reynolds, J.C., Short, M.J. and Leigh, R.J., 2004. Development of population control strategies for mink *Mustela vison*, using floating rafts as monitors and trap sites. *Biological Conservation*, *120*(4), pp.533-543.
39 Bryce, R., Oliver, M.K., Davies, L., Gray, H., Urquhart, J. and Lambin, X., 2011. Turning back the tide of American mink invasion at an unprecedented scale through community participation and adaptive management. *Biological Conservation*, *144*(1), pp.575-583.
40 Stefansson RA, von Schmalensee M, Skorupski J (2016) A tale of conquest and crisis: invasion history and status of the American mink (Neovison vison) in Iceland. Acta Biol 23:87–100
41 Lambin XL, Cornulier T, Oliver MK and Fraser EJ, 2014. Analysis and future application of Hebridean Mink Project data. Scottish Natural Heritage Commissioned Report No. 522
42 Robertson, P.A., Adriaens, T., Lambin, X., Mill, A., Roy, S., Shuttleworth, C.M. and Sutton-Croft, M., 2017. The large-scale removal of mammalian invasive alien species in Northern Europe. *Pest Management Science*, *73*(2), pp.273-279.
43 Velatta, F. & Ragni, B. 1991: La popolazione di nutria del lago Trasimeno. Consistenza, struttura e controllo numerico. - In: Spagnesi, M. & Toso, S. (Eds.); Proceedings of II National Congress of Wildlife Biologists. Supplementi alle Ricerche di Biologia della Selvaggina, XIX, pp. 311-326.
44 Henderson, I., 2009. Progress of the UK Ruddy Duck eradication programme. *British Birds*, *102*(12), p.680.
45 La Haye, M. (2020). Pallas' squirrel eradication in the Netherlands. Case study in: Invasive Alien Species Colonisation Prevention: Your guide to early detection and rapid response.
46 De Sousa, T. (2019). Plan d'action pour espèces exotiques envahissantes au Grand-Duché de Luxembourg: le Ragondin, Myocastor coypus (Molina, 1792). In L. Administration de la nature et des forêts (Ed.), (Vol. 20 pp).
47 Forvaltningsplan for mink, mårhund og vaskebjørn i Danmark (2020). https://mst.dk/media/191343/netversion-miljoestyrelsen_forvaltningsplan_2020-mink-maarhund-og-vaskebjoern.pdf
48 De Sousa, T. (2019). Plan d'action pour espèces exotiques envahissantes au Grand-Duché de Luxembourg: le Raton laveur, Procyon lotor (Linnaeus, 1758). In L. Administration de la nature et des forêts (Ed.), (13/12/2019 ed.).
49 Bekendtgørelse om bekæmpelse af bisamrotter - BEK nr. 819 (1987). https://www.retsinformation.dk/eli/lta/1987/819
50 De Sousa, T. (2019). Plan d'action pour espèces exotiques envahissantes au Grand-Duché de Luxembourg: la Tortue de Floride, Trachemys scripta ssp. (Schoepff, 1792). In L. Administration de la nature et des forêts (Ed.), (13/12/2019 ed.).
51 Bekendtgørelse om vildtskader - BEK. 1006 (2020). https://www.retsinformation.dk/eli/lta/2020/1006
52 Sibirisches Streifenhörnchen – Management- und Maßnahmenblatt zu VO (EU) Nr. 1143/2014 (2018). https://neobiota.bfn.de/unionsliste/art-19-management.html
53 De Sousa, T. L., Louis. (2019). Plan d'action pour espèces exotiques envahissantes au Grand-Duché de Luxembourg: le Rat musqué, Ondatra zibethicus (Linnaeus, 1766). In L. Administration de la nature et des forêts (Ed.), (13/12/2019. ed.).
54 Buchstaben-Schmuckschildkröte – Management- und Maßnahmenblatt zu VO (EU) Nr. 1143/2014 (2018). https://neobiota.bfn.de/unionsliste/art-19-management.html

Appendix 20. Neck-hold, and snares

1. Measure name			
1.1. English:	Neck-hold traps, and snares		
1.2. Lethal or non-lethal:	Non-lethal		
1.3. Other languages (if available):			
Bulgarian	Капани и примки за врата и за крака за живо улавяне	Italian	Trappole per il collo e lacci
Croatian	Zamke s omčama i stupice	Latvian	Kakla slazdi un cilpas
Czech	Oka a za krk držící pasti	Lithuanian	Smaugiamieji spąstai ir kilpos
Danish	Snare fælde	Maltese	
Dutch	Strikken en draadvallen	Polish	Zaciskowe pułapki duszące i wnyki
Estonian	Poovad lõksud ja silmused	Portuguese	Armadilhas e laços para segurar o pescoço
Finnish	Kaulanaru- ja jalkanaruansat	Romanian	Laţuri
French	Collets et pièges à fil	Slovak	Oká a za krk držiace pasce
German	Schlingen und schlingfallen	Slovenian	Zanke
Greek	Βρόγχοι αυχένα και θηλιές	Spanish	Trampas para capturar por el cuello y lazos
Hungarian	Nyakszorító csapdák és hurkok	Swedish	Snaror
Irish			

2. Technical details of measure
2.1.a. Measure description
These are a group of unenclosed traps and restraints designed for live capture of animals, including IAS, which are left unattended in the field. In 2008 an Agreement on International Humane Trapping Standards (AIHTS) between the EU, Canada, Russia and the US, came into force, establishing humane trapping standards to ensure a sufficient level of welfare of trapped animals. According to the standards, countries are committed to establishing appropriate processes for testing and certifying trapping methods following the international humane trapping standards. Furthermore, the use of traps that are not certified in accordance with humane trapping standards must be prohibited. However, according to Council Regulation (EEC) 3254/91 adopted in 1991 the use of leg-hold traps is already prohibited within the EU (Implementation of humane trapping in the EU, https://ec.europa.eu/environment/biodiversity/animal_welfare/hts/index_en.htm). According to this Regulation, 'leg-hold trap means a device designed to restrain or capture an animal by means of jaws which close tightly upon one or more of the animal's limbs, thereby preventing withdrawal of the limb or limbs from the trap' (Art. 1). Therefore, leg-hold traps are not considered here and only snares traps and similar are considered. According to the Habitats Directive (Council Directive 92/43/EEC), Annex VI lists the means of capture and killing prohibited under Article 15 which include traps which are non-selective according to their principle or their conditions of use. This provision transposes a similar provision of the Convention on the Conservation of European Wildlife and Natural Habitats (Bern Convention) into EC law. If a trap is designed to kill certain animal

species, they cannot be considered as non-selective according to their principle of use. However, their conditions of use must ensure selectivity, i.e. they must be set in a manner which does not allow non-target species to be trapped." (see https://www.europarl.europa.eu/doceo/document/E-8-2016-000336-ASW_EN.html). Snare traps can be designed to improve selectivity maintaining effectiveness [15].

Here we consider leg-hold snares, neck-hold traps/snares, and non-lethal stop-snares (neck hold traps), which consist of wire loops that tighten around the leg (placed horizontally) or neck (placed vertically) of the target species but do not close beyond a certain diameter so as not to kill the animal and are attached to the ground or an anchor by a chain or cable [1,2]. However, snares are permitted only in four EU Member States: Ireland, France, Spain and Belgium.

Snares are widely used for restraining and capturing mammals, especially predators such as foxes [9,12], but also marmots [10] and beavers [11]. Neck hold traps and leg hold snares have both been used for capturing raccoons [1]. As the coati is closely related to the raccoon (same family: Procyonidae) and the raccoon dog is morphologically quite similar to the raccoon, the measure may also apply to the two latter species. However, modified neck-hold snares are not recommended to manage animal populations due to welfare concerns [2].

A particular case is the egg trap, which is a live restraint device constructed of durable white nylon and steel with an enclosed trigger and casing that encapsulates the foot of an animal [6]. It was designed especially for the humane capturing of raccoons and resulted to be highly effective [6,7,8]. These traps could be considered a sort of leg-snares traps, and therefore are legal in Europe. Egg traps are considered more effective than cage traps for both raccoon genders and particularly for males [8]. However, injuries are common with these traps. Applying a scale of injuries evaluation to 62 egg trapped raccoons with a score system where >50 points indicates serious damage and >125 points severe damage, 63% showed no or limit injuries, 23% serious injuries and 14% severe damage, including self-mutilation (3%) [8]. According to [6], egg traps do not cause serious injuries to raccoons if the duration of entrapment is less than 12 hours, after which the risk of self-mutilation increases.

Individuals caught in non-lethal snare traps often sustain injuries from the wire ligature, which can in turn lead to tissue necrosis (pressure necrosis) [2]. Moreover, snares capture and stress a large number of individuals of non-target species and can cause them serious injuries which could affect survival [2,3,4]. There is a largely negative public perception against the use of non-lethal snares due to the high rate of injuries they could cause [5]. For instance, Iossa et al. [1] reported the percentage of injury for different species, with leg-hold snares affecting 18% of raccoons, and another study showed that coypu survival after one year was 50% lower in animals trapped in leg-hold traps in respect to cage traps [13]. However, expert staff could reduce injury or deaths with appropriate snare design, selection of locations, and correct procedure used [4].

2.1.b. Integration with other measures

Snares traps can be used for lethal or non-lethal control; here we consider only non-lethal control. When animals are live-trapped, they should then be dispatched with another method, e.g. gas or chemical euthanasia.

Snares are widely used to capture mammals for research, however management experience is quite limited. The only eradication project retrieved, which was on raccoon control, was based on the integrated use of cage-traps with more limited use of egg traps.

2.2.a. Availability - species and objectives										
Objective	**Unknown objective**		**Rapid Eradication**		**Management**					
					Eradication		**Control**		**Containment**	
Species	Avail.	Ref(s).	Avail.	Ref(s).	Avail.	Ref(s).	Avail.	Ref(s).	Avail.	Ref(s).
Acridotheres tristis										
Alopochen aegyptiaca										
Callosciurus erythraeus										
Corvus splendens										
Herpestes javanicus	P	1,9,12,14			P	14	P	14	P	14
Lepomis gibbosus										
Lithobates catesbeianus										
Muntiacus reevesi										
Myocastor coypus	P	13								
Nasua nasua	P	1,9,12,14			P	14	P	14	P	14
Nyctereutes procyonoides	P	1,9,12,14	P		P	14	P	14	P	14
Ondatra zibethicus										
Oxyura jamaicensis										
Perccottus glenii										
Plotosus lineatus										
Procyon lotor	P	6,7,8			A	14	A	14	A	14
Pseudorasbora parva										
Sciurus carolinensis										
Sciurus niger										
Tamias sibiricus										
Threskiornis aethiopicus										
Trachemys scripta										

2.2.b. Application – EU Member States and objectives										
Objective	**Unknown objective**		**Rapid Eradication**		**Management**					
					Eradication		**Control**		**Containment**	
Country	Avail.	Ref(s).	Avail.	Ref(s).	Avail.	Ref(s).	Avail.	Ref(s).	Avail.	Ref(s).
Austria										
Belgium										
Bulgaria										
Croatia										
Cyprus										
Czech Republic										
Denmark										
Estonia										

Finland										
France										
Germany										
Greece										
Hungary										
Ireland										
Italy					X	14	X	14	X	14
Latvia										
Lithuania										
Luxembourg										
Malta										
Netherlands										
Poland										
Portugal										
Romania										
Slovakia										
Slovenia										
Spain										
Sweden										
United Kingdom*										

* Not an EU Member State

3. Humaneness of the measure			
3.1. Welfare for all measures			
Measure type (if applicable):	**Humaneness impact categories**		
Domain	**No impact**	**Mild-Moderate**	**Severe - Extreme**
1: Water deprivation, food deprivation, malnutrition		Animals are usually trapped in the evening or during the night and traps are checked early in the morning. Animals could then remain from a few hours to the whole night without water and food.	

2: Environmental challenge		In cold areas animals could be exposed to low temperatures which are outside their thermoneutral range.	
3: Injury, disease, functional impairment		According to available literature, moderate injuries are frequent, including damage to tendons or ligaments and amputation of a digit.	Severe to extreme injuries are possible, with luxation or fractures of the limbs or amputation. In some cases, the wire can twist and tighten, becoming self-locking and leading to strangulation or severe injuries. Some animals attempt to gnaw through the wire, causing it to fray so that it cannot run [1, 4, 6, 15].
4: Behavioural, interactive restriction			Animals trapped in snares often perform severe abnormal and self-directed behaviour, such as chewing/biting of feet and limbs when restrained; this could lead in some cases to amputation. Trapped animals try to run, jump or scrabble, often for several hours or more. Trapped animals are visible and attackable by predators; they cannot escape, and defence is difficult.
5: Anxiety, fear, pain, distress, thirst, hunger etc.			Retrained animals feel anxiety, fear, pain, sickness, breathlessness that may lead to self-amputation of the limbs, with high risk of dead.

3.2. Mode of death (if relevant)			
Measure type (if applicable):	**Immediate death (i.e. no suffering)**	**Not immediate death (mild - moderate suffering)**	**Not immediate death (severe - extreme suffering)**
Rationale:	N/A		

3.3. Humaneness summary	There is a great concern on the humaneness of snares traps. Individuals caught in non-lethal snare traps often sustain injuries from the wire ligature, which can in turn lead to tissue necrosis (pressure necrosis) and sometimes experience self-mutilation or strangulation. Use of trap alarms is recommended to minimize time in the trap. Moreover, snares capture and stress a large number of individuals of non-target species and can cause them serious injuries which could affect survival after release.

4. Costs and effectiveness of the measure	
General effectiveness of the measure	Snare traps are usually used for trapping predators, but information on their effectiveness in controlling species of European Concern is scanty. A study [7] showed that egg traps were more effective in trapping raccoons than cage traps (the standard for capturing these species in Europe, where leg-hold traps are not allowed).

4.1. Case studies	
CASE STUDY #1	
Measure type (if relevant):	
Species:	*Procyon lotor*
Objective:	Eradication
Use of measure	Egg Traps were used to trap raccoon
Combined with other measure(s):	Egg traps (The Egg Trap Company, North Dakota U.S.A.) were used in addition to wire cage traps (model 2005, Tomahawk Live Trap Co., Tomahawk, Wisconsin, U.S.A.). Most of the effort was based on cage traps. As an example, from September 2016 to May 2017 trapping was performed in 59 sites covering an area of about 30 km^2: wire cages were used in 52 sites and Egg Traps only in 7 sites.
Country(ies) of application:	Italy
Geographic scale (km^2) and/or population size measure applied to:	120 km^2
Time period:	September 2016 to July 2019
Effort:	6739 + 54 trap nights; the relative effort between cage and Egg traps is not stated.

Costs:	Overall costs:
	Personnel costs:
	Equipment and infrastructure:
	Other, including overheads:
Effectiveness:	The project achieved eradication of the target species. However, most of the effort was based on cage traps and therefore it is not possible to evaluate the effectiveness of Egg traps for eradicating raccoons.

4.2. Costs effectiveness summary	The only available project of management of IAS of Union concern using this kind of traps actually achieved eradication of racoons, but was largely based on cage traps and used egg traps only in few areas. Therefore it is not possible to evaluate the effectiveness of egg traps for eradicating raccoons.

5. Side effects	
Non-target native species, their habitats and the broader environment:	Positive:
	Negative:
	Snares can capture and stress a large number of individuals of non-target species, mammals in general and particularly carnivores, and cause them serious injuries which could affect survival.
Other invasive alien species:	Positive:
	When present in the same areas, raccoon, raccoon dog and coati may be captured.
	Negative:
Public health and well-being:	Positive:
	Negative:
	There is a largely negative public perception against the use of non-lethal snares due to the high rate of injuries they could cause and the fact that animals feel stressed and fight to get released.
Economic:	Positive:
	Negative:

6. Conclusion

Overall assessment of the measure (qualitative)	
Snare traps are rarely used in Europe to manage IAS of Union concern. Their use is legal only in four Member States and most of the literature comes from North America. Individuals caught in non-lethal snare traps often sustain injuries from the wire ligature, which can in turn lead to tissue necrosis, strangulation and sometimes self-mutilation. Snares can also capture and stress several non-target species, causing serious injuries which can affect survival. Therefore, there is a great concern on their humaneness and a largely negative perception against the use of non-lethal snares due to the high rate of injuries they can cause. Though injuries could be reduced by well trained staff and egg traps were considered more effective than cage traps in a comparison study, the only IAS management project in Europe using this type of traps (on the eradication of raccoon) used mainly cage traps with a limited effort with egg traps.	
Assessor:	Sandro Bertolino
Reviewer 1:	Ana Nunes
Reviewer 2:	Ilaria Di Silvestre

7. References

[1] Iossa, G., Soulsbury, C. D., & Harris, S. (2007). Mammal trapping: a review of animal welfare standards of killing and restraining traps. Animal Welfare-Potters Bar Then Wheathampstead-, *16*(3), 335-352.

[2] IUCN. 2017. Information on non-lethal measures to eradicate or manage vertebrates included on the Union list. Technical note prepared by IUCN for the European Commission.

[3] Witmer, G. W. (2005). Wildlife population monitoring: some practical considerations. *Wildlife Research*, *32*(3), 259-263.

[4] Short, M. J., Weldon, A. W., Richardson, S. M., & Reynolds, J. C. (2012). Selectivity and injury risk in an improved neck snare for live-capture of foxes. *Wildlife Society Bulletin*, *36*(2), 208-219.

[5] Charlton, J. (2014) Code of practice for the use of vertebrate traps. Natural England, Animal Health and Veterinary Laboratories Agency.

[6] Proulx, G., Onderka, D. K., Kolenosky, A. J., Cole, P. J., Drescher, R. K., & Badry, M. J. (1993). Injuries and behavior of raccoons (*Procyon lotor*) captured in the Soft Catch™ and the EGG™ traps in simulated natural environments. *Journal of Wildlife Diseases*, *29*(3), 447-452.

[7] Austin, J., Chamberlain, M. J., Leopold, B. D., & Burger Jr, L. W. (2004). An evaluation of EGGTM and wire cage traps for capturing raccoons. *Wildlife Society Bulletin*, *32*(2), 351-356.

[8] Hubert Jr, G. F., Hungerford, L. L., Proulx, G., Bluett, R. D., & Bowman, L. (1996). Evaluation of two restraining traps to capture raccoons. *Wildlife Society Bulletin*, 699-708.

[9] Lovari, S., Valier, P., & Lucchi, M. R. (1994). Ranging behaviour and activity of red foxes (*Vulpes vulpes*: Mammalia) in relation to environmental variables, in a Mediterranean mixed pinewood. *Journal of Zoology*, *232*(2), 323-339.

[10] Sala, L., Sola, C., Spampanato, A., Tongiorgi, P., & Magnanini, M. (2014). Capture and identification techniques of marmot on Mount Cimone (Northern Apennines). *Journal of Mountain Ecology*, *1*: 14-16

[11] McNew Jr, L. B., Nielsen, C. K., & Bloomquist, C. K. (2007). Use of snares to live-capture beavers. *Human-Wildlife Conflicts*, *1*(1), 106-111.

[12] Muñoz-Igualada, J.A.I.M.E., Shivik, J. A., Domínguez, F. G., Lara, J., & González, L. M. (2008). Evaluation of cage-traps and cable restraint devices to capture red foxes in Spain. *The Journal of Wildlife Management*, *72*(3), 830-836.

[13] Chapman, J. A., Willner, G. R., Dixon, K. R., & Pursley, D. (1978). Differential survival rates among leg-trapped and live-trapped nutria. *The Journal of Wildlife Management*, *42*(4), 926-928.

[14] Mazzamuto, M. V., Panzeri, M., Bisi, F., Wauters, L. A., Preatoni, D., & Martinoli, A. (2020). When management meets science: adaptive analysis for the optimization of the eradication of the Northern raccoon (*Procyon lotor*). *Biological Invasions*, 1-12.

Appendix 21. Live decoy traps

1. Measure name			
1.1. English	Live decoy traps		
1.2. Lethal or non-lethal:	Non-lethal		
1.3. Other languages (if available):			
Bulgarian	Капани тип клетка с отделение за жива примамка	Italian	Trappole con esche vive
Croatian	Zamke sa živim mamcima	Latvian	Dzīvo mānekļu slazdi
Czech	Pasti s živou návnadou	Lithuanian	Spąstai su gyvais masalais
Danish	Fælder med levende lokkemad	Maltese	
Dutch	Vallen met levende lokkers	Polish	Pułapki z żywą przynętą
Estonian	Elava peibutisega püünised	Portuguese	Armadilhas de isco ou presa vivos
Finnish	Elävät houkuttimet	Romanian	Capcane tip cușcă cu animale folosite ca momeală vie
French	Pièges à leurres vivants	Slovak	Pasce so živou návnadou
German	Lebendköderfalle	Slovenian	Pasti z živo vabo
Greek	Παγίδες με ζωντανό κράχτη	Spanish	Trampas con cebo vivo o presa viva
Hungarian	Élő csaliállatos csapdák	Swedish	Fångstredskap med levande lockfågel
Irish			

2. Technical details of measure
2.1.a. Measure description
This method is the use of a live capture cage trap containing a live animal as a decoy to attract other members of the same species to enter. A wide variety of designs are available for use on different species. The most common designs involve a two compartment cage, with a live decoy housed in one, while the second is akin to a traditional cage trap, with a funnel or triggered door which allows the target animal to enter, but then not leave. Some decoy trap designs include multiple capture cages so that more than one animal can be caught without resetting. Decoy traps have been used for the capture of a number of bird species. Decoy traps play on the social or antagonistic behavior of the species to attract animals to enter the trap. For some social species, the presence of a decoy can act as an attractant and reduce trap avoidance behaviours. For territorial species, the presence of the decoy placed within their territory can act as a challenge, eliciting antagonistic behavior from the resident animal. The utility of decoy traps varies widely dependent on species behaviour, but can provide a highly effective and selective method of capture and may be of use for other species than those listed here, although there is only limited evidence of their wider use.

There are two separate welfare considerations associated with decoy traps, the welfare of the decoy animal, and of the individuals attracted into the cage. The use of decoy animals brings particular responsibilities, complications, and potential welfare concerns. Decoy birds require food, water, perches, space to stretch and shelter when in the trap and care is needed to ensure their welfare. Advice on the use of decoys emphasises the benefits of a healthy decoy animal which will be more active, vocal and visible, increasing its effectiveness [1]. It is also good practice to replace decoy birds at regular intervals. This is also likely to improve trapping success [2]. As with all capture methods, decoy traps must be regularly checked and captured animals removed. In some EU Member States there are legal requirements to check traps on at least a daily basis, but more frequent checking is recommended to reduce welfare risks and to increase trap effectiveness. Trap checking frequency should also consider the requirements of any non-target species at risk of capture.

Live decoy traps are relevant for the control of three of the listed species. There is a body of experience of their use for the Common Myna (*Acridotheres tristis*), their use has been trialled for use on Egyptian Goose (*Alopochen aegyptiaca*) while experience with native corvids suggests they may also be useful for the House Crow (*Corvus splendens*), different trap designs are needed for each. They may also be of use for other alien bird species, such as the Crested Myna (*Acridotheres cristatellus) although wider experience of their use on other listed or alien species is limited*.

Acridotheres tristis

For *Acridotheres tristis*, there is considerable experience of the use of decoy traps worldwide and in Europe. This is the main control method used to eradicate small populations of this species and it also contributes to the control and containment of more well established populations. Decoy traps have been successfully used to support control and eradications in New Zealand, Australia, Pacific Islands, the Seychelles and Azores, supplemented by shooting and the use of toxins. [3,4,5,6]. A review of Myna control on islands [7], largely based on decoy trapping, including successful eradications from the Spanish European islands of Tenerife, Gran Canaria and Mallorca. The most extensive use of this method [6] was the eradication of Common Mynas from the 1-2km^2 St Denis island in the Seychelles, removing 1186 birds over 5 years, mainly by decoy trapping.

Reports [3,8) describe wire netting cage-traps with walk-in funnel entrances that can also be used to contain a decoy bird. More recent accounts [6,7,9]) describe a variety of decoy trap designs and their practical use. A wide range of Myna traps based on these designs are commercially available and described on-line (search term ‘myna trap’).

The most effective locations for traps are sites with low levels of disturbance and open sites at least 3m from vegetation that can harbour ground predators. Experience of trap placement is required for their effective use and this can take time to develop. Mynas are intelligent birds and will quickly learn to avoid traps if they are not used carefully. Some studies recommend setting up and checking traps during darkness so the birds do not associate them with people. A proportion of birds may become trap-shy and eradication programmes should also have other methods available to deal with this eventuality.

Alopochen aegyptiaca

Experience of the use of decoy traps on this species is limited to trials which have assessed effectiveness and compared designs placed on land or on floating platforms [10,11,12]. Although they are not widely used at present, they are proposed to form part of any future coordinated control of this species in Belgium [13] as a supplement to the main control method based on shooting.

The design applied to this species is a large cage with a spring activated trap door on the side that will close behind any bird heavy enough to enter the trap which can allow multiple captures. A previously captured Egyptian goose is placed in the decoy compartment, and this attracts other birds. The trap can be baited with food to enhance trapping success. Although technical information is available [10], no commercial supply of this design is currently available, although they can readily be constructed. Traps can be transported on a trailer and can be operated by one or two people.

Egyptian geese are highly territorial in certain periods of the year. Trials identified the spring months as the best time to deploy decoy traps [12] when traps were placed in breeding areas close to the nest.

These decoy traps also caught non target species of similar or larger body weight. For example, when using floating decoy traps, over a period of 860 trap days, 80 Egyptian geese and 68 non- target species (including 17 non-target invasive bird species) were caught [15]. These non-target species can be released back into the wild if appropriate.

Corvus splendens
There are descriptions of the invasion history and global spread of this species, including a range of individuals and small populations established in Europe [15]. Shooting appears to be the most effective method of control, but can usefully be supplemented by trapping [16]. Crow traps have been used to reduce *Corvus splendens* numbers in Malaysia, Kenya, Tanzania and South Africa, although populations have continued to spread in these regions [15]. In the Netherlands an eradication programme has taken place in Hoek van Holland. This population originated from a ship-assisted introduction of two birds in 1994. By 2012, between 23 and 30 birds were reported as present [17,18]. The eradication was initially performed by capture, but later by shooting as remaining birds became trap shy.
Decoy traps have been used in a small number of cases to assist the control of *Corvus splendens*, but when used alone they are not considered suitable to achieve total eradication as a proportion of birds become wary and trap-shy [13,16]. Control in Yemen [19] has used decoy birds in large traps (known as Ladder or Australian traps), typically used to catch social corvids such as rooks and jackdaws, but with limited success.

For *Corvus splendens* there may also be benefit in considering other decoy designs, such as Larsen Traps, which are used to attract territorial birds. Larsen traps are most usefully deployed by placing them into bird territories while these are being actively defended by a pair, and then moved to the next territory when these birds have been captured, although they can also be used throughout the year. Slightly different designs of Larsen Trap are advocated for different species of corvid, with top-entry doors favoured for Magpies which prefer to land on top of the trap and drop into the enclosure; and side-entry doors for crows which tend to land next to the trap and walk in.

Evidence from the use of decoy traps on native corvids
Larsen Traps are the most widely used decoy traps in Europe, mainly used for the control of native corvids such as Magpies (*Pica pica*) and Crows (*Corvus corone*). Plans for the construction of a decoy trap for corvids have been published [20]. Comparing traps with or without a decoy, the presence of the decoy increased capture rates by ten times for magpies and 15 times for crows [21]. The most detailed accounts of the use [22,23], non-target captures and humaneness of Larsen Traps [24] relate to their use on these two native species. Surveys of Larsen trap use in Scotland to catch native corvids provide details of effectiveness, non-target capture rates and the welfare status of decoy birds.

Surveys [22] of the corvid trapping activities of 27 operatives undertaking corvid control in Scotland on over 16,000 trap days, recording details of 4,500 target bird captures, 119 non-target birds (11 species), and 9 non-target mammals (8 species). Larsen traps were the most common design used. Carrion crows and magpies were the most common target species caught, pheasants were the commonest non-target bird and domestic cats the commonest mammal caught. Live decoys were the most effective attractant used in traps, plastic decoys were generally ineffective. Of 35 decoy birds visually inspected during the study, the majority were in ‘very good’ or ‘good’ condition, two decoy birds had been killed by fox predation. From discussion with the operatives, nineteen other incidents of decoy fatality were reported over the study, nine of which were attributable to predation events [22].

Studies [24] have also used video surveillance of active Larsen Traps over 1601 trap days to identify a total of 138 corvids, nine buzzards, four blackbirds, one small passerine, and one squirrel triggering traps. Of these, 12 corvids, two buzzards, three blackbirds, the small passerine and the squirrel escaped. These traps were therefore considered relatively selective. Traps containing a decoy bird had the highest capture rates, compared to those containing only baits. Veterinary examination of all euthanized corvids determined that 21% suffered no injury prior to death; 78% had minor or moderate injuries, not believed to be detrimental to their survival had they escaped; and 1% had serious (life threatening) injuries. The most common injury was minor bill abrasion (70% of birds), which was probably related to the trap mesh size and construction. The most serious welfare issue arose when a bird was attacked by a fox while in the trap overnight. In the majority of cases, no serious injury concerns were raised by the traps used, irrespective of the time left in the trap (within the legal restrictions).

2.1.b. Integration with other measures

The use of decoy traps requires initial decoy birds to be caught using another method. For the species described here, these include live capture cage traps without a decoy, mist nets [25], noose traps [26] or the use of animals already held in captivity.

Captured birds can be removed from the traps and humanely dispatched. For *Acridotheres tristis,* designs have also been produced which enclose the trap in a canvas sleeve which could then be flooded with CO_2 to humanely kill captured birds [8,27].

The effectiveness of decoy traps varies between species and a proportion of any population are likely to be, or may become trap-shy. For species such as *Acridotheres tristis* or *Corvus splendens*, there are suggestions that birds become increasingly wary of traps if they observe other animals being captured and handled.
It is expected that alternative methods should also be available to supplement the use of decoy traps, particularly if the objective is eradication. Decoy traps appear to be the primary method used to eradicate or control *Acridotheres tristis* [7,28]. Decoy traps are recommended as a supplement to shooting for *Alopochen aegyptiaca* [13]. Drawing on experience of their use on other native corvids, decoy trap designs such as Larsen Traps may have potential for use on *Corvus splendens* which are normally controlled by shooting.

2.2.a. Availability - species and objectives

Objective	Unknown objective		Rapid Eradication		Management					
					Eradication		Control		Containment	
Species	Avail.	Ref(s).	Avail.	Ref(s).	Avail.	Ref(s).	Avail.	Ref(s).	Avail.	Ref(s).
Acridotheres tristis	A		A	7	A	7	A	9	A	9
Alopochen aegyptiaca	A		A		A		A	12	A	12
Callosciurus erythraeus										
Corvus splendens	P		P		P	19	P			
Herpestes javanicus										
Lepomis gibbosus										
Lithobates catesbeianus										
Muntiacus reevesi										
Myocastor coypus										
Nasua nasua										
Nyctereutes procyonoides										

Ondatra zibethicus										
Oxyura jamaicensis										
Perccottus glenii										
Plotosus lineatus										
Procyon lotor										
Pseudorasbora parva										
Sciurus carolinensis										
Sciurus niger										
Tamias sibiricus										
Threskiornis aethiopicus										
Trachemys scripta										

2.2.b. Application – EU Member States and objectives

Objective	Unknown objective		Rapid Eradication		Management					
					Eradication		Control		Containment	
Country	Avail.	Ref(s).	Avail.	Ref(s).	Avail.	Ref(s).	Avail.	Ref(s).	Avail.	Ref(s).
Austria										
Belgium							X	12	X	12
Bulgaria										
Croatia										
Cyprus										
Czech Republic										
Denmark										
Estonia										
Finland										
France										
Germany										
Greece							X	29		
Hungary										
Ireland										
Italy										
Latvia										
Lithuania										
Luxembourg										
Malta										
Netherlands										
Poland										
Portugal										

Romania										
Slovakia										
Slovenia										
Spain			X	7	X	7	X	30	X	30
Sweden										
United Kingdom*							X	22,23,24	X	22,23,24

* Not an EU Member State

3. Humaneness of the measure			
3.1. Welfare for all measures			
Measure type (if applicable):	**Humaneness impact categories**		
Domain	**No impact**	**Mild-Moderate**	**Severe - Extreme**
1: Water deprivation, food deprivation, malnutrition		Decoy animals should be provided with suitable food, water and shelter while in the trap which should limit the impact of captivity on their needs. Food and water may also need to be provided for captured animals dependent on the species, trap checking frequency and local conditions. If traps are regularly checked then any captured animals should be removed before their experience is significantly outside usual tolerance levels. If bait and water are provided in the trap then this may further reduce the risk to their welfare	

2: Environmental challenge		Decoy birds should be provided with shelter and not left exposed to severe weather, avoiding exposure to deleterious environmental conditions. Traps should not be set in severe weather conditions and decoys should be removed. Provided traps are regularly checked then any captured animals should be removed before their experience is significantly outside usual tolerance levels.	
3: Injury, disease, functional impairment		The only available data relates to the use of decoys to catch native corvids, no comparable data related to the listed alien species was found. Following visual inspection of 35 decoy birds [21] the majority were in 'very good' or 'good' physical condition. Veterinary examination of euthanised corvids captured in decoy traps [23] determined that 21% suffered no injury prior to death; 78% had minor or moderate injuries, not believed to be detrimental to their survival had they escaped. The most common injury was minor bill abrasion (70% of birds), which was probably related to the trap mesh size and construction. Serious (life threatening) injuries were found in 1% of cases. when a bird was attacked by a fox while	The only available data relates to the use of decoys to catch native corvids, no comparable data related to the *Corvus splendens* was found. There are records of captive animals being predated by foxes while in the trap [21,23] Careful trap placement and frequent checking should reduce this risk.

		in the trap overnight. In the majority of cases, no serious injury concerns were raised by the traps used, irrespective of the time left in the trap (within the legal restrictions)	
4: Behavioural, interactive restriction		The design of decoy traps should include sufficient space and facilities for birds to perch and stretch.	There are risks that animals may not be able to undertake normal defensive or escape reactions to the visibility or presence of predators. However, this risk can be reduced by the placement of traps and regular checking. Some designs of decoy traps, particularly Larsen traps, are particularly effective when birds are displaying territorial behaviour which can coincide with the breeding season, bringing the risk that trapped birds may be prevented from brooding eggs or feeding nestlings with potentially severe welfare consequences. Territorial behaviour can be linked to breeding, but not in all species, many are territorial throughout much of the year. Even where territorial behaviour is linked to breeding, it typically commences prior to pair formation, and there can be some weeks or months before eggs are laid and well developed, and young are produced. For precocial species, the young leave the nest after hatching and accompany the adults which may also reduce the risks. This sequence varies greatly between species and

			the risk to young must be assessed on a species by species basis. Decoy traps can also be used during periods where animals are not territorial, although different designs may be required, for example larger traps to catch social species. This risk to dependent young can be reduced by avoiding trapping during periods of the year when dependent young are considered vulnerable.
5: Anxiety, fear, pain, distress, thirst, hunger etc.		Decoy animals in traps need to be provided with food, water, perches, space to stretch and shelter and protected from severe weather. If these requirements are met, and the risks of predation are reduced, then their welfare may not be seriously compromised	There is a risk to dependent young if adult birds are trapped during the breeding season, This risk can be reduced by avoiding trapping during periods of the year when dependent young are considered vulnerable.

3.2. Mode of death (if relevant)			
Measure type (if applicable):	**Immediate death (i.e. no suffering)**	**Not immediate death (mild - moderate suffering)**	**Not immediate death (severe - extreme suffering)**
Rationale:	N/A		

3.3. Humaneness summary	Decoy birds must be provided with food, water, perches, space to stretch and shelter and protected from severe weather. Captured animals should be removed quickly based on regular inspection of the traps. Care is needed, for example in the placement of traps, to reduce the risks of predation. Care is also needed to avoid trapping during periods when dependent young would be at risk should the parent be trapped. If these requirements are met then the majority of birds should only suffer mild to moderate impacts on their welfare. Animals held in cages are vulnerable to predators, and cases of predation occur in the literature. These cases constitute severe to extreme impacts on their welfare. Careful trap placement and more frequent checking should reduce this risk.

	Decoy traps capture animals alive, they may then be released, brought into captivity or dispatched by a variety of means.

4. Costs and effectiveness of the measure

4.1. Case studies

CASE STUDY #1

Measure type (if relevant):	Live Decoy Traps	
Species:	Common Myna *Acridotheres tristis*	
Objective:	Rapid Eradication and Eradication	
Use of measure	Live capture using decoy traps have been the primary method used to eradicate small populations of this species from Spanish islands during the early phase of establishment [7,28].	
Combined with other measure(s):	Worldwide, decoy traps are often used in association with shooting or toxins. However, experience in Europe appears to be based solely on the use of decoy traps without the support of these other methods.	
Country(ies) of application:	Spain	
Geographic scale (km^2) and/or population size measure applied to:	To date, experience of the use of decoy traps has been in relation to the rapid eradication of small, geographically isolated populations. In Mallorca 12-17 birds were removed from a number of small pockets distributed over an area of 150km^2. On Gran Canaria, ten birds were removed from an area of around 1ha. In Tenerife ten birds were removed from an area of 25ha [7,28].	
Time period:	The time taken to remove birds in these examples appeared to reduce as operator experience was gained. Removal took five months on Tenerife, ten days on Gran Canaria, and 30 days on Mallorca [7,28].	
Effort:	The rapid eradication on Tenerife involved five months of active trapping by one person. On Gran Canaria it required ten days active trapping by one person. The programme on Mallorca involved 30 days trapping effort by one person.	
Costs:	Overall costs:	
	No financial details are available	
	Personnel costs:	
	No financial details are available, but if a daily rate per person was assumed, then an estimate of cost could be made from the number of days' effort described above.	
	Equipment and infrastructure:	
	No financial details are available, but decoy traps suitable for this species are available online at prices of €20-80 per trap. Other costs will include food for the decoy and potentially bait for attracting target birds.	
	Other, including overheads:	

	No financial details are available
Effectiveness:	In Europe, decoy trapping has been successfully used to rapidly eradicate this species from the Spanish islands of Tenerife, Gran Canaria and Mallorca during the early stages of establishment [7,28].

CASE STUDY #2	
Measure type (if relevant):	Live Decoy Traps
Species:	Egyptian Goose *Alopochen aegyptiaca*
Objective:	Rapid eradication, Eradication, Control and Containment
Use of measure	To date, these traps have only been used on a trial basis [11,12]. Reports [13] describe a strategy to eradicate or limit the spread of this species in Belgium. This eradication consists of culling birds through a combination of shooting and decoy trapping. Shooting is the principal method proposed to control Egyptian geese, but this would be supplemented with trapping where required, e.g. at sites were shooting is not possible because of safety or disturbance issues, such as parklands, nature reserves, urban areas and private land. Trapping is to be performed year-round using land-placed, multi-capture traps containing a live decoy.
Combined with other measure(s):	The proposal is to combine decoy trapping with shooting [13].
Country(ies) of application:	Belgium
Geographic scale (km^2) and/or population size measure applied to:	Trials took place at 29 sites, widely distributed across Belgium [11,12].
Time period:	A year round trial of these traps was undertaken at a sample of sites [11,12]. While useful in all seasons, they appeared particularly effective during spring when this species is at its most territorial.
Effort:	Under typical conditions, it is likely that 10 traps can be checked by one person per day. Although capture rates will be influenced by the local abundance of the species, in trials land-based traps yielded an average of 0.7 animals/trap day while floating traps appeared less effective and only removed 0.09 geese/trap day ([11,12].
Costs:	Overall costs:
	No information available. These traps have only been used on a trial basis.
	Personnel costs:
	Under typical conditions, it is likely that 10 traps can be checked by one person per day.
	Equipment and infrastructure:
	Traps for this species are not commercially available, but they can be readily built from widely available materials and the cost is likely to be in the region of a few hundred euros per trap.
	Other, including overheads:

Effectiveness:	At present decoy traps for Egyptian geese have only been used in trials. Decoy traps have proven effective in removing small breeding groups of Egyptian geese in parts of Belgium [14]. A trial at 27 different locations showed that over 1 to 9 days (a total of 89 catching days), land-based decoy traps were able to remove all breeding birds from an area (where typically one to two breeding pairs were present). When expressing effectiveness as the number of Egyptian geese caught per trapping day, land-based traps yielded an average of 0.7 animals/trap day while floating traps appeared less effective and only removed 0.09 geese/trap day. The trial involved two full-time field control officers who operated traps at 19 locations with a maximum distance between two sites of <120 km [14]. It can be assumed that if capture sites are not spread out too widely, a single person could manage about 10 trap inspections/day. Decoy traps are proposed for use where the main control method of shooting was not considered feasible because of safety or disturbance issues, such as parklands, nature reserves, urban areas and private land.

4.2. Costs effectiveness summary	Decoy traps are the most widely used and cost effective method for the eradication and control of *Acridotheres tristis*. They have been successfully used to eradicate this species from a number of Spanish islands and in many other parts of the world. They are relatively cheap and the main costs are the manpower required for their placement and checking. They offer an alternative method to shooting to support the eradication and control of *Alopochen aegyptiaca* although they have not yet been deployed in large numbers for this species. Based on the widespread use of this method to control native corvids, it may be of use to assist with the removal and control of *Corvus splendens* although experience to date is limited (has been applied unsuccessfully in Socotra [19]).

5. Side effects	
Non-target native species, their habitats and the broader environment:	Positive:
	Decoy traps are typically more selective than other cage traps as the presence of the decoy adds an element of species selectivity. If non-target animals are captured, then the live capture nature of the traps should allow them to be released unharmed if appropriate. The frequency with which traps are checked should also consider the ecology and welfare of non-target species.
	Negative:
	When assessing decoy traps to capture native crows and magpies, studies [22] record a non-target capture rate of 2.6% with the risk of catching raptors increased when meat baits were used, while others [24] record a 6% non-target capture rate. The use of decoy traps on floating platforms to catch *Alopochen aegyptiaca* resulted in a 46% non-target capture rate, mainly of similarly sized waterfowl, although land-based traps appear more effective for catching *Alopochen aegyptiaca*.

Other invasive alien species:	Positive:	
	The use of decoy traps on floating platforms for *Alopochen aegyptiaca* resulted in significant captures of other alien bird species of similar size. These comprised 11.5% of the total captures of the target and non-target species [14]. The frequency with which traps are checked should also consider the ecology and welfare of non-target species.	
	Negative:	
Public health and well-being:	Positive:	
	Negative:	
Economic:	Positive:	
	Negative:	

6. Conclusion
Overall assessment of the measure (qualitative)
Decoy traps provide an effective and selective method to catch certain species and most experience of their use relates to birds. They rely on species specific social or antagonistic behaviors which limit their use to particular species. The presence of a decoy improves capture rates compared to the use of baited trap alone (Reynolds 1990), while rates of non-target captures, for some widely used designs at least, are low. They have been widely used worldwide and in Europe as the main method to eradicate and control *Acridotheres tristis*, there is developing experience of their use as a supplement to shooting to control *Alopochen aegyptiaca*, while experience of their use to control native corvids suggests they may have potential for use on *Corvus splendens*. Decoy traps are relatively cheap to manufacture, and designs for *Acridotheres tristis* are commercially available between € 20-80 per trap, although larger designs for other species may be more expensive. The main costs for their use relate to the personnel time needs for checking and removing animals, experience with *Alopochen aegyptiaca* suggests 10 widely dispersed traps can be checked per day by one person. The presence of the decoy animal brings specific responsibilities for their care and welfare. Decoys require food, water, perches, space to stretch and shelter when in the trap and care is needed to ensure their welfare. Advice on the use of decoys emphasises the benefits of a healthy decoy animal which will be more active, vocal and visible, increasing its effectiveness, best practice recommends that decoys should be regularly replaced.. Like all live capture traps, they should be regularly checked, at least every 24 hours if not more frequently. Observations of the welfare of the decoys and other captured animals, mainly based on their use to capture native corvids, suggest that their physical condition is typically good, and that most trapping related injuries are minor and not life threatening. However, there are cases where birds in traps have been predated, raising particular concerns not only about predation but also about fear and distress as a result of predators investigating traps containing birds. The careful siting of traps and their regular checking should reduce these and other risks. Care is also needed to avoid trapping during periods when dependent young would be at risk of severe consequences should the parent be trapped. Food and water may need to be provided for captured as well as decoy birds, dependent on the species concerned and trap checking frequency.

The safe and effective use of decoy traps requires some experience and skill, which can take time to develop. The data described in this review derives from their use by experienced, or professionally trained operatives. If they are used by untrained and inexperienced operatives, then their effectiveness is likely to be reduced and the risks of compromising the welfare of the animals increased.

Decoy traps have been successfully used to support the eradication of *Acridotheres tristis* from areas of 1-2km^2 and populations numbering over 1000 individuals [6]. Their use is proposed to form part of national control strategies for *Alopochen aegyptiaca*. The use of decoy traps to control native corvids is widespread in some regions, such as Scotland, and forms the most common control method in use. Based on this experience with other corvids, it may provide a useful method for use on *Corvus splendens*, although more experience is required for this species.

Assessor:	Peter Robertson
Reviewer 1:	Sandra Baker
Reviewer 2:	Riccardo Scalera

7. References

1. Game and Wildlife Conservation Trust. 2020 Using Call Birds Factsheet https://www.gwct.org.uk/advisory/guides/larsen-traps-england-scotland/using-call-birds/ Accessed 18 June 2020
2. https://consult.defra.gov.uk/natural-england/general-and-class-licences/supporting_documents/Annex%20F%20%20Trapping%20Birds%20under%20General%20LicenceCode%20of%20PracticeFeb%202014.pdf
3. Tindall SD (1996). Ecological impacts and management of the common myna *Acridotheres tristis* p109. Thesis MSc, School of Biological Sciences, University of Auckland, New Zealand.
4. Wilson PR (1973). The ecology of the common myna (*Acridotheres tristis* L.) in Hawke's Bay. p 228, PhD Thesis, School of Biological Sciences, Victoria University of Wellington, New Zealand.
5. Canning G (2011) Eradication of the invasive common myna, *Acridotheres tristis*, from Fregate Island, Seychelles Phelsuma 19); 43-53
6. Feare, C.J., van der Woude, J., Greenwell, P., Edwards, H.A., Taylor, J.A., Larose, C.S., Ahlen, P.A., West, J., Chadwick, W., Pandey, S. and Raines, K., 2017. Eradication of common mynas *Acridotheres tristis* from Denis Island, Seychelles. *Pest Management Science*, *73*(2), pp.295-304.
7. Saavedra S (2010) Eradication of invasive Mynas from islands. Is it possible? Aliens 29
8. Tidemann C (2005) October. Indian mynas–can the problems be controlled. In Proceedings of the 15th National Urban Animal Management Conference, Canberra (pp. 19-21).
9. Pham T, van Son J (2009) Indian Myna Handbook: Managing the invasion of Indian mynas on New South Wales mid-north coast. https://www.bellingerlandcare.org.au/wp-content/uploads/IndianMynaHandbook.pdf
10. Van Daele, P., Adriaens, T., Devisscher, S., Huysentruyt, F., Voslamber, B., De Boer, V., Devos, K. and Casaer, J. (2012) Beheer van Zomerganzen in Vlaanderen en Zeeuws-Vlaanderen. Rapporten van het Instituut voor Natuur- en Bosonderzoek 2012 (INBO.R.2012.58). Instituut voor Natuur- en Bosonderzoek, Brussel. (link)
11. Huysentruyt, Frank, Tim Adriaens, Kim De Bus, Karel Van Moer, Sofie Standaert, and Jim Casaer. "Testing the efficacy of a floating multicapture trap for invasive Egyptian geese (Alopochen aegyptiacus)." In *IUGB (International Union Of Game Biologists) Congress 2013*. 2013.
12. Huysentruyt F., Adriaens T., Van Moer K., Standaert S., De Bus K., Devisscher S. & Casaer J. 2014. Catching invasive Egyptian geese (Alopochen aegyptiacus): evaluation of the optimal deployment season for a floating Larsen trap. Science for The New Regulation, One day conference on Invasive Alien Species. Ghent, Belgium 02/04/2014
13. Adriaens, T., Branquart, E., Gosse, D., Reniers, J. and Vanderhoeven, S., 2019. Feasibility of eradication and spread limitation for species of Union concern sensu the EU IAS Regulation (EU 1143/2014) in Belgium. Report prepared in support of implementing the IAS Regulation in Belgium.
14. INBO Partner Report (2014) Field trials – Egyptian goose (*Alopochen aegyptiacus*)
15. Ryall C, 2016. Further records and updates of range extension in House Crow Corvus splendens. Bulletin of the British Ornithologists' Club, 136(1):39-45.

16. CABI - Invasive Species Compendium (2017). Datasheet : Corvus splendens. Downloaded from http://www.cabi.org/isc/datasheet/15463 on 18-06-2020.
17. Slaterus, R., Aarts, B., van den Bremer, L. (2009). De Huiskraai in Nederland: risicoanalyse en beheer. Beek-Ubbergen. 2009/08.
18. de Baerdemaeker, A., Klaassen, O. (2012). Huiskraaien in Hoek van Holland: is de groei eruit? Straatgras 24(4):78-79.
19. Suleiman, A.S. and Taleb, N.A.D.I.M., 2010. Eradication of the house crow *Corvus splendens* on Socotra, Yemen. *Sandgrouse*, *32*(2), pp.136-140.
20. Game and Wildlife Conservation Trust, 2008. Larsen trap construction (leaflet). Game and Wildlife Conservation Trust, Hampshire. Available at: http://www.gwct.org.uk/media/208824/larsen_trap_construction.pdf
21. Reynolds, J. 1990. *Crow and magpie control: the use of decoy birds in cage traps*. The Game Conservancy Trust Annual Review of 1989, Fordingbridge.
22. Hartley, F.G., Campbell, S.T. & Jamieson, S. 2016. Assessing the nature and use of corvid cage traps in Scotland: Part 2 of 4 – Field survey of trap use in Scotland 2014-15. *Scottish Natural Heritage Commissioned Report No. 932*.
23. Reynolds, J.C. 2016. Assessing the nature and use of corvid cage traps in Scotland: Part 1 of 4 – Questionnaire survey of corvid trap users in Scotland. *Scottish Natural Heritage Commissioned Report No. 931*.
24. Campbell, S.T., Hartley, F.G. & Fang, Z. 2016. Assessing the nature and use of corvid cage traps in Scotland: Part 3 of 4 – Trap operation and welfare. *Scottish Natural Heritage Commissioned Report No. 933*.
25. Sharp T. Saunders G (2004). Trapping of pest birds. NSW Department of Primary Industries, Australia.
26. Dhami MK (2009) Review of the Biology and Ecology of the Common Myna (*Acridotheres tristis*) and some implications for management of this invasive species. Maanaki Whenua Landcare Research, Canterbury, New Zealand. http://www.indianmynaaction.org.au/documents/New%20Zealand%20paper.pdf
27. Pierce RJ (2005) A review of interactions between introduced Mynas and indigenous vertebrates and control methods for Mynas. Contract Report 1180, Invasive Species Specialist Group, Auckland, New Zealand.
28. Saavedra, S.; Maraver, A.; Anadón, J. D. & Tella, J.L. (2015). A survey of recent introduction events, spread and mitigation efforts of mynas (*Acridotheres* sp.) in Spain and Portugal. *Animal Biodiversity and Conservation*. **38** (1): 121–127.
29. Tsachalidis, E.P., Sokos, C.K., Birtsas, P.K. and Patsikas, N.K., 2006. The Australian crow trap and the Larsen trap: their capture success in Greece. In *Proceedings of the 2006 Naxos International Conference on sustainable management and development of mountainous and island areas* (Vol. 2, pp. 325-329). Democritus University of Thrace Island of Naxos.
30. Díaz-Ruiz, F., García, J.T., Pérez-Rodríguez, L. and Ferreras, P., 2010. Experimental evaluation of live cage-traps for black-billed magpies Pica pica management in Spain. *European Journal of Wildlife Research*, *56*(3), pp.239-248.

Appendix 22. Cervical dislocation

1. Measure name			
1.1. English:	Cervical dislocation		
1.2. Lethal or non-lethal:	Lethal		
1.3. Other languages (if available):			
Bulgarian	Пречупване на гръбначния стълб в областта на врата	Italian	Dislocazione cervicale
Croatian	Cervikalna dislokacija	Latvian	Kakla skriemeļu dislokācija
Czech	Narušení míchy	Lithuanian	Sprando išsukimas
Danish	Cervikal disloker	Maltese	
Dutch	Cervicale ontwrichting	Polish	Dyslokacja kręgów szyjnych
Estonian	Tservikaalne dislokatsioon	Portuguese	Deslocamento cervical
Finnish	Kaularangan sijoiltaan meno	Romanian	Dislocare cervicală
French	Luxation cervicale	Slovak	Narušenie miechy
German	Zervikale dislokation	Slovenian	Izpah (dislokacija) vratu
Greek	Αυχενική εξάρθρωση	Spanish	Dislocación cervical
Hungarian	Nyaki törés	Swedish	Halsdislokation
Irish			

2. Technical details of measure
2.1.a. Measure description
Cervical dislocation is the manual, rapid separation of the cervical vertebrae with accompanying lethal trauma to the spinal cord. The technique involves immobilization of the head and rapid disarticulation of cervical vertebrae. It may be performed manually by quick and firm traction and pulling of the body in a direction away from the head or it could be facilitated by a cervical dislocator. It is a technique normally used for the euthanasia of small animals, typically manually applied to those weighing less than 200 g, e.g. small birds, poultry, mice, immature rats, and rabbits [1]. For heavy rats and rabbits, the large muscle mass in the cervical region makes manual cervical dislocation physically more difficult [1]. Manual cervical dislocation may be appropriate for rodents < 200 g, rabbits < 1 kg, and birds < 3 kg, [1, 4]. If used on birds, the wings should be secured to avoid involuntary flapping [3]. Mechanical cervical dislocation has been used for larger species, such as mute swan *Cygnus olor* [5]. When performed correctly by well-trained individuals, cervical dislocation appears to be a humane method of euthanasia, which may induce rapid loss of consciousness and ensures a rapid death (loss of cortical function following cervical dislocation is rapid and occurs within 5 to 10 seconds [6,7]). On the other hand, cervical dislocation may be aesthetically displeasing to personnel performing or observing the method and requires mastering technical skills to ensure the loss of consciousness is rapidly induced [1].

Small to medium fish are killed by inserting a rod or thumb into the mouth, holding the fish with the opposite hand, and displacing it dorsally [3]. It is feasible and effective in small fish but should be confirmed by exsanguination or destruction of the brain.

Cervical dislocation is reported as being of potential use for the killing of many small to medium-sized vertebrates, but very few projects have been found applying it during control or eradication activities. Mechanical cervical dislocation has been used to dispatch live-captured feral mute swans. The method was planned with veterinarians and the entire process from the time of capture until a single bird was humanely killed averaged about 30 seconds [5].

2.1.b. Integration with other measures

Cervical dislocation must be applied to a restrained animal. The animal must have been caught and/or restrained using another method, e.g. **cage traps**. The technique involves immobilization of the head and rapid disarticulation of cervical vertebrae, so it may entail brief periods of handling.

2.2. Availability

Species	Availability	Reference(s)
Acridotheres tristis	P	4
Alopochen aegyptiaca	P	4
Callosciurus erythraeus	A	
Corvus splendens	P	4
Herpestes javanicus	P	
Lepomis gibbosus	P	4
Lithobates catesbeianus	P	
Muntiacus reevesi		
Myocastor coypus		
Nasua nasua		
Nyctereutes procyonoides		
Ondatra zibethicus		
Oxyura jamaicensis	P	4
Perccottus glenii	P	4
Plotosus lineatus	P	4
Procyon lotor		
Pseudorasbora parva	P	4
Sciurus carolinensis	A	
Sciurus niger	A	
Tamias sibiricus		
Threskiornis aethiopicus	P	4
Trachemys scripta	P	

3. Humaneness of the measure			
3.1. Welfare for all measures			
Measure type (if applicable): Cervical dislocation	**Humaneness impact categories**		
Domain	**No impact**	**Mild-Moderate**	**Severe - Extreme**
1: Water deprivation, food deprivation, malnutrition	No intrinsic effect on the animal.		
2: Environmental challenge	No intrinsic effect on the animal.		
3: Injury, disease, functional impairment	Applied correctly ensures a rapid death, without risk of injury or functional impairment.		
4: Behavioural, interactive restriction		There will be short-term physical restraint while the animal is being handled.	
5: Anxiety, fear, pain, distress, thirst, hunger etc.		The brief period of handling and restraint necessary to apply the method will almost certainly produce fear, but it will be very short (seconds).	

3.2. Mode of death (if relevant)			
Measure type (if applicable):	**Immediate death (i.e. no suffering)**	**Not immediate death (mild - moderate suffering)**	**Not immediate death (severe - extreme suffering)**
Rationale:	Loss of cortical function following cervical dislocation is rapid and occurs within 5 to 10 seconds as measured by a significant reduction in amplitude recordings of visual evoked responses and EEG [1,2,6,7]. Electrical activity in the brain persists for 13 seconds following cervical dislocation in rats [1].		

3.3. Humaneness summary	Cervical dislocation is a technique normally used for the euthanasia of small animals, typically those weighing less than 200 g (e.g. small birds, poultry, mice, immature rats, and rabbits [1]). A cervical dislocation should not be used for birds of over 3 kg or some older birds where the neck is difficult to pull quickly [4]. Small to medium fish are killed by inserting a rod or thumb into the mouth, holding the fish with the opposite hand, and displacing it dorsally [3]. When performed correctly by well-trained individuals, cervical dislocation appears to be a humane method of euthanasia, which may induce rapid loss of consciousness and ensures a rapid death (loss of cortical function following cervical dislocation is rapid and occurs within 5 to 10 seconds [1,2,6]). In most cases, the period of fear whilst being removed from the trap and handled for dispatch will be very short (seconds). On the other hand, cervical dislocation may be aesthetically displeasing to personnel performing or observing the method and requires mastering technical skills to ensure a loss of consciousness is rapidly induced [1].

4. Costs and effectiveness of the measure

4.1. Case studies	
CASE STUDY #1	
Measure type (if relevant):	Cervical dislocation mechanically performed
Species:	Mute swan *Cygnus olor* (flightless, nonbreeding mute swans and smaller numbers of failed breeding pairs)
Objective:	Control
Combined with other measure(s):	Birds were captured with a swan pole after pursuit by boat and then euthanized with mechanical cervical dislocation. Live capture and euthanasia were used to remove molting swans at 6 of the 11 molting sites where culling by shooting using 12-gauge shotguns was inappropriate because of the proximity to waterfront residential homes. The authors used mechanical cervical dislocation as the preferred method of field euthanasia for captured mute swans where shooting was not possible because it (1) was considered efficient and humane by consulting veterinarians given the field conditions; (2) was consistent with the guidelines for euthanasia of free-ranging wildlife (AVMA 2000); (3) minimized distress to captured swans associated with alternative methods of euthanasia; (4) was practical under field conditions (marine habitat from boats), (5) reduced worker safety risks; and (6) allowed for burial of tissues free of chemical contamination.
Country(ies) of application:	USA
Geographic scale (km^2) and/or population size measure applied to:	

Time period:	2005-2008	
Effort:	A boat was used to drive flightless swans from the protective cover into open water. Once in the open, swans were slowly herded by 2–3 additional capture teams in joint boats to deeper offshore waters (1.2–3.7 m) where they were easier to capture and where the operation was less visible from waterfront homes.	
Costs:	Overall costs:	
	Total cost incurred during the 24 live-capture cull operations was $40,259.74. Mean cost per swan culled was $28.84.	
	Personnel costs:	
	Staff hours was the most expensive part of culling operations. Salaries of staff ($29,699) for 1,181 staff hours composed 74% of the total project costs.	
	Equipment and infrastructure:	
	Personnel $29,699, Vehicles $4,629, Boats $4,687, Other $1,481	
	Other, including overheads:	
Effectiveness:	1,396 flightless mute swans were capture during 24 live-capture culling operations. Mean cull size was 58 swans per operation (range 6–199) for the 4-year period (2005–2008). Culling operations lasted between 1.0–3.5 hrs for all 4 years combined (44 hours total) and cull success averaged 32 swans per hour. This culling method frequently resulted in removal of all flightless mute swans in the area.	
Reference(s):	[5]	

4.2. Costs effectiveness summary	While no case studies on the measure application could be found for the species of Union concern, a case study is available for the mute swam. This is a large bird (8-12 kg) and researchers applied a mechanical cervical dislocation. Overall, 1,396 mute swans were captured and euthanized at 40,359 $ (28.84 $/bird); 74% of the total project costs were related to personnel cost, and the rest to vehicles, boats, and other material. Though costly, the technique allowed managers to remove swans in highly developed areas where shooting would not have been appropriate.

5. Conclusion	
Overall assessment of the measure (qualitative)	
Cervical dislocation provides reliable and humane euthanasia of small to medium animals caught in traps or restrained with another method. It is a method potentially available for small mammals, birds, and fish of Union concern and already used for laboratory animals. However, practical applications with wild animals are scanty. This may be due to the need for well-trained personnel or because it may be aesthetically displeasing. **Cranial depression** is much more used for the euthanasia of restrained animals.	
Assessor:	Sandro Bertolino
Reviewer 1:	Ana Nunes
Reviewer 2:	Kevin Smith

6. References

[1] AVMA (2020). AVMA Guidelines for the Euthanasia of Animals: 2020 Edition

[2] Cartner, S. C., Barlow, S. C., & Ness, T. J. (2007). Loss of cortical function in mice after decapitation, cervical dislocation, potassium chloride injection, and CO2 inhalation. *Comparative medicine*, *57*(6), 570-573.

[3] Clifford DH (1984) Preanesthesia, anesthesia, analgesia, and euthanasia. In: Laboratory Animal Medicine (Fox JC, Cohen BJ, Loew FM, edsl. Orlando: Academic Press, pp 527-62

[4] Close, B., Banister, K., Baumans, V., Bernoth, E. M., Bromage, N., Bunyan, J., ... & Morton, D. (1997). Recommendations for euthanasia of experimental animals: Part 2. *Laboratory animals*, *31*(1), 1-32.

[5] Hindman, L. J., Harvey IV, W. F., Walbridge, H. R., Hooper, M., & Driscoll, C. P. (2015). Efficient Method of Capture and Field Euthanasia of Flightless Mute Swans. Proceedings of the 16th Wildlife Damage Management Conference. (L.M. Conner, M.D. Smith, Eds). 2016. Pp. 55-64.

[6] Iwarsson, K., & Rehbinder, C. (1993). A study of different euthanasia techniques in guinea pigs, rats and mice: animal response and postmortem findings [decapitation, cervical dislocation]. *Scandinavian Journal of Laboratory Animals (Denmark)*.

[7] Vanderwolf, C. H., Buzsaki, G., Cain, D. P., Cooley, R. K., & Robertson, B. (1988). Neocortical and hippocampal electrical activity following decapitation in the rat. *Brain Research*, *451*(1-2), 340-344.

Appendix 23. Cranial depression (dispatch by destruction of the brain)

1. Measure name	
1.1. English:	Cranial depression (dispatch by destruction of the brain)
1.2. Lethal or non-lethal:	Lethal
1.3. Other languages (if available):	

Bulgarian	Механичен удар върху черепа, включително с прихванат болт (проникващ или непроникващ)	Italian	Commozione cerebrale
Croatian	Kranijalna depresija	Latvian	Galvaskausa saspiešana
Czech	Promáčknutí lebky	Lithuanian	Kaukolės suspaudimas
Danish	Kranie brud (slag I hovedet / boltpistol)	Maltese	
Dutch	Indrukken van de kop	Polish	Zmiażdżenie czaszki
Estonian	Kolju depressioon	Portuguese	Depressão craniana
Finnish	Kallopainauma	Romanian	Comoție cerebrală
French	Fracture du crâne	Slovak	Devastácia lebky
German	Craniale sepression, schädeldepression (schlagschuss)	Slovenian	Udarec v glavo (kranialna depresija)
Greek	Κρανιακή σύνθλιψη	Spanish	Depresión cranaeal
Hungarian	Koponyatörés	Swedish	Slag mot huvudet
Irish			

2. Technical details of measure
2.1.a. Measure description
Dispatch by destruction of the brain (otherwise cranial depression) is the very rapid application of force to the skull of an animal to induce instantaneous insensibility and death. Includes; • Manual blunt force trauma • Captive bolts (penetrating or non-penetrating) Cranial depression is the principal and intended mechanism for most humane **spring-operated kill traps** (where striking the head or neck is anticipated) as well as automatically resetting traps such as **Goodnature traps** (both assessed separately). Insensibility is produced by percussive shock to the brain. Destruction of the brain is produced by percussive shock (especially to the brain stem) and/or direct penetrative damage to the skull and brain. Methods are often adapted from their use with livestock (e.g. [12,14]), and as such are highly refined and considered very reliable if undertaken by competent operators. Alternatively, some have a very long tradition of use with wildlife (e.g. euthanasia of moribund animals or game fish landed by sporting anglers) where they also have been proven to be simple, effective and are considered humane. Most efficient or cost-effective methods vary

dependent on species and size and in principle would produce a humane death for all listed IAS, though the larger or more aggressive wild species may make restraint difficult and suggest alternative methods. As most methods of cranial depression, especially manual blunt force, require skill, precision, dexterity and some strength to use effectively a number of hazards may occur which make the method less efficient, and by extension potentially less humane. For example if operators are required to kill animals for extended periods or the equipment is in a poor state of maintenance. Projects should ensure that training, equipment and staff resource are in place to ensure that the method remains humane, e.g. [15,16]. As such this method is most suited to the dispatch of individual small animals or very small groups *in situ* (i.e. trap-side) where these are found away from public view.

In combination with shooting restrained animals (which might also be considered as a type of destruction of the brain; using a free bullet), cranial depression can be used for any species as a 'trap-side' method of dispatch, avoiding welfare issues of moving animals into processing stations for subsequent dispatch, or the greater burdens of onward transport to processing facilities for euthanasia in groups. Unlike shooting restrained animals, cranial depression requires the animal to be removed from its cage/restraint and presented to an operator. For small animals or birds this might be as simple as using workers trained to handle the animal (with appropriate personal protective equipment; PPE such as thick gloves). For ***Ondatra zibethicus*** and ***Herpestes javanicus***, the measure is not be recommended due to the difficulty of handling these species necessary to ensure an efficient and humane outcome. Larger animals may require sedation or anesthesia before using a captive bolt gun and might be more suited to shooting with a free bullet without sedation. For these larger species, especially ***Muntiacus reevesi*** Muntjac deer, it is also unlikely the manually applied blunt force trauma would be suitable, though non-penetrative captive bolt guns have a potential role in stunning animals humanely prior to dispatch with other methods, or alternatively killing them (penetrative captive bolt).

2.1.b. Integration with other measures

This measure must be applied to a restrained animal. Animal must have been caught and/or restrained using another method, e.g. **cage traps**. Access to the head/skull is necessary for the application of blunt force or captive bolts so may entail brief periods of handling.

2.2. Availability

Species	Availability	Reference(s)
Acridotheres tristis	A	[5,6]
Alopochen aegyptiaca	A	
Callosciurus erythraeus	U	
Corvus splendens	P	
Herpestes javanicus	P	With suitable restraint
Lepomis gibbosus	P	[12]
Lithobates catesbeianus	A	[13]
Muntiacus reevesi	A	Only captive bolt with suitable restraint [12]
Myocastor coypus	P	Only captive bolt with suitable restraint
Nasua nasua	P	Only captive bolt with suitable restraint
Nyctereutes procyonoides	P	Only captive bolt with suitable restraint
Ondatra zibethicus	P	With suitable restraint
Oxyura jamaicensis	P	
Perccottus glenii	P	[12]

Plotosus lineatus		
Procyon lotor	P	Only captive bolt with suitable restraint [12]
Pseudorasbora parva	P	
Sciurus carolinensis	A	[7,8,9,10]
Sciurus niger	A	
Tamias sibiricus		
Threskiornis aethiopicus	P	
Trachemys scripta	P	

3. Humaneness of the measure			
3.1. Welfare for all measures			
Measure type (if applicable):	**Humaneness impact categories**		
Domain	**No impact**	**Mild-Moderate**	**Severe - Extreme**
1: Water deprivation, food deprivation, malnutrition	No intrinsic effect on the animal.		
2: Environmental challenge	No intrinsic effect on the animal.		
3: Injury, disease, functional impairment	Applied correctly produces instantaneous insensibility without recovery. Even if animals are injured during the brief period of restraint necessary to deliver the measure, suffering is likely to be very brief.		
4: Behavioural, interactive restriction		There will be short-term physical restraint while the animal is being handled.	
5: Anxiety, fear, pain, distress, thirst, hunger etc.		The brief period of handling and restraint necessary to apply the method will almost certainly produce fear in wild species (listed IAS) but will be very short (seconds).	

3.2. Mode of death (if relevant)			
Measure type (if applicable):	**Immediate death (i.e. no suffering)**	**Not immediate death (mild - moderate suffering)**	**Not immediate death (severe - extreme suffering)**
Rationale:	Produces immediate insensibility and usually immediate death. Requirement for a trained / competent operator able to immediately re-apply measure to insensible but living animals guarantees successful humane outcomes.		

3.3. Humaneness summary	After shooting in the cage, cranial depression represents one of the most humane methods of dispatch for restrained animals when applied by trained operatives. Manual blunt force trauma can be used for smaller and for neonatal animals with a thin cranium [12]. For the smaller species and the birds, competence in the use of the tool can be acquired very quickly with proficiency requiring only a little more effort. For most animals the period of fear whilst being removed from the trap and presented for dispatch will be very short (seconds), and instantaneous insensibility guaranteed by the presence of a competent operative. Death is usually also instantaneous and can be guaranteed by competent operators. Penetrating captive bolts are most appropriate for euthanasia of medium and large mammals [12]. Adequate restraint is important to ensure proper placement of captive bolts. Note that within a livestock slaughter setting personnel who have to perform manually applied blunt force trauma to the head to large numbers of animals can become fatigued, which can lead to inconsistency in application, creating humane concerns about its efficacious application (AVMA 2020). However, it is unlikely that this method would be used within an invasive alien species management programme if such a high volume of animals needed to be dispatched over a short time period. Cranial depression may be perceived by some stakeholders to be distasteful as it may involve the use of brute force on animals, may produce blood or other obvious injury or may be upsetting to watch.

4. Costs and effectiveness of the measure	
General effectiveness of the measure	Cranial destruction using manual blunt force trauma or captive bolts is regularly used to dispatch animals in a research/lab setting [12] and its use has been adopted in wildlife management programmes. More importantly, it has been extensively developed to be used in livestock slaughter where the effectiveness of the measure, its ability to reliably deliver instantaneous insensibility and death, as well as its general superiority to other methods of dispatch for restrained small animals and birds (i.e. more humane) has been the subject of quantitative research (see case studies below). Manual blunt force trauma can be used for smaller and for neonatal animals with a thin cranium, and when properly performed, loss of consciousness is rapid making it inexpensive and effective (AVMA 2020). Penetrative captive bolts, are an effective method of euthanasia for medium and large animals for example for ruminants, horses, dogs etc. and is often applied in the livestock sector (AVMA 2020).

4.1. Case studies		
CASE STUDY #1		
Measure type (if relevant):	Use of portable captive bolt guns and/or blunt force on livestock in farm settings	
Species:	Applicable to all IAS which can be removed from traps easily (all birds, all herptiles, all mammals <3kg except *Ondatra zibethicus* and *Herpestes javanicus* due to difficulty of handling animals)	
Objective:		
Combined with other measure(s):	Combined with any live capture measure. Can also be used as a method of euthanasia for any living animals pinned (but not killed) in kill traps.	
Country(ies) of application:	any	
Geographic scale (km^2) and/or population size measure applied to:		
Time period:		
Effort:		
Costs:	Overall costs:	
	Personnel costs:	
	Equipment and infrastructure:	
	Equipment is dependent on context and species. Varies between the exceptionally cheap (wooden baton or metal bar) to state-of-the-art portable pneumatic captive bolt pistols. Suitable captive bolt systems vary with species and include spring powered devices, pneumatic devices (powered by compressed gas), or bolt systems powered by the ignition of a gas or powder charge (i.e. cartridge).	
	Other, including overheads:	

Effectiveness:	Here we discuss a number of studies comparing the humaneness and effectiveness of cranial depression in the euthanasia of mammal and bird livestock species comparable to IAS. Generally this method becomes progressively more challenging with larger animals (size produced by age or species) so we choose examples for birds and mammals representing those listed IAS for which the method might be recommended as a first choice. However, cranial depression (when applied using a captive bolt) is routinely used as a method of stunning and killing species exceeding the heaviest listed IAS (10-15Kg; Reeves muntjac, *Muntiacus reevesi*). Thus here we discuss its potential use for mammals <3Kg and birds with heads similar to the largest listed IAS (1.5-3Kg; Egyptian goose, *A. aegyptiaca*). We discuss turkeys, small pigs, rabbits and guinea pigs (despite the first being considerably heavier than wild geese). Erasmus et al. (2010) [1] compared the humanness and efficiency of three methods of killing on a range of size classes of turkeys (3-13 Kg); a pneumatically powered captive bolt pistol, blunt force trauma as well as manual and mechanically assisted cervical dislocation. A number of comparative trials found that the captive bolt (discharged twice in immediate succession) and blunt trauma (single hit) were similarly effective at consistently causing immediate insensibility. Conversely, neither method of cervical dislocation reliably caused immediate insensibility. In a related report [2], damage to the brain and skulls of the birds were investigated, indicating that the captive bolt method was superior to even blunt force trauma in reliably producing damage which would lead to immediate death, though both were considered effective and reliable. Conversely cervical dislocation often failed to damage the brain directly (its intention) but rather resulted in neck stretching and neck crushing which probably resulted in death from cerebral hypoxia and ischaemia in some cases (i.e. these methods were slower and less reliable). The OIE Terrestrial Animal Health code (28th edition, May 2019; section 7.5.7 (2)) states that " Captive bolts powered by cartridges, compressed air or spring can be used for poultry. The optimum position for poultry species is at a right angle to the frontal surface. Firing of a captive bolt in accordance with the manufacturers' instructions should lead to immediate destruction of the skull and the brain and, as a result, immediate death." Working with statistically robust numbers of piglets (*Sus scrofa*) in a farm setting (mean weight 2Kg, max weight 3.75 Kg) Grist *et al.* [3] found that the mean handling time for each piglet was 6.45 seconds (pen to insensibility and death). In one trial all animals treated with a single shot of a captive bolt gun (207 piglets) showed an immediate cessation of brainstem reflexes though one was given a second shot as a precaution, and the damage to the brain in a sub-sample of animals suggested that death had been immediate. The method was considered to reliably produce a humane death in animals of this size. A statistically robust comparative assessment of the humaneness of blunt force, non-penetrating captive bolt, a novel mechanical cervical dislocation device on rabbits of a range of sizes, including groups with target weights at around 1.5 kg and 3.5 kg [11]. In this study blunt force was established as the worst performing method, failing to cause instant insensibility and death in 22% of cases, attributable to a poor performance with adult rabbits over 3 Kg at one facility. The report states that they were difficult to restrain and present for dispatch. In contrast, the captive bolt method, using pneumatic pistol achieved instant death and insensibility for 100% of attempts (63 rabbits). We note that the method of application of blunt

	force in this study deviates from that anticipated for 3Kg animals. That notwithstanding, the non-penetrating bolt gun was reliable and effective. The humaneness of four different methods of stunning/euthanasia were tested on statistically robust numbers of farmed guinea pigs (*Cavia porcelus*) of around 1Kg [4]; cervical dislocation and penetrative captive bolts (stunning and killing), and stunning using electric shock and carbon dioxide. Most animals subjected to cervical dislocation (97%) showed some signs of sensibility or absence of death and the method was considered unsuitable. Use of a spring powered captive bolt produced immediate insensibility and death in all but one animal (subsequently considered to be due to operator error). Animals stunned using one of two CO_2 filling rates (20% and 30% per minute) all took at least 91 seconds to become insensible, with some at the slower fill rates taking up to 380 seconds (time to death was also recorded but is not important). The mean duration of heaving breathing (assumed to indicate substantial distress) was 60 seconds and 48 seconds (for slow and fast fill rates respectively).

CASE STUDY #2	
Measure type (if relevant):	Use of cranial depression for an eradication program
Species:	Common Myna (*Acridotheres tristis*)
Objective:	Eradication
Combined with other measure(s):	Trapping, shooting and poison
Country(ies) of application:	Spain (Canary Islands and one Balearic Islands); UK (St Helena and Ascension Island as British Overseas Territory)
Geographic scale (km^2) and/or population size measure applied to:	Whole islands or Island archipelagos
Time period:	10 years
Effort:	Unreported but clearly varied across six different island projects reported on. Some detail of one 53-day program on Ascension Island is given by [6].
Costs:	Overall costs:
	Personnel costs:
	Equipment and infrastructure:
	Other, including overheads:

Effectiveness:	Saavedra [5,6] describes how cranial depression was used as a humane method of euthanasia in six eradication and control programs against the common myna on Atlantic islands (Tenerife, Gran Canaria, Fuerteventura, Ascension and St. Helena) as well as Mallorca (Balearic Islands). Birds were trapped in a variety of live capture traps and euthanized by placing them in an appropriate bag (pillow case) and striking it firmly against a hard surface (ground, rock or concrete). As traps were placed away from public areas, euthanasia could be trap- side. No comment on failures of the method were made. Eradication was successful on two islands with control achieved on three, only one was considered to have failed due in inadequate effort.

CASE STUDY #3	
Measure type (if relevant):	Use of cranial depression for managing grey squirrels in the UK
Species:	Grey squirrel (*Sciurus carolinensis*)
Objective:	Eradication (Isle of Anglesey), control and containment (rest of UK)
Combined with other measure(s):	Varies slightly with context. Mainly trapping with some shooting.
Country(ies) of application:	UK (England and Wales)
Geographic scale (km^2) and/or population size measure applied to:	Eradication across 710Km^2 (Isle of Anglesey, Wales)
Time period:	15 years
Effort:	Detail provided in [7,8,9]
Costs:	Overall costs:
	Personnel costs:
	Details confounded by the extensive use of volunteers
	Equipment and infrastructure:
	Other, including overheads:
Effectiveness:	Grey squirrels were trapped in woodlands in a range of contexts and euthanized using a blow to the head (blunt force); this method is recommended by the state agency responsible for protecting trees (where squirrels are recognized pests) [10]. Substantial trapping is done by voluntary groups across northern England to provide some control and containment to avoid the spread of the grey squirrel further endangering the red squirrel. Further trapping (with cranial depression as the recommended means of dispatch) is used across England and Wales to control the density of grey squirrels where they become a pest of trees.

	This case study is of the successful eradication of the grey squirrel from the 710 km^2 area of the Isle of Anglesey (Wales; UK), and continued operations to maintain its grey squirrel free status by undertaking a squirrel containment operation on the mainland (protecting the two bridges to the island). Across 13 years of operation, 100,000s of traps days were conducted by volunteers and professional operatives [9], (52,000 trap days in 2007 alone) [8,9] catching and dispatching 6400 animals. Squirrels were run from the trap into a strong bag (e.g. hessian sack) placed on the ground, corralled with its head in the corner of a bag and struck firmly with a hard and heavy object [10]. Insensibility is instantaneous, as is death. If there is any doubt as to the success of the first blow, a successive second blow can be delivered, guaranteeing a humane outcome. The act of transferring animals from trap to bag and preparing for the blow takes a few seconds when used by competent operatives. This method avoids soiling the trap with blood which might prejudice subsequent trap use (aversive to new animals).

4.2. Costs effectiveness summary	The use of cranial depression to provide a reliable and humane euthanasia of animals restrained in traps appears extremely successful. Both case examples with listed IAS (squirrels and mynas) used variations of the measure which could be used easily by volunteers or local operatives in their local contexts, after a little training as well as costing very little to deploy (rocks, sticks, bars and bags). Presumably this permitted extra resource to be used for other activities promoting the success of the projects. In both cases, trapping (followed by the use of cranial depression) represented the majority of the removal activity, though shooting and poisoning (mynas) also occurred. The measure is potentially applicable to larger species (including all terrestrial and avian IAS of Union concern) if more potent, though costly tools are used such as captive bolt devices. The more expensive state-of-the-art tools (pneumatically powered captive bolt pistols) ensure the powerful and accurate blow necessary to guarantee instant insensibility and death as well as a very humane death for the animal. Only the applications of the method to livestock have produced the quantitative and experimental comparisons necessary to evaluate the comparative humaneness of the method. When applied appropriately by competent operatives, blunt force is seen to be as effective as captive bolt tools to provide reliable instantaneous insensibility, with both outperforming comparator methods (gas, cervical dislocation, drugs etc.), either at producing insensibility or combining this with the destruction of the brain necessary to guarantee death.

5. Conclusion

Overall assessment of the measure (qualitative)

Many wildlife control programs using live-traps or other methods of restraint are surprising reticent about communicating the details of how animals are dealt with once captive. Many simply state 'animals were euthanized using a humane method'. As such, it has been surprisingly difficult to identify with confidence the method of dispatch used for all significant coordinated eradication/containment/control programs applied to listed IAS. It is more challenging still to find comment indicating the advantages or disadvantages of any method.

All methods of destroying the brain should produce reliable and instantaneous insensibility and death, demonstrated by the favorable comparison with methods of euthanasia and slaughter used for livestock (case study 1). Even when the first strike/blow/shot fails to produce instantaneous death, insensibility and humaneness is assured, and death is guaranteed by the immediate re-application of the method.

As well as representing one of the most humane means of dispatch, these methods are often very cheap to deploy and often very easy to carry out into the field to be used alongside traps where they are placed well away from roads and people. In addition, they often require limited training to ensure competence and can be used by local staff or volunteers where this is appropriate for the program of control. Importantly, they can usually be applied trap-side, avoiding any burdens to welfare of captive animals associated with moving them to any sort of processing station or facility.

However, these methods must be skillfully executed to ensure a quick and humane death, because failure to do so can cause substantial suffering (AVMA, 2020). Recent commentary by EFSA on the killing of rabbits both for slaughter [15] and for other purposes [16] identifies a number of hazards in the use of both blunt force trauma and captive bolt systems as well as describing remedies to ensure that poor practice does not lead to unacceptable or unnecessary problems for animal welfare. These included training and competence of operators, maintenance of appropriately specified equipment and avoiding problems caused by operator fatigue. These principles can be extended to the use of these methods on many of the IAS of Union concern. Also, current legislation limits the use of some skilled manual methods of on-farm slaughter (incl. manual blunt force trauma for rabbits and manual cervical dislocation for poultry) to 70 animals per day and this might be taken as a working limit where manual blunt force is used on appropriate IAS of Union concern.

Their major disadvantage is not technical but social, with the methods being seen by some workers and some stakeholders in society as messy, brutal and distasteful (denying objective comparative assessments of their humaneness). As such programs may be reluctant to promote their use of the method, preferring instead less humane, but more societally appealing tools such as gassing or drugs.

The two measures assessed here (blunt force and captive bolts) compare to shooting (dispatch of a restrained animal in a cage using a free bullet) in effectiveness but may produce different operational consequences which are indicated in the table below.

Table: Summarized comparisons between two assessed methods of cranial depression with shooting as an additional method of destroying brains			
Consideration	**Blunt force**	**Captive bolts**	**Free-bullet**
Capital cost/consumable costs	Very cheap/free	Depends on application (cheap to costly)/moderate	Depends on application (expensive)/ Depends on application (cheap to expensive)
Administration costs	None	Variable but low	Variable to moderate (i.e. permits and mandatory training)
Handling/stress	For animals where this is appropriate (limited – seconds)	Depends on application (may be longer than blunt force – seconds)	Minimal as the handler needs approach the trap which may cause stress
Portability	Very portable	Depends on application (very portable to least portable portable)	Portable
Operator skill required	For animals where this is appropriate (lowest)	Intermediate	Highest
Operator welfare	Depends on application (probably intermediate)	Depends on application (probably worst)	Probably best
Noise	Quiet	Quiet to moderately quiet	Depends on species (air weapons moderately quiet; firearms noisy)
Societal perception (aesthetic)	Lowest	Highest	Intermediate
Societal perception (humaneness)	Variable but probably lowest	Variable but probably highest	Intermediate
Societal perception (security)	Variable but probably highest	intermediate	Variable but probably lowest

Assessor:	James Aegerter
Reviewer 1:	Kevin Smith
Reviewer 2:	Sandro Bertolino
Reviewer 3:	Ilaria Di Silvestre

6. References

[1] Erasmus, M. A., et al. (2010). "Using time to insensibility and estimated time of death to evaluate a nonpenetrating captive bolt, cervical dislocation, and blunt trauma for on-farm killing of turkeys." Poultry science **89**(7): 1345-1354.

[2] Erasmus, M. A., et al. (2010). "Using time to insensibility and estimated time of death to evaluate a nonpenetrating captive bolt, cervical dislocation, and blunt trauma for on-farm killing of turkeys." Poultry science **89**(7): 1345-1354.

[3] Grist, A., Knowles, T., & Wotton, S. (2018). Humane Euthanasia of Neonates II: Field study of the effectiveness of the Zephyr EXL Non-Penetrating Captive Bolt system for euthanasia of new-born and weaned piglets. *Animal Welfare, 27*, 319-326.

[4] Limon, G., Gonzales-Gustavson, E. A., & Gibson, T. J. (2016). Investigation Into the Humaneness of Slaughter Methods for Guinea Pigs (Cavia porcelus) in the Andean Region. *Journal of Applied Animal Welfare Science, 19*(3), 280-293.

[5] Saavedra, S. (2010). Eradication of invasive mynas from islands. Is it possible? *Aliens Invasive Species Bull, 29*, 40-47.
[6] Saavedra, S. (2009). *First control campaign for common myna (Acridotheres tristis): Final report*. Retrieved from Live Arico Invasive Species Department:
[7] Robertson, P. A., Adriaens, T., Lambin, X., Mill, A., Roy, S., Shuttleworth, C. M., & Sutton-Croft, M. (2017). The large-scale removal of mammalian invasive alien species in Northern Europe. Pest Management Science, 73(2), 273-279. doi:10.1002/ps.4224
[8] Schuchert, P., Shuttleworth, C. M., McInnes, C. J., Everest, D. J., & Rushton, S. P. (2014). Landscape scale impacts of culling upon a European grey squirrel population: can trapping reduce population size and decrease the threat of squirrelpox virus infection for the native red squirrel? Biological Invasions, 16(11), 2381-2391. doi:10.1007/s10530-014-0671-8
[9] Jones, H., White, A., Lurz, P., & Shuttleworth, C. (2017). Mathematical models for invasive species management: Grey squirrel control on Anglesey. Ecological Modelling, 359, 276-284.
[10] Mayle, B. A., Ferryman, M., & Pepper, H. W. (2007). Controlling grey squirrel damage to woodlands. (0855385944). Forestry Commission: Edinburgh (United Kingdom): Forestry Commission.
[11] Walsh, J. L., Percival, A., & Turner, P. V. (2017). Efficacy of Blunt Force Trauma, a Novel Mechanical Cervical Dislocation Device, and a Non-Penetrating Captive Bolt Device for On-Farm Euthanasia of Pre-Weaned Kits, Growers, and Adult Commercial Meat Rabbits. Animals, 7(12), 100
[12] Leary, S., Underwood, W., Anthony, R., Cartner, S., Grandin, T., Greenacre, C., . . . Miller, D. (2020). AVMA Guidelines for the Euthanasia of Animals: 2020 Edition. AVMA American Veterinary Medical Association: Schaumburg, IL, USA.
[13] Kamoroff, C., Daniele, N., Grasso, R. L., Rising, R., Espinoza, T., & Goldberg, C. S. (2020). Effective removal of the American bullfrog (Lithobates catesbeianus) on a landscape level: long term monitoring and removal efforts in Yosemite Valley, Yosemite National Park. Biological Invasions, 22(2), 617-626.
[14] EU Regulation 1099/2009
[15] EFSA Panel on Animal Health and Welfare, Saxmose Nielsen, S., Alvarez, J., Bicout, D. J., Calistri, P., Depner, K., . . . Spoolder, H. (2020). Stunning methods and slaughter of rabbits for human consumption. EFSA Journal, 18(1), e05927. doi:10.2903/j.efsa.2020.5927
[16] EFSA Panel on Animal Health and Welfare, Saxmose Nielsen, S., Alvarez, J., Bicout, D. J., Calistri, P., Depner, K., . . . Spoolder, H. (2020). Scientific opinion concerning the killing of rabbits for purposes other than slaughter. EFSA Journal, 18(1), e05943. doi:10.2903/j.efsa.2020.5943

Appendix 24. Decapitation

1. Measure name	
1.1. English:	Decapitation
1.2. Lethal or non-lethal:	Lethal
1.3. Other languages (if available):	

Bulgarian	Обезглавяване	Italian	Decapitazione
Croatian	Dekapitacija	Latvian	Galvas noņemšana (dekapitācija)
Czech	Dekapitace	Lithuanian	Galvos pašalinimas
Danish	Halshugning (Decapitation)	Maltese	
Dutch	Onthoofding	Polish	Dekapitacja
Estonian	Dekapitatsioon (pea eemaldamine)	Portuguese	Decapitação
Finnish	Dekapitaatio	Romanian	Decapitare
French	Décapitation	Slovak	Dekapitácia
German	Dekapazitation, enthauptung	Slovenian	Obglavljenje
Greek	Αποκεφαλισμός	Spanish	Decapitación
Hungarian	Lefejezés	Swedish	Halshuggning
Irish			

2. Technical details of measure
2.1.a. Measure description

Decapitation is caused by severing the head from the body using heavy shears or a guillotine. Decapitation appears to induce a rapid loss of consciousness [3,4]. It has been demonstrated that electrical activity in the brain persists for 13 to 14 seconds following decapitation [2]. The electrical activity does not imply that pain is perceived and recent studies conclude that loss of consciousness develops rapidly [3,4]. The maximum time the pain and distress could be perceived in rats is 2.7 seconds [5].

However, the handling and restraint required to perform decapitation may be distressful for animals. Personnel performing this method should recognize the inherent danger of the guillotine and take precautions to prevent personal injury. Decapitation may be aesthetically displeasing to personnel performing or observing the method [1]. According to AVMA [1], decapitation using scissors or sharp blades is acceptable with conditions for altricial neonates. Some rodent neonates, whether altricial or precocial, may have a tissue mass that is too large for scissors, so appropriate decapitation tools such as a guillotine should be selected. The use of plastic cones to restrain animals appears to reduce distress from handling, minimizes the chance of injury to personnel, and improves positioning of the animal [1].
Decapitation is used for small laboratory animals and is considered acceptable with conditions for euthanasia of small (< 200 g) birds [1]. Decapitation is acceptable with conditions for the euthanasia of poultry and should be executed with a sharp instrument, ensuring rapid and unobstructed severing of the head from the neck [1]. Decapitation alone is not considered a humane approach to euthanasia of fish; pithing of the brain after

decapitation helps ensure rapid loss of brain function and death for these species [1]. In amphibians and reptiles, decapitation should be preceded by an injectable anesthetic and followed by pithing or another method of destroying the brain [1]. Despite decapitation being considered humane and studies on laboratory animals indicating a rapid loss of consciousness and death, as far as we know it is not used in wildlife control programs and no evidence of its application could be found for the species of Union concern.
2.1.b. Integration with other measures
Decapitation must be applied to restrained animals. The animal must have been caught and/or restrained using another method, e.g. **cage traps**. The technique involves immobilization of the animal and the use of a scissors or a guillotine. The handling and restraint required to perform decapitation may be distressful.

2.2. Availability		
Species	**Availability**	**Reference(s)**
Acridotheres tristis	P	1
Alopochen aegyptiaca	P	1
Callosciurus erythraeus	P	1
Corvus splendens	P	1
Herpestes javanicus		
Lepomis gibbosus	P	1
Lithobates catesbeianus	P	1
Muntiacus reevesi		
Myocastor coypus		
Nasua nasua		
Nyctereutes procyonoides		
Ondatra zibethicus		
Oxyura jamaicensis		
Perccottus glenii	P	1
Plotosus lineatus	P	1
Procyon lotor		
Pseudorasbora parva	P	1
Sciurus carolinensis	P	1
Sciurus niger	P	1
Tamias sibiricus	P	1
Threskiornis aethiopicus	P	1
Trachemys scripta	P	

3. Humaneness of the measure			
3.1. Welfare for all measures			
Measure type (if applicable):	**Humaneness impact categories**		
Domain	**No impact**	**Mild-Moderate**	**Severe - Extreme**
1: Water deprivation, food deprivation, malnutrition	No intrinsic effect on the animal.		
2: Environmental challenge	No intrinsic effect on the animal.		
3: Injury, disease, functional impairment	It ensures a rapid death, without risk of injury or functional impairment.		
4: Behavioural, interactive restriction		There will be short-term physical restraint while the animal is being handled.	
5: Anxiety, fear, pain, distress, thirst, hunger etc.		The brief period of handling and restraint necessary to apply the method will almost certainly produce fear, but it will be very short (seconds). During decapitation the pain and distress perceived in rats lasts for 2.7 seconds.	

3.2. Mode of death (if relevant)			
Measure type (if applicable):	**Immediate death (i.e. no suffering)**	**Not immediate death (mild - moderate suffering)**	**Not immediate death (severe - extreme suffering)**
Rationale:	Decapitation appears to induce a rapid loss of consciousness. Electrical activity in the brain persists for 13 to 14 seconds; however, this activity does not imply that pain is perceived, and recent studies conclude that loss of consciousness develops rapidly. The		

	maximum time the pain and distress could be perceived in rats is 2.7 seconds.		

3.3. Humaneness summary	Decapitation is considered a humane method of dispatching animals. A large body of research on laboratory animals shows that it induces a rapid loss of consciousness and death. The method is used in laboratories with small mammals and in farms with poultry. In heterothermic vertebrates, it should be accompanied by brain destruction. However, the method is not used in wildlife management, possibly because it is aesthetically displeasing.

4. Costs and effectiveness of the measure

4.1. Case studies	
CASE STUDY #1	
Measure type (if relevant):	
Species:	
Objective:	
Combined with other measure(s):	
Country(ies) of application:	
Geographic scale (km^2) and/or population size measure applied to:	
Time period:	
Effort:	
Costs:	Overall costs:
	Personnel costs:
	Equipment and infrastructure:
	Other, including overheads:

Effectiveness:	
Reference(s):	

4.2. Costs effectiveness summary	No case studies could be found of the measure application to any of the species of Union concern, or even as part of a broader wildlife management programme.

5. Conclusion

Overall assessment of the measure (qualitative)

Decapitation is considered a humane method of dispatching animals and there is a large body of research on laboratory animals. The method is used to euthanize animals in laboratories and farms. However, the method is not used in wildlife management, possibly because it is aesthetically displeasing. When animals are captured during control operations, other killing methods are preferred, e.g. **shooting**, **cervical dislocation**, **use of gases**.

Assessor:	Sandro Bertolino
Reviewer 1:	Ana Nunes
Reviewer 2:	Kevin Smith

6. References

[1] AVMA (2020). AVMA Guidelines for the Euthanasia of Animals: 2020 Edition

[2] Mikeska, J. A., & Klemm, W. R. (1975). EEG evaluation of humaneness of asphyxia and decapitation euthanasia of the laboratory rat. *Laboratory Animal Science*, *25*(2), 175.

[3] Vanderwolf, C. H., Buzsaki, G., Cain, D. P., Cooley, R. K., & Robertson, B. (1988). Neocortical and hippocampal electrical activity following decapitation in the rat. *Brain Research*, *451*(1-2), 340-344.

[4] Holson, R. R. (1992). Euthanasia by decapitation: evidence that this technique produces prompt, painless unconsciousness in laboratory rodents. *Neurotoxicology and teratology*, *14*(4), 253-257.

[5] Derr, R. F. (1991). Pain perception in decapitated rat brain. *Life Sciences*, *49*(19), 1399-1402.

Appendix 25. Electrocution

1. Measure name	
1.1. English:	Electrocution
1.2. Lethal or non-lethal:	Lethal

1.3. Other languages (if available):

Bulgarian	Електрически ток (електрошок)	Italian	Elettrolocuzione
Croatian	Elektrokucija	Latvian	Elektrostrāvas trieciens
Czech	Zabití elektrickým proudem	Lithuanian	Elektros smūgis
Danish	Elektrisk aflivning	Maltese	
Dutch	Elektrocutie	Polish	Porażenie prądem
Estonian	Elektrilöök	Portuguese	Eletrocussão
Finnish	Sähköllä lopettaminen	Romanian	Electrocutare
French	Électrocution	Slovak	Usmetenie elktrošokom/elektrickým prúdom
German	Elektrokution, tötung mittels stromschlag	Slovenian	Električni udar
Greek	Ηλεκτροπληξία	Spanish	Electrocución
Hungarian	Halálos áramütés	Swedish	Elektrifiering
Irish			

2. Technical details of measure

2.1.a. Measure description

The purpose of electrocution is to produce cardiac fibrillation and arrest, which kill the animal by stopping the heart from pumping blood around the body. If this happens, the brain will be starved of oxygen and will rapidly die. When an appropriate electric current is passed through the heart it goes into a state known as ventricular fibrillation. However, animals do not lose consciousness for 10 to 30 seconds or more after the onset of cardiac fibrillation, it is, therefore, necessary that animals be unconscious and insensible to pain before being electrocuted. Unconsciousness can be induced by passing a current through the brain [1,2].
There are two methods for euthanasia. In the 1-step head-to-body approach an electrical current is simultaneously passed through both the brain and the heart. This simultaneously induces unconsciousness through a grand mal epileptic seizure and electrocutes the animal by inducing cardiac arrest. In the 2-step method, an electrical current is passed through the head to induce unconsciousness, then a second current is passed through either the side of the body or the brisket to induce cardiac arrest [1,3,4]. Current electricity is used to attract fish and amphibians in water, but this is considered in **Electrofishing.**

Electrocution is considered humane if the animal is first rendered unconscious [1]. On the other hand, it is difficult to apply in animals that are difficult to restrain if not sedated. Sometimes it may not result in the death of small animals (< 5 kg), because ventricular fibrillation and circulatory collapse do not always persist after cessation of current flow, or in dehydrated animals [1]. Electrocution may be hazardous to personnel or be ineffective in animals, therefore personnel must be familiar with appropriate placement of electrodes and use of equipment.

Alternating current has been used to euthanize dogs, cattle, sheep, goats, swine, chickens, foxes, farmed minks, and fish [1]. Electric shock used to euthanize farmed blue fox (*Alopex lagopus*) was considered acceptable for humane killing [5,6].

Electrocution traps are commercially available for mice, but it is not clear if they cause unconsciousness before killing them [8]. The use of electricity to trap and kill possums was considered unacceptable on humanitarian grounds [7], however, it was further developed to a commercial product [10]. In Australia, an electric grid system has been used for flying foxes (*Pteropus* species) [9].

2.1.b. Integration with other measures

Electrocution is generally applied to restrained animals. The animal must have been caught and/or restrained using another method, e.g. **cage traps**. The technique requires periods of handling to immobilize the animals and apply the technique (e.g. place electrodes and electric power on). Electrocution traps are available for small and medium mammals, in this case animals are directly electrocuted inside the trap.

2.2. Availability

Species	Availability	Reference(s)
Acridotheres tristis	P	1
Alopochen aegyptiaca	P	1
Callosciurus erythraeus	P	1,5,6,9,10
Corvus splendens	P	1
Herpestes javanicus	P	1,5,6,9,10
Lepomis gibbosus	P	1
Lithobates catesbeianus		
Muntiacus reevesi	P	1,5,6,9,10
Myocastor coypus	P	1,5,6,9,10
Nasua nasua	P	1,5,6,9,10
Nyctereutes procyonoides	P	1,5,6,9,10
Ondatra zibethicus	P	1,5,6,9,10
Oxyura jamaicensis	P	
Perccottus glenii	p	1
Plotosus lineatus	P	1
Procyon lotor	p	1,5,6,9,10
Pseudorasbora parva	p	1
Sciurus carolinensis	p	1,5,6,9,10
Sciurus niger	p	1,5,6,9,10
Tamias sibiricus	p	1,5,6,9,10
Threskiornis aethiopicus	p	1
Trachemys scripta	P	

3. Humaneness of the measure			
3.1. Welfare for all measures			
Measure type (if applicable):	**Humaneness impact categories**		
Domain	**No impact**	**Mild-Moderate**	**Severe - Extreme**
1: Water deprivation, food deprivation, malnutrition	No intrinsic effect on the animal.		
2: Environmental challenge	No intrinsic effect on the animal.		
3: Injury, disease, functional impairment	Applied correctly ensures a rapid death, without risk of injury or functional impairment.		
4: Behavioural, interactive restriction		There will be short-term physical restraint while the animal is being handled.	
5: Anxiety, fear, pain, distress, thirst, hunger etc.			The animal should be handled and prepared for electrocution and this will require from a few to several minutes according to species and staff training. Before applying electrocution, the animal should be unconscious and insensible to pain. Preparing the animals and passing a current through the brain will stress the animal and inflict pain.

3.2. Mode of death (if relevant)			
Measure type (if applicable):	**Immediate death (i.e. no suffering)**	**Not immediate death (mild - moderate suffering)**	**Not immediate death (severe - extreme suffering)**
Rationale:		Animals do not lose consciousness for 10 to 30 seconds or more after onset of cardiac fibrillation, it is therefore necessary to induce unconsciousness passing a current through the brain before the animal is electrocuted. The method should not be applied without ensuring immediate unconsciousness.	

3.3. Humaneness summary	The method is considered humane and used for farmed animals and in a few occasions also with wild animals. Since animals do not lose consciousness for 10 to 30 seconds the method should not be applied without ensuring a rapid unconsciousness passing a current through the brain before the animal is electrocuted. This is a method used to kill restrained animals which were trapped before, the stress produced by cage traps or other methods should thus be also considered. However, 'electrical and electronic devices capable of killing or stunning' are prohibited under Annex VI of the Habitats Directive (Council Directive 92/43/EEC).

4. Costs and effectiveness of the measure

4.1. Case studies	
CASE STUDY #1	
Measure type (if relevant):	
Species:	
Objective:	
Combined with other measure(s):	
Country(ies) of application:	
Geographic scale (km^2) and/or population size measure applied to:	
Time period:	

Effort:		
Costs:	Overall costs:	
	Personnel costs:	
	Equipment and infrastructure:	
	Other, including overheads:	
Effectiveness:		
Reference(s):		

4.2. Costs effectiveness summary	No case studies on the measures application to species of Union concern could be mobilized.

5. Conclusion	
Overall assessment of the measure (qualitative)	
If properly applied the method is considered humane and is already used for dispatching farmed animals. The method is difficult to apply in animals that are not immobilized, therefore the application to wild animals is not easy and they should be trapped and immobilized beforehand. Electrocution may be hazardous to personnel or be ineffective in animals, therefore the method must be applied only by personnel familiar with appropriate placement of electrodes and use of equipment. Experience with wild animals is limited, probably due to the limitations of the method. Traps that kill animals with electricity without the need of human intervention have been developed for possums in New Zealand and flying foxes in Australia.	
Assessor:	Sandro Bertolino
Reviewer 1:	Ana Nunes
Reviewer 2:	Kevin Smith

6. References

[1] AVMA (2020). AVMA Guidelines for the Euthanasia of Animals: 2020 Edition

[2] Pascoe, P. J. (1986). Humaneness of an electroimmobilization unit for cattle. *American Journal of Veterinary Research*, *47*(10), 2252-2256.

[3] Vogel, K. D., Badtram, G., Claus, J. R., Grandin, T., Turpin, S., Weyker, R. E., & Voogd, E. (2011). Head-only followed by cardiac arrest electrical stunning is an effective alternative to head-only electrical stunning in pigs. *Journal of animal science*, *89*(5), 1412-1418.

[4] Weaver, A. L., & Wotton, S. B. (2009). The Jarvis Beef Stunner: Effects of a prototype chest electrode. *Meat science*, *81*(1), 51-56.

[5] Korhonen, H. T. (2016). Comparative electrophysiology for euthanasia in mink (*Neovison vison*) and blue fox (*Vulpes lagopus*). In *XIth International Scientific Congress in Fur Animal Production* (Vol. 1665, No. 2298, p. 383).

[6] Korhonen, H. T., Cizinauskas, S., & Viitmaa, R. (2009). Evaluation of the traditional way of euthanasia of farmed foxes from an animal welfare point of view. *Annals of Animal Science*, *9*(1), 73-87.

[7] Dix, I. D., Jolly, S. E., Bufton, L. S., & Gardiner, A. I. (1994). The potential of electric shock for humane trapping of brushtail possums, *Trichosurus vulpecula*. *Wildlife Research*, *21*(1), 49-51.

[8] Mason, G. J., & Littin, K. E. (2003). The humaneness of rodent pest control.

[9] Jones, B., Australia, R. S. P. C. A., Cathles, H., & Landholder, W. J. (2003, February). Solutions for achieving humane vertebrate pest control. In *Proceedings of the 2003 RSPCA Australia scientific seminar*.

[10] DOMIGAN, Ian R. *An integrated design methodology for inventing animal traps in accordance with NAWAC guidelines*. 2011. PhD Thesis. Lincoln University.

Appendix 26. Freezing – rapid freezing and cooling (hypothermia)

1. Measure name			
1.1. English:	Freezing – rapid freezing and cooling (hypothermia)		
1.2. Lethal or non-lethal:	Lethal		
1.3. Other languages (if available):			
Bulgarian	Замразяване – бързо замразяване и охлаждане (хипотермия)	Italian	Congelamento
Croatian	Zamrzavanje	Latvian	Sasaldēšana
Czech	Zamrazení	Lithuanian	Sušaldymas
Danish	Hypotermi – forfrysning	Maltese	
Dutch	Invriezen - snel invriezen en afkoelen (onderkoeling)	Polish	Zamrażanie
Estonian	Külmutamine	Portuguese	Congelamento
Finnish	Jäädytys	Romanian	Congelare – congelare rapidă și răcire (hipotermie)
French	Congélation	Slovak	Zmrazovanie
German	Schnelles Erfrieren und Herunterkühlen (Hypothermie)	Slovenian	Zamrznitev
Greek	Πάγωμα	Spanish	Congelación
Hungarian	Lefagyaztés	Swedish	Nedfrysning
Irish			

2. Technical details of measure
2.1.a. Measure description
<u>**Freezing to euthanize**</u> **Rapid freezing** (e.g. immersion in liquid nitrogen) has been used to euthanize very small animals, and embryos and fetuses, particularly within the scientific research sector as it preserves tissues for clinical examination [1]. According to the American Veterinary Medical Association Guidelines for the Euthanasia of Animals [1], for **rodents** (laboratory and wild caught) rapid freezing in liquid N_2 is only acceptable for mouse and rat foetuses and neonates < 5 days of age. The guidelines also state that for **poultry**, eggs that have been incubated for less than 80% of the full incubation time can be destroyed by cooling (at < 4°C for 4 hours), or freezing. The use of **hypothermia** to euthanize by placing the animal in very cold temperatures such as deep freezers, has also been used to euthanize small animals, however it is no longer an acceptable method for any animal [2,3] and evidence of its current use could not be found.

Two step cooling – cooling (to anesthetize) followed by freezing (to euthanize)
Hypothermia (by cooling and freezing) is also known to act as an anaesthetic agent for ectothermic animals such as amphibians, reptiles and fish. For example, cooling has been used to anaesthetize fish prior to slaughter for decades [4]. This has led to a two-step approach, cooling (to anaesthetize) followed by freezing (to euthanize), being adopted for **amphibians** and **small reptiles** [5], and also for some **warm water fish** species.

Mammals and birds
No evidence could be found for the use of cooling or freezing with any of the mammalian or avian IAS of Union concern, and based on the limits of their use set out by the AVMA these methods are unlikely to be suitable for use as part of an IAS mammal or bird eradication, control, or containment programme.

Fish
In relation to fish, the AVMA [1] state that freezing may be used for euthanasia providing the animal is unconscious prior to freezing (e.g. fish can be anaesthetized by immersion in solutions of MS 222, which is an anaesthetic agent), also that an additional follow-on euthanasia method can be used to ensure death. However, slow chilling or freezing of un-anesthetized animals, including placing fish into a freezer without prior anesthesia, is an unacceptable method according to the AVMA guidelines [1]. Cucherousset et al. [6] used anaesthetic followed by freezing in a slurry of ice water to euthanise pumpkin seed (***Lepomis gibbosus***) as part of a study into the invasiveness of the species across north-west Europe (Norway, The Netherlands, UK, Belgium, and France). Lambooij et al. [7] report on using rapid cooling followed by freezing [hypothermia] in brine (-18°C for 15 minutes) as a form of euthanasia for eels (*Anguilla anguilla*), and Mathews and Varga [4] report on anaesthesia using MS-222 followed by rapid freezing by immersion in liquid nitrogen for Zebrafish (*Danio rerio*) in biomedical research. However, the measure may not be suitable for the Amur sleeper or Chinese sleeper, ***Percottus glenii***, which according to Bogutskaya & Naseka [8] go into torpor at close to 0°C and their ability to 'freeze into ice and then to 'melt' is well known", and they may have a natural 'antifreeze'. As a result, when these fish are frozen in ice during winter conditions, no internal freezing occurs and ice crystals do not destroy their body tissues.

Reptiles and amphibians
According to the AVMA [1], rapid freezing can be used to euthanize reptiles and amphibians < 4g (note the AVMA < 4g limit is based on rodent models) when it results in immediate death; it is likely that this can be achieved by placing animals (< 4 g) in liquid N_2. The guidelines also note that the technique should not be used for species that have adapted freeze tolerance strategies, as this method may not result in instant death. The use of cooling and freezing were formerly accepted means of anesthesia and euthanasia for ectothermic tetrapods, however such practices are now largely banned around the world due to concerns that ice-crystals may form, causing significant pain, while the animal is still conscious [5,9]. The AVMA guidance for reptiles and amphibians is based on a mammalian-biased perspective and a number of studies provide evidence that cooling followed by freezing is in fact a humane measure for amphibians and reptiles larger than 4g, and recommend that the guidelines be reevaluated [5,9,10]. This is discussed further in the humaneness section below.

Cooling followed by freezing has been applied to ***Trachemys scripta elegans*,** where individuals were placed in a fridge for 45 minutes and then in a freezer for 1 hour before decapitation [10]. Their rationale for using **hypothermia** to induce anesthesia is based on the turtle's natural history, as cold temperatures induce a behaviorally torpid state in turtles. Orchard [11] also used freezers as a two-step euthanasia procedure as part of a bullfrog (***Litobates catesbeianus***) eradication programme on Vancouver Island, Canada. Here, the bullfrogs' core body temperatures were lowered to just below 2°C by placing them in a chest freezer for at least 12 hours, then they were transferred to a deep freezer (for 48 hours) to quickly freeze the animals to euthanize them. Shine et al. [5] placed cane toads (*Rhinella marina*) into a standard household refrigerator, and once the toad's core reached fridge temperature (~5°C), it was transferred to a household freezer for 30 min, resulting in mortality.

2.1.b. Integration with other measures
The measure can be combined with any measure that captures individuals alive, for example electrofishing (for fish and bullfrogs). The animals (>4 g for amphibians and reptiles) must be fully anaesthetized before rapid freezing. For ectothermic animals this can be achieved by cooling and this is discussed as part of the measure. Also where cooling is used to anaesthetize animals, other dispatch measures can be used, such as decapitation.

2.2. Availability

Species	Availability	Reference(s)
Acridotheres tristis		
Alopochen aegyptiaca		
Callosciurus erythraeus		
Corvus splendens		
Herpestes javanicus		
Lepomis gibbosus	A	[6]
Lithobates catesbeianus	A	[11]
Muntiacus reevesi		
Myocastor coypus		
Nasua nasua		
Nyctereutes procyonoides		
Ondatra zibethicus		
Oxyura jamaicensis		
Perccottus glenii		
Plotosus lineatus		
Procyon lotor		
Pseudorasbora parva	P	[1]
Sciurus carolinensis		
Sciurus niger		
Tamias sibiricus		
Threskiornis aethiopicus		
Trachemys scripta	A	[10]

3. Humaneness of the measure			
3.1. Welfare for all measures			
Measure type (if applicable): Cooling (as an anesthesia) prior to freezing	**Humaneness impact categories**		
Domain	**No impact**	**Mild-Moderate**	**Severe - Extreme**
1: Water deprivation, food deprivation, malnutrition	[The measure is not suitable for any of the IAS of mammals or birds and so the potential impact to these groups is not assessed.] This largely depends upon the period of time the animal is cooled for before it is rendered unconscious. In addition, for ectothermic animals, cooling will slow down metabolism. Keifer and Zheng [10] cooled *T. scripta* individuals for 45 minutes, which is well within their tolerance levels for time without food and water.	Orchard [11] cooled *L. catesbeianus* for 12 hours and it is not known whether this is outside usual tolerance levels for food and water given the reduction in metabolism it will induce. There is therefore a possible mild-moderate impact for species that require longer periods of cooling.	
2: Environmental challenge		Especially for cold tolerant species, the effects of cooling may be reversed by restoration of normal ambient conditions, which could mean that the measure could be classed as a moderate impact.	This depends upon the life history/physiology of the species, but at least for warm water fishes, and tropical amphibians and reptiles the cooling stage is likely to be significantly outside the normal temperature range of the species. In addition, while the aim of the measure is to render unconsciousness through exposure to extreme cold, the impact is likely to be classed as severe especially if the measure causes permanent injury (see domain 3).

3: Injury, disease, functional impairment		For amphibians and reptiles in the absence of information it is presumed that whether the cooling process leads to injury, and to what magnitude, will be species-specific depending on physiology. For example, if the species is cold tolerant, the measure may not lead to permanent injury before the animal is unconscious.	
4: Behavioural, interactive restriction		Due to the nature of cooling on ectothermic animals it will not induce untoward physiological or psychological effects. However the handling and placing the animal into the cooling apparatus may lead to mild impacts.	
5: Anxiety, fear, pain, distress, thirst, hunger etc.		The crux of the issue is whether throughout the cooling process the animal can feel pain. This is likely for fish, as eels have been shown to display distress and elevated heart rates during the cooling stage [7]. Amphibians and reptiles lack behavioural or physiological means of demonstrating pain or distress while hypothermic [1]. However, whether they feel pain or distress during cooling (and freezing) is under debate (see humaneness summary below) as their nerve conduction may be fully blocked during the cooling process before ice crystal formation [10]. In addition there is likely to be a mild-moderate impact due in relation to human contact due to the minimum of physical handling needed.	

3.2. Mode of death (if relevant)			
Measure type (if applicable): Freezing following on from anesthetic (incl. cooling)	**Immediate death (i.e. no suffering)**	**Not immediate death (mild - moderate suffering)**	**Not immediate death (severe - extreme suffering)**
Rationale:	For small (< 4 g) amphibians and reptiles, death by rapid freezing (e.g. in liquid N_2) is likely to be almost immediate.	For larger **amphibians** and **reptiles**, following anesthesia (which could include by cooling), death will not be immediate. However, the time taken to death is dependent upon different factors, include size of the animal. If following best practice, all animals should be fully anaesthetized before freezing so there should be no suffering. Shine et al. [5] placed *Rhinella marina* individuals into a freezer for 30 minutes to euthanize following cooling. Orchard [11] placed bullfrogs in a deep freeze for 48 hours (though time to death is not stated). For **fish**, also time to death will not be immediate, however as recommended by the AVMA [1] freezing is an acceptable measure assuming the animal is already fully anesthetised (e.g. using MS 222).	For **fishes** it is possible that cooling as a step to anesthetize is not effective and would lead to significant pain during the subsequent freezing process due to ice crystal formation, assuming noxious stimuli could be conducted to their brains at that temperature. For example Lambooji et al. [7] cooled eels to body temperature of < 5 °C, followed by freezing to euthanize eels in brine (-18°C) for 15 minutes. However, 5% of the eels were not effectively aneathetised by the cooling process and it took more than 27 seconds before unconsciousness was induced through freezing.

3.3. Humaneness summary	
	The AVMA [1] guidance for **amphibians** and **reptiles** (>4 g), states that “hypothermia reduces amphibians’ tolerance for noxious stimuli and there is no evidence that it is clinically efficacious for euthanasia. In addition, it is believed that freezing can result in the formation of ice crystals in tissues that may cause pain. Consequently, because amphibians and reptiles lack behavioural or physiological means of demonstrating pain or distress while hypothermic, generalized prohibitions on hypothermia for restraint or euthanasia are appropriate. Localized cooling in frogs may reduce nociception, but this localized effect is not appropriately applied to the whole body as a part of euthanasia procedures. Freezing of deeply anaesthetized animals may be justified under circumstances where human safety could be compromised.” The AVMA [1] also note that “little information is

	available on the sensory capacity of amphibians and reptiles at the egg stage of development. Freezing is likely appropriate for newly oviposited eggs, as would be methods of maceration that result in instantaneous death. Later stages may be destroyed using methods that are acceptable for adult animals”. However, the AVMA [1] guidelines are based on a mammalian-biased perspective and there has been little discussion of the scientific evidence underlying these current guidelines [9]. Shine et al. [5] showed that brain activity in cane toads (*Rhinella marina*) declines smoothly during freezing (following on from cooling), with no indication of pain perception. Therefore, they concluded that cooling followed by freezing can offer a humane method of killing cane toads, and may be widely applicable to other ectotherms (especially small species that are rarely active at low body temperatures). Keifer and Zheng [10] tested the effect of hypothermia (as an anesthetic prior to euthanasia by decapitation) on the in vitro eye blink reflex for ***Trachemys scripta elegans***. They found that before the point that ice crystals would form, noxious stimuli fail to be conducted to the brain and there is minimal overall brain activity, making it impossible for the perception of painful stimuli after induction of hypothermia. They noted that the temperature at which nerve conduction was largely blocked was 5–6°C, and that core temperature following treatment was -1 °C (they also noted that, for ***Lithobates catesbeianus,*** nerve conduction failure occursat 0–2°C). Therefore, it was highly unlikely that the formation of ice crystals during induction of hypothermia caused pain [10]. Lillywhite et al. [9] propose that rapid cooling and freezing is a humane form of euthanasia for numerous species of smaller (up to several kilogrammes) amphibians and reptiles. Note that ***Lithobates catesbeianus*** individuals can weigh up to 800 g [12], and while references for ***Trachemys scripta*** weight could not be found, it is likely that fully grown adults can reach up to c. 3 kg. These research findings indicate that a re-evaluation of AVMA guidelines is required [5,10]. Shine et al. [13] conclude that the use of cooling followed by freezing is a more humane alternative to blunt trauma [cranial compression] that is known to be used in some large scale invasive amphibian species control (e.g. cane toad control in Australia) where there is easy access to domestic fridges and freezers. In relation to **fish**, no research on the welfare impacts of the measure on species of Union of concern could be found. However according to Close [3] putting fish into a freezer or crushed ice prolongs the period of consciousness and does not reduce the ability to feel pain; therefore it should not be used as a method of euthanasia. Close [3] also noted that in marine fish ice crystals will form in the cells before the sea water freezes, thereby causing the fish extreme pain. In addition, Lambooij [7] assessed slaughter methods of farmed eels (*Anguilla anguilla*). This consisted of chilling until their body temperature was < 5 °C for stunning, and subsequently placing them in cold brine at -18 °C for 15 min for killing. They found that transferring the individuals from water at 18 °C to ice water (0.0 +/- 0.1 °C) elicited behavior and an irregular heart rate which indicated that they were stressed. In addition, 5% of the eels were not effectively stunned by the cooling process. While placing eels in brine water at -18 °C is an effective method to kill the eels before evisceration and processing, they state it cannot be recommend to place conscious eels in cold brine water, as it takes more than 27 seconds before unconsciousness may be induced. They conclude that from an animal welfare point of view, it is therefore not recommended to stun eels by live chilling.

	Shine et al. [13] conclude that "*euthanasia of large reptiles would likely best be performed with drugs, but small amphibians or reptiles could perhaps be more humanely euthanized with methods involving hypothermia. That said, it remains possible that species inhabiting areas where ambient temperatures fall to very low levels at night may maintain neuronal activity under thermal conditions that would abolish such activity in species inhabiting warmer regions. We need additional research on a wider variety of ectothermic species to establish guidelines for best practice.*" More research is needed on this measures application to allow best practices to be developed for different species to ensure that the cooling process leads to full unconsciousness and that nerve conduction is totally blocked, before the animal is frozen. In relation to fishes, cooling may not be an effective anesthesia process at least for all cold water species, but if animals are made fully unconscious through other means (e.g. MS 222) freezing is a humane method for euthanasia.

4. Costs and effectiveness of the measure	
General effectiveness of the measure	No case studies could be found that provided sufficient detail on the use of the measures as part of an eradication or control programme for the IAS of Union concern. Orchard [11] noted its use as part of an eradication programme of ***Lithobates catesbeianus*** at two sites on Vanvouver Island, Canada. Also see Keifer and Zheng [10] for a study of the measure's humaneness for ***Trachemys scripta***. Lillywhite et al. [9] also note that "rapid cooling and freezing as a form of euthanasia for smaller amphibians and reptiles has advantages including reduced risks to health and safety of investigators (e.g., bites from venomous snakes), the efficiency of costs, and the research-associated advantages of leaving animals without gross damage to body form and without the chemical contamination of body fluids ".

4.1. Case studies	
CASE STUDY #1	
Measure type (if relevant):	
Species:	
Objective:	
Combined with other measure(s):	
Country(ies) of application:	
Geographic scale (km^2) and/or population size measure applied to:	

Time period:		
Effort:		
Costs:	Overall costs:	
	Personnel costs:	
	Equipment and infrastructure:	
	Other, including overheads:	
Effectiveness:		

5. Conclusion

Overall assessment of the measure (qualitative)

No evidence could be found for the use of cooling or freezing with any of the mammalian or avian IAS of Union concern, and based on the limits of their use set out by the AVMA (<4g) these methods are unlikely to be suitable for use as part of an IAS mammal or bird eradication, control, or containment program.

Rapid freezing (e.g. in liquid N_2) of very small amphibians and reptiles seems to be an effective and humane measure, however it is not clear which weight thresholds should be considered for it application as part of an eradication or management programme for IAS of Union concern. For example, it is not clear whether the recommended weight limit of 4g, which is based on rodent models [1] would be applicable to amphibians and reptiles, which have a completely different physiology in relation to the response to lower body and environment temperatures.

Cooling followed by freezing of small amphibians and reptiles and fish seems to have been a widely applied measure for euthanasia as part of wildlife management programmes, and food production, as it offers a cost effective way to kill a large number of individuals quickly. However this practice seems to have been broadly stopped for amphibians and reptiles due to welfare concerns, although studies suggests that this decision and the AVMA guidelines need revision and that more research is needed. Lillywhite et al. [9] conclude that "amphibians and reptiles are incapable of experiencing pain at temperatures when ice crystals form in tissues during whole-body cooling, or in the case of cold-tolerant and freeze-tolerant species, there is no pain attributable to whole-body hypothermia *per se*." In addition, Shine et al. [13] state that "*the evidence to date suggests that cooling of amphibians and reptiles can virtually eliminate brain activity, eliminating the ability to per-ceive nociceptive stimuli. In addition, there are no signs of increased activity, as would be expected if the animals were in pain*". Therefore, the measure may well provide a cost-effective and

humane way to euthanise American bullfrogs ***Lithobates catesbeianus*** and red-eared sliders ***Trachemys scripta*** (and potentially any amphibian or reptile up to several kilogrammes [9]). Cooling followed by freezing for fish is also a widely applied approach, however little evidence could be found on its welfare impact or on its application to any of the IAS of Union concern, with only one study on its use for pumpkinseed ***Lepomis gibbosus*** [6]. Based on this and the AVMA [1] guidance, it seems that freezing is an appropriate measure assuming that the animal is fully anesthetized first, but cooling may not be an effective or appropriate way of doing this at least for all cold-water species.	
Assessor:	Kevin Smith
Reviewer 1:	Riccardo Scalera
Reviewer 2:	Sandra Baker

6. References

[1] AVMA. 2020. Guidelines for the Euthanasia of Animals: 2020 Edition. American Veterinary Medical Association

[2] Close, B., Banister, K., Baumans, V., Bernoth, E., Bromage, N., Bunyan, J., Erhardt, W., Flecknell, P., Gregory, N., Havkbarth, H., Morton, D., & Warwich, C. (1996). Recommendations for euthanasia: Part 1. *Laboratory Animals*, 30: 293-316.

[3] Close, B., Banister, K., Baumans, V., Bernoth, E., Bromage, N., Bunyan, J., Erhardt, W., Flecknell, P., Gregory, N., Havkbarth, H., Morton, D., & Warwich, C. (1997). Recommendations for euthanasia: Part 2. *Laboratory Animals*, 31: 1-32.

[4] Mathews, M., & Varga, Z. M. (2012). Anesthesia and Euthanasia in Zebrafish. *ILAR Journal*, 53(2): 192–204.

[5] Shine, R., Amiel, J., Munn, A. J., Stewart, M., Vyssotski, A. L., & Lesku, J.A. (2015). Is "cooling then freezing" a humane way to kill amphibians and reptiles? *Biology Open,* 4: 760-763. doi:10.1242/bio.012179

[6] Cucherousset, J., Copp, G. H., Fox, M. G., Sterud, E., van Kleef, H. H., Verreycken, H., & Zahorska, E. (2009). Life-history traits and potential invasiveness of introduced pumpkinseed Lepomis gibbosus populations in northwestern Europe. *Biological Invasions*, 11: 2171-2180.

[7] Lambooij, E., van de Vis, J. W., Kloosterboer, R. J., & Pieterse, C. (2002). Welfare aspects of live chilling and freezing of farmed eel (*Anguilla anguilla* L.): neurological and behavioural assessment. *Aquaculture,* 210 159–169.

[8] Bogutskaya, N.G. & Naseka, A. M. (2002). *Percottus glennii* Dybowski, 1977. Freshwater fishes of Russia.

[9] Lillywhite, H. B., Shine, R., Jacobson, E., Denardo, D. F., Gordon, M.S., Navas, C. A., Wang, T., Seymour, R. S., Storey, K. B., Heatwole, H., Heard, D., Brattstrom, B., & Burghardt, G.M. (2017). Anesthesia and Euthanasia of Amphibians and Reptiles Used in Scientific Research: Should Hypothermia and Freezing Be Prohibited? *BioScience*, 67(1): 53-61.

[10] Keifer, J., & Zheng, Z. (2017). Cold block of in vitro eyeblink reflexes: evidence supporting the use of hypothermia as an anesthetic in pond turtles. *Journal of Experimental Biology*, 220, 4370-4373. doi:10.1242/jeb.168427

[11] Orchard, S. A. (2011). Removal of the American bullfrog *Rana (Lithobates) catesbeiana* from a pond and a lake on Vancouver Island, British Columbia, Canada. Veitch, C. R.; Clout, M. N. and Towns, D. R. (eds.). *Island invasives: eradication and management*. IUCN, Gland, Switzerland pp 217-221

[12] FAO. 2020. Cultured Aquatic Species Information Programme. Rana catesbeiana. Cultured Aquatic Species Information Programme. Text by Flores Nava, A. In: FAO Fisheries Division [online]. Rome. Updated 9 February 2005. [Cited 8 September 2020].

[13] Shine, R., Lesku, J.A. and Lillywhite, H.B. (2020). Assessment of the cooling-then-freezing method for euthanasia of amphibians and reptiles. *Journal of the American Veterinary Medical Association*, 255(1): 48-50.

Appendix 27. Injection euthanasia

1. Measure name	
1.1. English:	Injection euthanasia
1.2. Lethal or non-lethal:	Lethal
1.3. Other languages (if available):	

Bulgarian	Евтаназия с инжекция	Italian	Eutanasia per iniezione
Croatian	Injekcijska eutanazija	Latvian	Eitanāzija ar nāvējošu injekciju
Czech	Euthanasie injekcí	Lithuanian	Mirtina injekcija
Danish	Injektions eutanasi (dødbringende injection)	Maltese	
Dutch	Euthanasie door injectie	Polish	Eutanazja iniekcyjna
Estonian	Keemiline eutanaasia (anesteetikumi üledoos)	Portuguese	Eutanásia com injeção
Finnish	Injektio eutanasia	Romanian	Eutanasiere prin injectare
French	Euthanasie	Slovak	Injekčná eutanázia
German	Injektionseuthanasie	Slovenian	Evtanazija z injekcijo
Greek	Ευθανασία με ένεση	Spanish	Inyección, euthanasia
Hungarian	Eutanázia injekcióval	Swedish	Dödlig injektion
Irish			

2. Technical details of measure
2.1.a. Measure description
While this measure focuses on injectable drug overdoses for euthanasia, it also covers the use of drugs applied via water in order euthanize fish species. The use of injectable euthanasia agents is one of the most rapid and reliable methods of performing euthanasia [1], commonly used for euthanasia of experimental animals [2]. The method consists of the administration of a drug overdose either by the intravenous, intracardiac, or intraperitoneal routes. Drugs such as barbiturates can depress the central nervous system leading to death following ceased breathing and heart function. It results in a smooth loss of consciousness before the cessation of cardiac and/or respiratory function, minimizing pain and distress to the animal. Applying an intravenous or intracardiac injection, pre-euthanasia drugs (tranquillisers, sedatives, immobilisers or general anaesthetics) may be required to facilitate safe and humane handling of animals before euthanasia. Injectable solutions of a drug can be used for humane euthanasia of most vertebrates [2,3,4,20]. Intravenous injection of an overdose of barbiturate is the preferred method for euthanasia of mammals and birds [1]. For fish and amphibians, drugs are best administered by dissolving them in the water in which the animals are placed; this reduces stress from handling and injection [1,2, 20]. Use of injectable drugs is suggested for most vertebrates by

guidelines produced in many countries worldwide for euthanasia of animals under field conditions, e.g. in Australia [15] and Canada [14, 20] and for animals used in scientific research [2,15].

When intravenous administration is considered impractical or impossible, intraperitoneal (into the body cavity) administration of a non-irritating agent is acceptable. Intracoelomic, intracardiac, or intraosseous administration of barbiturate are painful and stressful in conscious animals and are only acceptable in fully anaesthetised animals. These routes should never be used in a conscious animal, or when sedation alone is used. These methods are acceptable when intravenous access would cause distress, be dangerous, or impractical (e.g. birds smaller than 400g).

Accurate administration of drugs, especially regarding intracardiac and intravenous injection, requires significant training and experience, particularly for use in field situations [1]. Expertise is also required to inform under what conditions and for what species a given method will be suitable. It is also very important to ensure the confirmation of death, that the technique has been performed correctly and that the animal has not simply been sedated without being euthanized. It is therefore preferable, and in many countries mandatory, that such methods are administered by a qualified veterinarian. Only approved euthanasia drugs may be used; in Europe, these chemical compounds are regulated.

Drug injection has already been used to anesthetize vertebrate IAS of Union concern in field research: e.g. **coypu** [5,6], **coati** [7], **raccoon dogs** [8], **muskrat** [9, 10], **raccoon** [11]. Therefore, procedures for trapping and handling animals are already established until the moment just before euthanasia with injection. Intensive removal of **grey squirrels** to study recovery dynamics was conducted through live-trapping and euthanasia with a 2.5ml injection of the barbiturate Euthatal (sodium pentobarbitone) into its abdominal cavity [12].

For fish species the use of Eugenol, isoeugenol, and clove oil is common among researchers as anaesthetic. Whenever possible, products with standardized, known concentrations of essential oils should be used so that accurate dosing is possible. Concentrations required for anaesthesia will vary depending on species and other factors, but may be as low as 17 mg/L for some species. Greater concentrations (10 times the upper range for anaesthesia) will be required for euthanasia [17]. These oils are not very water soluble; injecting the solution through a syringe and fine-gauge needle under the water in the container used for euthanizing is helpful in ensuring dispersal in the water. Fish should be left in the anaesthetic solution for a minimum of 10 minutes after cessation of opercular movement [20].

Tricaine methanesulfonate, buffered (MS 222, TMS) can also be used to euthanize fish. An aversive response to MS 222 has been demonstrated for zebrafish and medaka, while carp, fathead minnow, and rainbow trout showed no aversion [18]. Solutions must be buffered and concentrations required for euthanasia may vary depending upon the species, life stage, and water chemistry parameters. A concentration of 250 to 500 mg/L, or 5 to 10 times the anesthetic dosage, is effective for most species [17, 18, 20]. Fish that are too large for practical or cost-effective immersion in lethal doses of buffered MS 222 can be euthanized by applying the concentrated, buffered solution directly to the gills [17, 19].

2.1.b. Integration with other measures

Injectable euthanasia agents are used to euthanised a restrained animal. The animal must have been caught and/or restrained using another method, e.g. **cage traps or nets.** The technique requires the immobilization of the animals, their sedation or anesthesia according to the injection route, and then the injection of the drug. Therefore, it may entail a period of handling which may not be short.

2.2. Availability		
Species	**Availability**	**Reference(s)**
Acridotheres tristis	P	1,2,3,4
Alopochen aegyptiaca	P	1,2,3,4
Callosciurus erythraeus	A	1,2,3,4,12
Corvus splendens	P	1,2,3,4
Herpestes javanicus	P	1,2,3,4
Lepomis gibbosus	P	17,18,19
Lithobates catesbeianus	A	16
Muntiacus reevesi	P	1,2,3,4
Myocastor coypus	P	1,2,3,4,5,6
Nasua nasua	P	1,2,3,4,7
Nyctereutes procyonoides	P	1,2,3,4,8
Ondatra zibethicus	P	1,2,3,4,9,10
Oxyura jamaicensis	P	1,2,3,4
Perccottus glenii	P	17,18,19
Plotosus lineatus		
Procyon lotor	P	1,2,3,4,11
Pseudorasbora parva	P	17,18,19
Sciurus carolinensis	A	1,2,3,4,12
Sciurus niger	A	1,2,3,4,12
Tamias sibiricus	P	1,2,3,4,12
Threskiornis aethiopicus	P	1,2,3,4
Trachemys scripta	A	1,2,3,4

3. Humaneness of the measure			
3.1. Welfare for all measures			
Measure type (if applicable): Injection euthanasia	**Humaneness impact categories**		
Domain	**No impact**	**Mild-Moderate**	**Severe - Extreme**
1: Water deprivation, food deprivation, malnutrition	No intrinsic effect on the animal.		
2: Environmental challenge	No intrinsic effect on the animal.	In terms of overdose of anaesthetic to fish via water body, as the fish will be kept in containers (with water) while the drugs take affect there will be short term exposure to environmental conditions outside their normal range.	
3: Injury, disease, functional impairment	Applied correctly ensures a rapid death, without risk of injury or functional impairment.		
4: Behavioural, interactive restriction	No intrinsic effect on the animal.	In terms of overdose of anaesthetic to fish via water body, there will be short term interference with the behavioural needs as the animals are kept in containers while the drugs take effect.	
5: Anxiety, fear, pain, distress, thirst, hunger etc.	In terms of overdose of anaesthetic to fish via water body, the use of clove oil has been shown not to increase cortisol levels in zebra fish *Danio rerio* [21].	The use of injectable euthanasia agents results in a smooth loss of consciousness before the cessation of cardiac and/or respiratory function, minimizing pain and distress to the animal. However, intracardiac, or intraosseous injection is painful and stressful in conscious animals and are only acceptable in fully anaesthetised	

		animals. Sedation may be required in any case to facilitate the safe and humane handling of animals before euthanasia. Therefore, animals may experience fear and pain until sedation or anaesthesia, which will take a mild to moderate period. In terms of overdose of anaesthetic to fish via water body, there is evidence that some species display an aversion response to MS222 (whether buffered or not). In experiments on euthanasia of zebra fish *Danio rerio*, MS222 led to higher levels of cortisol than use of clove oil [21].	

3.2. Mode of death (if relevant)			
Measure type (if applicable):	**Immediate death (i.e. no suffering)**	**Not immediate death (mild - moderate suffering)**	**Not immediate death (severe - extreme suffering)**
Rationale:	The use of injectable euthanasia agents results in a smooth loss of consciousness, which then leads to death. Therefore, this technique in itself does not bring suffering and is considered one of the most rapid and reliable methods of performing euthanasia. Time to death using clove oil can be within 1 minute if correct doses used and has been shown not to lead to increased stress in individuals of zebrafish [21].	Time to death using MS222 can be within 1 minute if correct doses used, however some species display an aversion response [18, 21].	

3.3. Humaneness summary	This technique does not bring suffering and is considered one of the most rapid and reliable methods of performing euthanasia. Sedation or anaesthesia is required, either to facilitate the handling of animals or to avoid fear and pain. Injection euthanasia is applied to restrained animals; therefore pain and stress are related to the technique employed to capture the animals (e.g. trapping) and to handle them before sedation. In terms of overdose of anaesthetic to fish via water body, as the fish will be kept in containers with water while the drugs take affect there may be some stress experienced by the animals. A study on zebra fish *Danio rerio* concluded that the use of clove oil is more humane than using MS222, as it results in significantly lower levels of distress (cortisol levels) in the animals [21].

4. Costs and effectiveness of the measure

4.1. Case studies	
CASE STUDY #1	
Measure type (if relevant):	Injection of 2.5ml barbiturate euthatal (sodium pentobarbitone) into abdominal cavity
Species:	*Sciurus carolinensis*
Objective:	Population control to study recolonization after management
Combined with other measure(s):	Live-trapping and euthanasia with injection
Country(ies) of application:	Ireland
Geographic scale (km^2) and/or population size measure applied to:	Two 12 ha woodlands
Time period:	1997-1998
Effort:	Five grey squirrel removal exercises (or culls), were carried out; three in Ardmulchan and two in Beau Parc. Trap density in each site was between 1.08 and 1.75 traps/ha. Culls lasted 5 days and squirrels removed during the five periods were: 40, 28, 19 12, 20
Costs:	Overall costs:
	Personnel costs:
	Equipment and infrastructure:

	Other, including overheads:
Effectiveness:	This was a study aimed at studying recolonization after removal. Time to recover to pre-cull level varied between 3-10 weeks and only in one area it was longer. A cull of five days using intensive live-trapping was sufficient to remove most, if not all, of the squirrels within the woodlands concerned. This was evident from the very low numbers of previously marked animals in the postcull samples.
Reference(s):	[12]

4.2. Costs effectiveness summary	Drug injection has already been used to anesthetize vertebrate IAS of Union concern during field research (e.g. **coypu** [5,6], **coati** [7], **raccoon dogs** [8], **muskrat** [9, 10], **raccoon** [11]), where the goal was not related to management. Therefore, procedures for trapping and handling animals are already established until the moment just before euthanasia with injection. Only in one case **grey squirrels** were removed as part of a study aimed at studying recolonization [12]. The method was considered effective, since most if not all squirrels were removed in 5 days trapping. The areas were however limited to 12 ha.

5. Conclusion

Overall assessment of the measure (qualitative)

The use of injectable euthanasia agents is one of the most rapid and reliable methods of performing euthanasia and is commonly used with experimental animals for its humaneness. The administration of drugs requires significant training and experience. Expertise is also required to evaluate conditions and doses for every species and situations. It is also fundamental to ensure the confirmation of death, and that the animal has not simply been sedated without being euthanized. It is therefore preferable, and in many countries mandatory, that such methods are administered by a qualified veterinarian. Euthanasia drugs are generally regulated and a register is needed. The veterinarian can either be present at the time the animals are captured, or be at the place where the animals will be transported to. Transportation will greatly increase fear and pain. Anyhow, the presence of a veterinarian will increase the cost of the project.

Drug injection has already been used to anesthetize listed IAS in field research with generally low sample sizes low. In one case grey squirrels were effectively removed from two small woodlands (12 ha) and euthanised with injection. Data from large-scale projects is not available.

In relation to application euthanasia agents directly to water for fish, MS222 and clove oil can both result in rapid death of individuals, however clove oil has been found to be more humane than MS222 in a study on zebrafish *Danio rerio*.

Assessor:	Sandro Bertolino
Reviewer 1:	Ana Nunes
Reviewer 2:	Kevin Smith

6. References

[1] AVMA (2020). AVMA Guidelines for the Euthanasia of Animals: 2020 Edition

[2] Close, B., Banister, K., Baumans, V., Bernoth, E. M., Bromage, N., Bunyan, J., ... & Morton, D. (1997). Recommendations for euthanasia of experimental animals: Part 2. *Laboratory animals*, *31*(1), 1-32.

[3] Department of Parks and Wildlife (2013). Humane killing of animals under field conditions in wildlife management. https://www.cdu.edu.au/files/2019-11/dpaw-sop15.1-humane-killing-of-animals.pdf

[4] Charbonneau, R., Niel, L., Olfert, E., von Keyserlingk, M., & Griffin, C. (2010). CCAC guidelines on: euthanasia of animals used in science. *Ottawa: Canadian Council on Animal Care*.

[5] Bó, R. F., Palomares, F., Beltrán, J. F., Villafañe, G. D., & Moreno, S. (1994). Immobilization of coypus (*Myocastor coypus*) with ketamine hydrochloride and xylazine hydrochloride. *Journal of Wildlife Diseases*, *30*(4), 596-598.

[6] Guichón, M. L., Doncaster, C. P., & Cassini, M. H. (2003). Population structure of coypus (*Myocastor coypus*) in their region of origin and comparison with introduced populations. *Journal of Zoology*, *261*(3), 265-272.

[7] Martins Santos, F., Carvalho de Macedo, G., Teixeira Gomes Barreto, W., Rodrigues Oliveira-Santos, L. G., Martins Garcia, C., Miranda Mourão, G. D., ... & Elisei de Oliveira, C. (2018). Outcomes of *Trypanosoma cruzi* and *Trypanosoma evansi* infections on health of Southern coati (*Nasua nasua*), crab-eating fox (*Cerdocyon thous*), and ocelot (*Leopardus pardalis*) in the Brazilian Pantanal. *PloS one*, *13*(8), e0201357.

[8] Kowalczyk, R., & Zalewski, A. (2011). Adaptation to cold and predation—shelter use by invasive raccoon dogs *Nyctereutes procyonoides* in Białowieża Primeval Forest (Poland). *European Journal of Wildlife Research*, *57*(1), 133-142.

[9] Sleeman, J., Stevens, R., & Ramsay, E. (1997). Field immobilization of muskrats (*Ondatra zibethicus*) for minor surgical procedures. *Journal of wildlife diseases*, *33*(1), 165-168.

[10] Proulx, G. I. L. B. E. R. T., & Gilbert, F. F. (1983). The ecology of the muskrat, *Ondatra zibethicus*, at Luther Marsh, Ontario. *Canadian field-naturalist. Ottawa ON*, *97*(4), 377-390.

[11] Belant, J. L. (2004). Field immobilization of Raccoons (*Procyon lotor*) with telazol and xylazine. *Journal of Wildlife Diseases*, *40*(4), 787-790.

[12] Lawton, C., & Rochford, J. (2007). The recovery of grey squirrel (*Sciurus carolinensis*) populations after intensive control programmes. In *Biology and Environment: Proceedings of the Royal Irish Academy* (pp. 19-29). Royal Irish Academy.

[13] Rowsell, H. C. (1991). The Canadian Council on Animal Care--its guidelines and policy directives: the veterinarian's responsibility. *Canadian journal of veterinary research*, *55*(3), 205.

[14] Care, C. C. O. A. (2003). CCAC guidelines on: the care and use of wildlife. *Canadian Council on Animal Care, Ottawa, Ontario, Canada*.

[15] Department of Biodiversity, Conservation and Attractions (2017) Standard Operating Procedure: Humane Killing of Animals under Field Conditions. Perth, WA: Department of Biodiversity, Conservation and Attractions.

[15] Charbonneau, R., Niel, L., Olfert, E., von Keyserlingk, M., & Griffin, C. (2010). CCAC guidelines on: euthanasia of animals used in science. *Ottawa: Canadian Council on Animal Care*.

[16] Franch, N., Queralt, J.M. & Vidal S. 2019. Pla d'erradicació de la granota toro Lithobates catesbeianus. Generalitat de Catalunya. 9 pp.

[17] Neiffer, D.L., Stamper, M.A. (2009). Fish sedation, anesthesia, analgesia, and euthanasia: considerations, methods, and types of drugs. *ILAR J 50*:343–360.

[18] Readman, G.D., Owen, S.F., Knowles, T.G. (2017). Species specific anaesthetics for fish anaesthesia and euthanasia. Sci Rep 7:7102

[19] Ross, L.G., Ross B. (2008). Anaesthetic and sedative techniques for aquatic animals. 3rd ed. Oxford, England: Blackwell

[20] Gourdon, J. and Jimenez, A. (2021). SOP 303.03 – Fish and aquatic amphibian euthanasia. Approved by the McGill University Animal Care Committee. Comparative Medicine & Animal Resources Centre

[21] Davies, D.J., Klug, J., Hankins, M., Doerr, H.M., Monticelli, S.R., Song, A., Gillespie, C.H. and Bryda, E.C. (2015). Effects of clove oil as a euthanasia agent on blood collection efficiency and serum cortisol levels in Danio rerio. *J Am Assoc Lab Anim Sci*., 54(5): 564–567.

Appendix 28. Keeping in captivity

1. Measure name	
1.1. English:	Keeping in captivity
1.2. Lethal or non-lethal:	Non-lethal

1.3. Other languages (if available):

Bulgarian	Отглеждане на затворено	Italian	Mantenere in cattività
Croatian	Držanje u zatočeništvu	Latvian	Turēšana nebrīvē
Czech	Držení v zajetí	Lithuanian	Laikymas nelaisvėje
Danish	Holde i fangeskab	Maltese	
Dutch	In gevangenschap plaatsen	Polish	Utrzymywanie w niewoli
Estonian	Vangistuses hoidmine	Portuguese	Manutenção em cativeiro
Finnish	Vangittuna pitäminen	Romanian	Menținere în captivitate
French	Garder en captivité	Slovak	Držba v zajatí
German	Haltung in Gefangenschaft	Slovenian	Ujetništvo
Greek	Διατήρηση σε αιχμαλωσία	Spanish	Mantenimiento en cautividad
Hungarian	Fogságban tartás	Swedish	Hålla i fångenskap
Irish			

2. Technical details of measure

2.1.a. Measure description

Once an individual of an IAS species has been captured alive it may be transferred and housed in permanent captivity for the remainder of its natural life. Such enclosures may be within a licensed zoo or wildlife park, authorised/licensed rescue centre or sanctuary or other authorised captive animal facility. Within the European Union, zoos, and in some cases, rescue centres/sanctuaries, are subject to licensing requirements which are laid down in the EU Zoos Directive (1999/22/EC), with the Zoos Directive Good Practices document [1] providing further details on best practice implementation for stakeholders and Member State authorities.

However, given capacity issues within zoos, authorised rescue centres and sanctuaries and the long-term costs of keeping captive animals, this measure has only been used to control very small isolated IAS populations [2]. The specific husbandry requirements of each of the 22 IAS species also needs to be taken into account and will vary significantly - with enclosure design, husbandry and enrichment plans, nutrition and veterinary care needing to be tailored to the individual species once in human care. Potentially, all IAS could be kept in captivity, but several circumstances limit the use of this method including long-term costs and space availability. For the assessment's purpose, we assume here that the captured animals are kept in captivity according to available best practices [8, 20].

Additionally, permits are required to be issued by Member State competent authorities for the keeping of species included on the List of Union Concern in line with Articles 7 and 8 of the IAS Regulation (1143/2014). According to the Regulation, measures should be taken to prevent the escape and breeding of the animals kept in captivity. Although the majority of the 22 IAS species which this project concerns itself with, have a history of being kept under captive management (i.e. in human care), we have only considered this measure as being 'Available' in Section 2.2. when it has the 'defined objective' of being used to control or manage feral/free-living IAS populations.
2.1.b. Integration with other measures
This measure can be viewed as a potential subsequent stage after **live capture in a cage trap**. Once captured, the animals would need to be housed appropriately to avoid potential escaping and any breeding- this may include **surgical or chemical sterilization** measures. Keeping in captivity as a measure has been used in conjunction with **lethal measures** in Italy to control red-eared sliders [3] and in the Netherlands to control Pallas squirrels [4]

2.2. Availability		
Species	**Availability**	**Reference(s)**
Acridotheres tristis	P	
Alopochen aegyptiaca	P	
Callosciurus erythraeus	A	4
Corvus splendens	P	
Herpestes javanicus	P	
Lepomis gibbosus	P	
Lithobates catesbeianus	P	
Muntiacus reevesi	P	
Myocastor coypus	P	
Nasua nasua	P	
Nyctereutes procyonoides	P	
Ondatra zibethicus	P	
Oxyura jamaicensis	P	
Perccottus glenii	P	
Plotosus lineatus		
Procyon lotor	A	5
Pseudorasbora parva	P	
Sciurus carolinensis	P	
Sciurus niger	P	
Tamias sibiricus	P	
Threskiornis aethiopicus	P	
Trachemys scripta	A	3

3. Humaneness of the measure			
3.1. Welfare for all measures			
Measure type (if applicable):	**Humaneness impact categories**		
Domain	**No impact**	**Mild-Moderate**	**Severe - Extreme**
1: Water deprivation, food deprivation, malnutrition	If animals are kept according to available species-specific best practices, no impact. See Domain 5 for self-imposed food deprivation. Coati (*Nasua nasua*) Information on the provision on appropriate food and water sources is readily available [6, 7, 8]. Red eared slider (*Trachemys scripta*) See Domain 3.		
2: Environmental challenge		Exposure to environmental conditions which are outside the normal range encountered by the animal are possible in captivity; however, if the facilities apply species-specific best practices, the impact is limited. For instance, coati (*Nasua nasua*) adapts well to life in captivity when kept in suitable conditions [9]. Procyonids are hardy animals and adapt to a variety of climates, however tropical species should be provided with indoor enclosures and heat during harsh winters [10]. Red eared slider (*Trachemys scripta*) Access to highly species-specific temperature ranges, UV light provision [11] and correct nutrition [12] is required in captivity to prevent husbandry related diseases	

3: Injury, disease, functional impairment		Coati (*Nasua nasua*) Obesity is a common problem in captive coatis due to relative inactivity and higher caloric intake compared with their wild counterparts [6]. Raccoon (*Procyon lotor*) Obesity caused by overeating and lack of exercise is also a common problem in captive raccoons [10].	
4: Behavioural, interactive restriction		Keeping wild-caught IAS animals in captivity inherently results in altering their ability to perform some of their natural behaviours. Some animals may adapt to captivity better than others, however this adaptation will be individual, species and institution specific. Coati (*Nasua nasua*) Abnormal behaviours, such as tail chewing, have been observed in coatis that do not have suitable foraging and mental and physical stimulation opportunities in captivity [6]. Chronic stress due to unfulfilled behavioural motivations may result in abnormal behaviours. The development of abnormal behaviours in wild-caught IAS animals, without a previous history of captivity, can be considered a negative welfare indicator, with these behaviours being a reflection of their inability to cope with their current environmental circumstances [13]. Procyonid species are good climbers and should be housed in enclosures with climbing structures [6]. Further information on appropriate enclosure size, design and construction husbandry is readily available [8].	
5: Anxiety, fear, pain, distress, thirst, hunger etc.		When transferred from the wild to captivity, animals will experience varying degrees of stress [14], for example self-imposed food deprivation may occur in response to capture and confinement in some species [15]. Further research is needed to quantify the welfare outcomes in the above Domains, associated with captivity on wild-caught individuals of the 22 listed IAS species.	

		Welfare outcomes need to be assessed and managed throughout the translocation, habituation and long-term care processes, as there will be differences in welfare outcomes based on the individual, species and institution.	

3.2. Mode of death (if relevant)			
Measure type (if applicable):	**Immediate death (i.e. no suffering)**	**Not immediate death (mild - moderate suffering)**	**Not immediate death (severe - extreme suffering)**
Rationale:			

3.3. Humaneness summary	IAS may be kept in captivity following their capture in live traps and in some cases also after undergoing surgical or chemical sterilization. In addition, any captured animals destined for a life in captivity will be confined in transport cages, transported and potentially quarantined. The cumulative impacts these methods have on animal welfare should also be taken into consideration. However, this assessment concerns only the impact of the keeping in captivity and overall, this impact is considered mild-moderate, taking into account species-specific characteristics and scientific knowledge supporting best practices. Application of best practices in a captive environment aim to provide for species/individual needs, and therefore should aim to reduce any negative welfare impacts and promote positive welfare opportunities throughout the animal's lifetime in captivity, noting that an animal's welfare state is dynamic, it may vary over different lifestages and any negative impacts may accumulate or depreciate over time. Consequently, more research is needed on the impact of the captivity on species included in the Union list to support development of robust, evidence-based best practice guidelines and promotion of positive animal welfare practices. Both will ultimately serve to safeguard animal welfare and guide future IAS management policies. The cumulative impact and opportunities offered by this method on animal welfare should be objectively assessed on a case-by-case basis.

4. Costs and effectiveness of the measure

4.1. Case studies

CASE STUDY #1	
Measure type (if relevant):	Kept in captivity
Species:	***Trachemys scripta*** (red-eared, yellow-bellied slider)
Objective:	Eradication
Combined with other measure(s):	Combined with **live trapping** as part of EU LIFE funded project (LIFEEMYS -LIFE 12 NAT/IT/000395) reintroducing European pond turtles (*Emys orbicularis*) and removing the IAS ***Trachemys scripta*** from specific habitats and areas [16]. 43 *T. scripta* (males only) were surgically sterilised within the project before being kept in captivity.
Country(ies) of application:	Italy
Geographic scale (km^2) and/or population size measure applied to:	600 *T. scripta* were removed from the environment with all of these animals being housed in a purpose-built enclosure at Pistoia zoo, Italy [16].
Time period:	The capture of the animals and their transfer to the zoo was undertaken over a period of 36 months. Although not specifically stated in the case study, the time period of captivity required in this case - where lethal measures were not applied, appropriate husbandry was provided, and further breeding prevented - would depend on the age of the individual concerned and the average life expectancy of the pond slider, which can up to 30 years [18].
Effort:	Information about the amount of effort required to meet the ongoing needs of these animals in captivity is not available. In total, an area of about 5,100m^2 has been restored.
Costs:	Overall costs:
	Creation of appropriate enclosures, maintenance and management of the *T. scripta* at Pistoia zoo, Italy. 30,000€ [17]. Ongoing maintenance and keeping of the animals is not costed in this figure.
	Personnel costs:
	From this example, there is no specific cost assigned to personnel required. As there is often more than one species cared for by a keeper or a team of keepers in a zoological setting it is also difficult to estimate the specific personnel requirements for the ongoing management of the species. We can determine that the addition of 600 *T. scripta* would increase the workload of zookeepers for the duration that the animals were kept at the facility and therefore this would have an ongoing personnel cost associated with their care.
	Equipment and infrastructure:

	Other, including overheads:
Effectiveness:	Concerns remain about whether all of the IAS population was successfully removed from the environment [17]. To our knowledge no escapes have occurred from the purpose built facility.

CASE STUDY #2	
Measure type (if relevant):	Kept in captivity
Species:	***Procyon lotor*** (raccoon)
Objective:	Eradication
Combined with other measure(s):	The method was combined with the use of **live traps** ("walk-in" traps with alarm when animals are captured) and hunting when the capture was not possible. These methods are not evaluated here.
Country(ies) of application:	Netherlands
Geographic scale (km^2) and/or population size measure applied to:	Around 50-100 raccoons have been estimated to be present in the province of Limburg (Sittard-Schinveld-Nuth area; total area covered by the project not specified). Captured raccoons have been moved to AAP (Animal Advocacy and Protection) rescue centre.
Time period:	October 2019- March 2020 A second phase is planned from September 2020 to March 2021 to complete the eradication of the estimated 50-100 raccoon population.
Effort:	A total of 17 raccoons have been captured during the first 6 months of the project, neutered and temporarily rehomed in AAP rescue centre. As AAP will relocate the animals in various zoos within 2 months in average, the total effort is impossible to estimate. In captivity a raccoon can live up to 20 years.
Costs:	Overall costs:
	Because raccoons are a potential vector of *Baylisascaris procyonis*, a dangerous parasite that can be transmitted to humans, all captured animals needed to be kept in quarantine infrastructures before being moved to their definitive enclosure, with additional costs involved. 85% of the raccoons captured by the project were infected by *Baylisascaris procyonis*. The following costs have been indicated by AAP: €15.04 per animal/day during the quarantine period (30.5 days); €7.60 per animal/day after quarantine. In addition, one-time costs include transport: (not estimated) and neutering: €100/animal. These costs don't include outplacement activities or additional veterinary treatments.
	Personnel costs:
	Equipment and infrastructure:
	Other, including overheads:

Effectiveness:	In 6 months of capture efforts, 17 raccoons have been captured and temporarily rehomed at AAP. Camera traps in the project area have detected the presence of at least 12 more raccoons, which should be captured during the second phase of the project. The target population is isolated. No animals escaped and all of them survived to the neutering and rehoming so far.
References	[5]

CASE STUDY #3		
Measure type (if relevant):	Kept in captivity	
Species:	***Callosciurus erythraeus*** (Pallas' squirrel)	
Objective:	Eradication	
Combined with other measure(s):	The method was combined with the use of **live traps** (not evaluated here).	
Country(ies) of application:	Netherlands	
Geographic scale (km^2) and/or population size measure applied to:	50 Km^2 near Weert, Ell and in the adjacent area	
Time period:	2011-2013	
Effort:	A total of 249 Pallas' squirrels have been captured during 3 years (winter and spring months only), neutered and temporarily rehomed in a rescue centre in Opglabbeek, Belgium. The animals have then been relocated in various zoos and sanctuaries. Consequently, the total effort is impossible to estimate. In captivity a Pallas' squirrel can live up to 15 years.	
Costs:	Overall costs:	
	This information is unavailable for keeping the animals in captivity. The overall cost of the capture phase is about EUR 330,000.	
	Personnel costs:	
	Equipment and infrastructure:	
	Other, including overheads:	
Effectiveness:	249 squirrels have been captured in 3 year. One more Pallas' squirrel has been detected in 2015 and captured. The population has been successfully eradicated. To our knowledge no escapes have occurred.	

References:	[4]

4.2. Costs effectiveness summary	A quantitative evaluation of the cost effectiveness of keeping IAS in captivity is impossible because of the lack of published data. Additionally cost-effectiveness is likely to vary widely amongst the IAS listed species and institutions/facilities. However, given capacity issues within zoos/rescue centres/sanctuaries and the long-term costs of keeping captive animals, this measure can only be effectively used to control small and isolated IAS populations.

5. Conclusion

Overall assessment of the measure (qualitative)

Given capacity issues within licensed zoos, authorised rescue centres and sanctuaries and the long-term costs of keeping captive animals, this measure has only been used to support control of very small isolated IAS populations [2] and the feasibility of this measure in other contexts is limited.

The welfare impact of keeping IAS species in captivity can be considered to be mild-moderate, however given a lack of objective data on this topic, more severe welfare outcomes may be experienced by some individuals/species in some circumstances, with the need to assess welfare over an animals lifetime. Given the non-lethal nature of this measure, consideration should be given to lifelong welfare provision in captivity compared to other control methods. Application of best practices in a captive environment, informed by ongoing research, which considers both species-specific and individual needs will aim to reduce negative welfare impacts and promote positive welfare opportunities throughout the animal's lifetime. Further research which may inform best practices, is needed to quantify welfare outcomes imposed by captivity on wild-caught individuals of the 22 listed IAS species.

An animal's affective state in captivity will vary depending on a variety of factors, therefore causing their welfare to fluctuate along a spectrum from positive to negative. All cases will vary depending on a myriad of variables such as species, individual, institutional practices, enclosure, social setting, season, veterinary intervention etc., and welfare will be in constant flux. The degree of flux and the primary welfare status (a reflection of the animals' affective state) can be managed, to an extent, by the knowledge and actions of industry professionals charged with managing the animals. Consideration should also be given to promotion of positive welfare states, such as contentment, relaxation, satiation, joy, curiosity, pleasure etc., which is necessary to place the animals in a positive welfare state, rather than to simply avoid the stated negative mental states, which will only serve to place animals into a neutral welfare status [19].

However, if the zoos/sanctuaries/rescue centres apply species-specific best practices, this method may represent a humane alternative to lethal measures in some limited contexts of small isolated populations.

Assessor:	Ilaria Di Silvestre, Sally Binding, Melissa Broadway and Allan Muir
Reviewer 1:	Kevin Smith
Reviewer 2:	Sandra Baker

6. References

[1] European Commission. (2015). EU Zoos Directive Good Practices Document.
[2] IUCN. (2017). Information on non-lethal measures to eradicate or manage vertebrates included on the Union list. Technical note prepared by IUCN for the European Commission.
[3] Ferri, V. (2019) Piano di controllo e gestione delle specie esotiche di testuggini palustri (Trachemys scripta ssp). https://www.regione.lombardia.it/wps/wcm/connect/85638e14-7506-4107-96d1-52d11f5df1bb/Piano+di+controllo+e+gestione+delle+specie+esotiche+di+testuggini+palustri+-+Azione+A7.pdf?MOD=AJPERES&CACHEID=ROOTWORKSPACE-85638e14-7506-4107-96d1-52d11f5df1bb-mM0b5g0
[4] La Haye, M. (2020). Pallas' squirrel eradication in the Netherlands. Case study in: Invasive Alien Species Colonisation Prevention: Your guide to early detection and rapid response.
[5] Mededeling portefeuillehouder stand van zaken bestrijding van de wasbeer (T8568), February 2020: Limburg Province.
[6] Whiteside, D. P. (2009). Nutrition and Behavior of Coatis and Raccoons. Veterinary Clinics of North America: Exotic Animal Practice, 12(2), 187–195. doi:10.1016/j.cvex.2009.01.002
[7] Shora, J., Myhill, M., & Brereton, J. E. (2018). Should zoo foods be coati chopped. *Journal of Zoo and Aquarium Research*, 6(1), 22-25.
[8] AZA Small Carnivore TAG 2010. Procyonid (Procyonidae) Care Manual. Association of Zoos and Aquariums, Silver Spring, MD.
[9] Mayol, J., Álvarez, C., and Manzano, X. (2009). Presència i control del coatí, Nasua nasua L., i d'altres carnívors introduïts en època recent a Mallorca. *Bolleti la Soc. d'Historia Nat. Les Balear*. 52, 183–191.
[10] Miller, R. E. (2015). *Fowler's Zoo and Wild Animal Medicine, Volume 8*. WB Saunders.
[11] Acierno MJ, Mitchell MA, Roundtree MK, Zachariah TT: Effects of ultraviolet radiation on 25-hydroxyvitamin D3 in red-eared slider turtles (Trachemys scripta elegans). Am J Vet Res 67:2046-2049, 2006
[12] Mans C, Braun J. Update on common nutritional disorders of captive reptiles. *Vet Clin North Am Exot Anim Pract*. 2014;17(3):369-395. doi:10.1016/j.cvex.2014.05.002
[13] Bacon, H. (2018). Behaviour-Based Husbandry—A Holistic Approach to the Management of Abnormal Repetitive Behaviors. Animals. 8. 103. 10.3390/ani8070103.
[14] Baker, M.L., Gemmell, E. and Gemmell, R.T. (1998), Physiological changes in brushtail possums, *Trichosurus vulpecula,* transferred from the wild to captivity. J. Exp. Zool., 280: 203-212. doi:10.1002/(SICI)1097-010X(19980215)280:3<203::AID-JEZ1>3.0.CO;2-R
[15] Price, E., O. (2002). Animal domestication and behaviour. CABI Publishing, 313 pp.
[16] LIFEEMYS - Ligurian Invasive Fauna Eradication pro indigenous Emys orbicularis restocking (LIFEEMYS -LIFE 12 NAT/IT/000395) https://ec.europa.eu/environment/life/project/Projects/index.cfm?fuseaction=search.dspPage&n_proj_id=4530#AD
[17] After LIFE Conservation Plan, 2017 (LIFEEMYS -LIFE 12 NAT/IT/000395) http://www.lifeemys.eu/wp-content/uploads/After-life-Conservation-Plan.pdf
[18] Frazer N, Gibbons JW, Greene JL. 1990. Life tables of a slider turtle population. In Life History and Ecology of the Slider Turtles, Gibbons JW (ed.). Smithsonian Institution Press: Washington, DC; 183–200.[19] Mellor, D.J., & Beausoleil, N.J., (2015) Extending the 'Five Domains' model for animal welfare assessment to incorporate positive welfare states. Animal Welfare 24: 241-253.
[20] AZA Small Carnivore TAG (2011) Mongoose, Meerkat, & Fossa (Herpestidae/Eupleridae) Care Manual. Association of Zoos and Aquariums, Silver Spring, MD.

Appendix 29. Modified atmospheres

1. Measure name			
1.1. English:	Modified atmospheres		
1.2. Lethal or non-lethal:	Lethal		
1.3. Other languages (if available):			
Bulgarian	Убиване с газообразни вещества	Italian	Soppressione di animali con sostanze gassose
Croatian	Plinsko gušenje	Latvian	Atmosfēras izmaiņas (t.i. nāvējošās gāzes vai vakuums)
Czech	Modifikované ovzduší	Lithuanian	Pakeistas oras
Danish	Gasning (kuldioxidm Argon, Nitrogen)	Maltese	
Dutch	Gewijzigde atmosfeer	Polish	Modyfikacja składu powietrza oddechowego
Estonian	Õhu koostise muutmine (nt. süsinikdioksiid, argoon, lämmastik)	Portuguese	Atmosferas modificadas (gases)
Finnish	Ilman koostumuksen muuttaminen (mm. Hiilidioksiditainnutus)	Romanian	Uciderea animalelor cu substanțe gazoase
French	Atmosphères modifiées	Slovak	Usmetenie plynom
German	Veränderte Atmosphären	Slovenian	Spremenjena atmosfera
Greek	Τροποποιημένη ατμόσφαιρα	Spanish	Modificación de atmósfera (gases)
Hungarian	Módosított nyomás	Swedish	Modifierade atmosfärer
Irish			

2. Technical details of measure
2.1.a. Measure description
A broad measure covering the removal/dispatch of any restrained animal using gasses or other means to modify their atmospheric environment. This includes the use of: • inert gasses to produce anoxia (e.g. **nitrogen**) • physiologically active gasses producing both anoxia and hypoxia (e.g. **carbon dioxide** and **carbon monoxide**) • Inhaled anesthetics or other neurological/physiological depressants (e.g. **vaporized Isoflurane**) • **Gasses delivered in foam** but not mechanical suffocation associated with medium/high density foams • New approaches are also under development for this measure, such as the use of **decompression** to produce anoxia A number of broad approaches are used depending on the application; these are not exclusive; • 'Trap-side' methods, suitable for deployment in situ where the animal is caught and restrained in a trap; often suitable for only one animal at a time.

- Transport of animals from their restraint in the field, to a facility or processing station for dispatch; often suitable for many animals, or larger species.
- Simple methods than can be deployed at low cost by amateurs / volunteers.
- Substantial gassing systems, often static or vehicle mounted, suitable only for coordinated professional deployment.

For applications where animals must be transported to a processing station (either a vehicle equipped with appropriate apparatus, or further transport to a facility), the additional burdens to welfare of transport and handling / lairage must also be considered.

Whilst often considered humane by society in general, and still in wide use in laboratories or animal breeding facilities none of these methods are considered the most humane methods to euthanize individual animals or small groups (a few animals at a time) [1,2]. However, the method is still either applied or is recommended where:

- stakeholder opinions/welfare are considered important (i.e. members of the public, landowners), relying either on the misconception that gassing is the most humane approach, or on live animals being carried away and euthanasia occurring at an 'emotional distance'.
- the emotional state/welfare of operators must be managed.
- methods rely less on operator expertise and more on suitable equipment.
- The rate of dispatch/volume of animals to be dispatched is a consideration.

The OIE still sanction gas-based methods for the rapid euthanasia of livestock in cases of virulent notifiable animal disease, where consequences of processing animals slowly using more humane methods are impractical and must be balanced against the burdens to welfare produced by distressing pathologies in many animals.

2.1.b. Integration with other measures

Animals must be restrained/captive (e.g. **live capture traps**) before introduced to a chamber. Some systems permit whole cages to be placed into chambers, some envelope cages with an impermeable cover to produce a chamber from the cage, whilst some systems require the animals to be handled/herded into large self-standing chambers.

2.2. Availability

Species	Availability	Reference(s)
Acridotheres tristis	A	[5]
Alopochen aegyptiaca	A	[4]
Callosciurus erythraeus	A	[6]
Corvus splendens	P	
Herpestes javanicus	P	
Lepomis gibbosus		
Lithobates catesbeianus		
Muntiacus reevesi		
Myocastor coypus	A	[3]
Nasua nasua	A	
Nyctereutes procyonoides	P	
Ondatra zibethicus	P	
Oxyura jamaicensis	P	

Perccottus glenii		
Plotosus lineatus		
Procyon lotor	P	[13]
Pseudorasbora parva		
Sciurus carolinensis	A	[14]
Sciurus niger	A	
Tamias sibiricus	P	
Threskiornis aethiopicus	P	
Trachemys scripta	P	

3. Humaneness of the measure			
3.1. Welfare for all measures			
Measure type (if applicable):	**Humaneness impact categories**		
Domain	**No impact**	**Mild-Moderate**	**Severe - Extreme**
1: Water deprivation, food deprivation, malnutrition	No intrinsic effect on the animal.		
2: Environmental challenge			Death is the result of hypoxia / anoxia. Even if animals are immersed into a completely anoxic atmosphere (impossible to achieve in practice) insensibility will be preceded by a period during which both physiological as well as sensory perceptions (in some species) cause increasing fear in the animal. In practice anoxic atmospheres are produced more slowly in order to shorten the duration of this period of fear. Regardless of the duration of fear and distress, the onset of insensibility is still considered to be preceded with the same sensations associated with mechanical suffocation (drowning).

3: Injury, disease, functional impairment	No intrinsic effect on the animal. Even if animals are injured during the brief period of restraint necessary to move groups of animals into large facilities, suffering is likely to be very brief.		
4: Behavioural, interactive restriction		There will be short-term physical restraint/handling.	
5: Anxiety, fear, pain, distress, thirst, hunger etc.		Trap-side applications - The brief period of handling and restraint necessary to apply the method will almost certainly produce fear in wild species (listed IAS) but will be short. Transport to processing stations – May extend the period the animal is held without food or water and will produce fear in wild species (listed IAS) – duration and mode of transport must be carefully considered.	

3.2. Mode of death (if relevant)			
Measure type (if applicable):	**Immediate death (i.e. no suffering)**	**Not immediate death (mild - moderate suffering)**	**Not immediate death (severe - extreme suffering)**
Rationale:		For trap-side deployments, limitations to equipment may result in sub-optimal experience, either in handing animals into the equipment, and/or in providing optimal rates of atmospheric modification. Where transport to a processing station is involved, the cumulative experience of handling / transport / lairage / handling must be considered.	Death is not instantaneous. For all deployment's animals will experience a period of increasing distress (the same sensations associated with mechanical suffocation i.e. drowning) until they become insensible. This is rarely <10 seconds, and may be over a minute for some species [4,5].

3.3. Humaneness summary	Society still considers the use of gasses as an acceptable means of dispatch (humane in the reflexive anthropocentric use of the word) avoiding as it does the public evidence of noise, blood, brutal force and the sight of animals in distress (gassing animals usually involves covering/enclosing them whilst operating or removing them from their public setting and working out of sight or elsewhere). As such it is still in common use across Europe to kill small mammals in laboratories, and is sanctioned (and occasionally deployed) to rapidly depopulate poultry units in cases of virulent disease. However, it is rarely the most humane method available and its use in veterinary settings and laboratories is being disparaged [1,2,6], and in common with many other methods of killing or dispatch, has the potential to be applied badly and produce much greater suffering, especially where it is applied in field settings. Best practice aims for the controlled use of a rising concentration of an odorless gas (for laboratory rats and mice, typically 10%-40% chamber replacement rate.min-1) maintaining a 70%-100% concentration until the animal is considered to have been dead for a number of minutes. Exposure in the animal's home cage is preferable (avoids neophobic stress associated with introduction to the chamber) but group exposure is now considered potentially inappropriate [6]. Death must be confirmed by the absence of reflex responses or the application of an additional measure. The most humane deployments involve gas chambers sufficiently large to take cages (avoiding the need to handle or transfer animals from the restraint device), enable the careful control of the delivery rate and target concentrations of gasses, and which use gasses known to be free of contaminants. Unfortunately these are often substantial apparatuses unsuited to trap-side deployments. In contrast, the measure is applied in some places by using exhaust gasses from conventional car engines (albeit with some constraints, e.g. [7]), or using homemade trap-side devices (e.g. [8]) where little control over the rate of introduction or final concentration of gas is achieved. That animals die in these devices does not imply that the death was either humane or anywhere optimal in terms of deployment. The use of gasses for euthanasia is further complicated by the likelihood that responses, and optimal combinations of gasses, filling rates and target concentrations are all likely to be species specific, with research required to find combinations that are most effective at producing rapid stress-free insensibility, and those that are least aversive, e.g. [4,9,10]. **Operator safety and environmental pollution** Nitrogen and carbon dioxide both present risks to operators where used in large volumes in enclosed spaces (gassing systems for larger animals, caged animals, or groups of animals), though neither is inherently toxic or pose significant risks to the environment. Carbon monoxide (CO), as a laboratory grade gas is toxic produces some small risks to the environment. However, many historical uses of the gas have relied on its generation by an internal combustion engine, appropriately detuned and with constraints [7], where the uncertain generation of sufficient CO to ensure rapid action, the presence of pollutants and contaminants from fuel or inappropriately maintained engines, and the requirement for the gasses to be filtered and cooled before deployment (all processes prone to failure if not maintained assiduously) all present scenarios where the welfare of animals can be severely compromised.

	Anesthetic gasses (e.g. halothane, halogenated ethers such as isoflurane) can also be used as a measure alone, or included with other gasses to improve welfare of animals. These also carry risks to operators (requiring scrubbers to be used on the exhausts/vents of gassing systems) as well as posing indirect risks to environments – all that might be used with animals are potent greenhouse gasses.

4. Costs and effectiveness of the measure

4.1. Case studies

CASE STUDY #1

Measure type (if relevant):	Dispatch of birds caught in mass participation wildlife control schemes in Australia
Species:	Common myna (*Acridotheres tristis*)
Objective:	control
Combined with other measure(s):	Live-trapping
Country(ies) of application:	Australia
Geographic scale (km²) and/or population size measure applied to:	
Time period:	
Effort:	
Costs:	Overall costs:
	Personnel costs:
	Equipment and infrastructure:
	Other, including overheads:
Effectiveness:	Many cities and neighborhoods in Australia run citizen myna control programs, encouraging the live-trapping and dispatch of common mynas in volunteers' gardens or property. The birds once caught must be euthanized and a well-advertised framework of advice on trapping and euthanasia is provided via state or national NGOs, e.g. [7,8] based on published evidence [5]. Cervical dislocation is disparaged in favor of killing using carbon dioxide or carbon monoxide. Assemble at home kits for the euthanasia of individual birds

	using domestically available carbon dioxide are advertised widely for the purpose, whilst a method is described to kill myna's using car exhaust (CO poisoning) underpinned by scientific study [5]. The method is not reported to be quick (e.g. 20-85 seconds [5]), and there appears to be much scope for the method to fail to operate as swiftly or reliably as anticipated; warnings are posted that if birds fail to die within an allotted period to seek advice.

CASE STUDY #2	
Measure type (if relevant):	Dispatch of squirrels live-trapped in an eradication scheme
Species:	Pallas' squirrel (*Callosciurus erythraeus*)
Objective:	Eradication
Combined with other measure(s):	Live-trapping
Country(ies) of application:	Belgium
Geographic scale (km^2) and/or population size measure applied to:	15 ha
Time period:	6 years
Effort:	
Costs:	Overall costs:
	Personnel costs:
	Equipment and infrastructure:
	Other, including overheads:
Effectiveness:	Pallas's squirrels caught as part of an eradication campaign in a city park [11]. Euthanasia was delivered using a vehicle mounted apparatus using carbon dioxide, with the costs of the vehicle and the apparatus being included in the report. Interestingly, costs are also included for an additional isoflurane vaporizer, which could have been used to explore the benefits of prior, or simultaneous use of a gaseous anesthetic to mitigate harmful effects produced by the rising concentration of gas. Unfortunately, no comment on the humanness of either gas protocol, or the use of anesthetic is made, neither is a detailed protocol describing the fill rate or duration of exposure. The use of a vehicle mounted processing station will have enabled operations to proceed discretely in a busy public space, close to trapping locations, minimizing the distance trapped animals need to have been carried from their trapping location to the site of their dispatch.

4.2. Costs effectiveness summary	Euthanasia using modified atmospheres in both cases is either done by the public, or in public places. Generally, the chamber where death occurs is concealed, ensuring that no objections can be drawn from the appearance of distressed animals during the last minutes of consciousness. In one case study (squirrels), a significant apparatus was provided, ensuring that the principles of best practice could be followed. In the other (mynas), volunteers were left to construct apparatus at home from everyday items (bin bags, garden hose and gaffer tape). Whilst ensuring that apparatus is cheap and potentially available at many trapping sites across a neighborhood, both the gassing chamber, its accoutrements, as well as the gas generator (car engine) are all likely to result in the principles of best practice for euthanasia using modified atmospheres are unachievable. Whilst death from hypoxia/anoxia is almost certainly going to produce extended periods of distress to animals even when done well (e.g. 60 seconds), this period is only likely to be extended for sub-optimal deployments e.g. [5].

5. Conclusion

Overall assessment of the measure (qualitative)

Euthanasia using modified atmospheres is never as quick as most other methods of dispatch, with all animals subjected to the method experiencing periods of distress lasting for some time (a number of 10s of seconds). Similar durations of distress in individual cases following other methods of dispatch, e.g. cranial dispatch, cervical dislocation or even many injected drugs would be considered a case of failure. Whilst animals do not commonly display gross signs of distress, the final stages of consciousness, especially for anoxiant gases such as nitrogen, argon and even carbon dioxide may produce distress similar to that produced by mechanical anoxia (strangling or drowning [12]) and the narcosis associated with hypercarbia may be of very variable expression in different species, only significantly mitigating the distress of anoxia in some.

Reviewing a large number of studies on the use of individual gasses, as well as gas mixtures (mainly carbon dioxide in variable mixtures with nitrogen, argon, xenon) it is clear that important differences in the experience of animals is produced by seemingly small changes in the mixture used to modify the atmospheres of animals which lead to insensibility and death. These differences are species specific, combinations which work well for geese [4] may cause significant and avoidable harm to another species. Thus one requirement for the use of modified atmospheres in dispatching such a diverse range of species as the IAS of Union concern would be research to determine, for each species, either the optimal combinations of gasses and filling rates (including the benefits of using an anesthetic gas as part of the gassing protocol), or to identify those combinations produce unnecessary additional harm.

It is also clear that the principle of humane euthanasia using modified atmospheres, a well-regulated supply of gas, of known purity, introduced at an appropriate temperature and rate to a chamber of known volume is incompatible with either trap-side euthanasia, or mass deployment using home-made equipment. For this measure it is likely that cheap tools are likely to be sub-optimal, thereby causing unnecessary suffering.

Assessor:	James Aegerter
Reviewer 1:	Kevin Smith
Reviewer 2:	Sandro Bertolino

6. References

[1] AVMA. (2020). AVMA Guidelines for the Euthanasia of Animals. Retrieved from
[2] NC3Rs. (2013). Improving rodent welfare during euthanasia. Retrieved from https://www.nc3rs.org.uk/improving-rodent-welfare-during-euthanasia
[3] Bertolino, S., & Cocchi, R. (2018). Piano di gestione nazionale della Nutria Myocastor coypus. Roma: Istituto Superiore per la Protezione e la Ricerca Ambientale.
[4] Gerritzen, M. A., Reimert, H. G. M., Lourens, A., Bracke, M. B. M., & Verhoeven, M. T. W. (2013). Killing wild geese with carbon dioxide or a mixture of carbon dioxide and argon. Animal Welfare, 22(1), 5-12. doi:10.7120/09627286.22.1.005
[5] Tidemann, C. R., & King, D. H. (2009). Practicality and humaneness of euthanasia of pest birds with compressed carbon dioxide (CO2) and carbon monoxide (CO) from petrol engine exhaust. Wildlife Research, 36(6), 522-527. doi:https://doi.org/10.1071/WR09039
[6] Boivin, G. P., Hickman, D. L., Creamer-Hente, M. A., Pritchett-Corning, K. R., & Bratcher, N. A. (2017). Review of CO_2 as a Euthanasia Agent for Laboratory Rats and Mice. Journal of the American Association for Laboratory Animal Science : JAALAS, 56(5), 491-499.
[7] RSPCA, A. (2017). RSPCA Information Paper – Management of common (Indian) myna birds. In.
[8] CVCIA. Catching & Euthanasing Indian Mynas. Retrieved from http://cvcia.org.au/common-mynas/catching-a-myna/
[9] Gerritzen, M. A., Reimert, H. G. M., Hindle, V. A., Mckeegan, D. E. F., & Sparrey, J. (2010). Welfare assessment of gas-filled foam as an agent for killing poultry. Retrieved from
[10] Sandilands, V., Raj, A. B. M., Baker, L., & Sparks, N. H. C. (2011). Aversion of chickens to various lethal gas mixtures. Animal Welfare, 20(2), 253-262.
[11] Adriaens, T., Baert, K., Breyne, P., Casaer, J., Devisscher, S., Onkelinx, T., . . . Stuyck, J. (2015). Successful eradication of a suburban Pallas's squirrel Callosciurus erythraeus (Pallas 1779) (Rodentia, Sciuridae) population in Flanders (northern Belgium). Biological Invasions, 17(9), 2517-2526. doi:10.1007/s10530-015-0898-z
[12] Ludders, J. W., Schmidt, R. H., Dein, F. J., & Klein, P. N. (1999). Drowning is not euthanasia. Wildlife Society Bulletin (1973-2006), 27(3), 666-670.
[13] Kato, T., Ichida, Y., Tei, K., Asano, M., & Hayama, S. I. (2009). Reproductive characteristics of feral raccoons (Procyon lotor) captured by the pest control in Kamakura, Japan. Journal of Veterinary Medical Science, 71(11), 1473-1478.
[14] Santicchia, F., Wauters, L. A., Piscitelli, A. P., Van Dongen, S., Martinoli, A., Preatoni, D., ... & Ferrari, N. (2020). Spillover of an alien parasite reduces expression of costly behaviour in native host species. Journal of Animal Ecology.

Appendix 30. Shooting to dispatch restrained animals

1. Measure name			
1.1. English:	Shooting to dispatch restrained animals		
1.2. Lethal or non-lethal:	Lethal		
1.3. Other languages (if available):			
Bulgarian	Отстрел на уловени животни	Italian	Sparo per l'abbattimento di animali catturati
Croatian	Pucanje – pogubljivanje zatočenih životinja	Latvian	Nošaušana –savaldīto dzīvnieku nonāvēšana
Czech	Zastřelení – odstranění zadrženého jedince	Lithuanian	Apsvaigintų gyvūnų nušovimas
Danish	Skydning til drab af fastholdte dyr	Maltese	
Dutch	Afschot van gevangen dieren	Polish	Odstrzał interwencyjny
Estonian	Mahalaskmine - kinnipüütud loomade eemaldamine	Portuguese	Tiro – eliminação de animais capturados
Finnish	Ampuminen – kiinniotettujen eläinten lopetus	Romanian	Eliminare animale capturate prin împușcare
French	Tir – envoi d'animaux immobilisés	Slovak	Streľba/lov – usmrtenie jedincai
German	Schuss zum Töten gefangener Tiere	Slovenian	Streljanje – usmrtitev ujetih živali
Greek	Πυροβολισμός-Θανάτωση συλληφθέντων ατόμων	Spanish	Disparo- eliminación de animals capturados
Hungarian	Lelövés – elfogott állatok elpusztítása	Swedish	Avlivning med vapen
Irish			

2. Technical details of measure
2.1.a. Measure description
This method is used for the euthanasia of animals that have been caught and/or restrained using another method, e.g. **cage traps**. Shooting an animal from distance by professional or voluntary hunters is considered in **Shooting – hunting**. A properly placed gunshot can cause immediate insensibility and humane death. The method is routinely used to euthanize animals severely injured while hunting or after collision with vehicles [1]. Animals caught in cages are not fully restrained and therefore it is not easy to hit them in vital points, preferably the head (at a point midway between the eyes and base of the ears). Thus, personnel should be trained in the correct and safe use of firearms and the anatomy of the species involved. A correct shot will instantly render the animal unconscious, however, muscle spasms, and bleeding may continue for a brief period. The safety of personnel, the public, and other animals that are nearby should be considered. State and local laws often restrict firearm use and should be checked. Shooting can be undertaken with a low calibre rifle, a pistol, or an **air rifle.** A close-range shot to the head with a high-velocity air rifle is used for the euthanasia of cage-trapped brushtail possums (*Trichosurus vulpecula*) and it is proposed as a suitable and humane approach to the euthanasia of small mammals caught in traps [2]. In comparison with blunt-force trauma, the air rifle method was quicker in dispatching possum (i.e. shorter

conscious handling and total time) and arguably had better animal welfare performance by significantly reducing the time to unconsciousness [2]. Rifles and guns are used to euthanise coypu [3], feral cats [4], grey squirrels [7], raccoons [8], minks [10], while air rifles have been used for killing trapped beavers [5] and minks [9,10]. Shooting is the preferred method of dispatching live-trapped grey squirrels in Northern England [7]. In some cases, trapped squirrels are transferred to another trap before being dispatched by shooting to avoid the first trap becoming contaminated with blood [7]. The method is also used for birds. Wild turkeys *Meleagris gallopavo* were eradicated from Santa Cruz Island by catching them with **drop nets** and dispatching the immobilized birds with a small-calibre firearm [6].

2.1.b. Integration with other measures

Shooting is used for the euthanasia of animals that have been caught and/or restrained using another method, e.g. **cage traps** or **drop nets.** A properly placed gunshot in a vital area can cause immediate insensibility and humane death. Personnel should be skilled in the correct and safe use of firearms and the anatomy of the species involved.

As an example in the UK live-trapping was the commonest method of controlling ***S. carolinensis*** supplemented by shooting free-ranging squirrels; shooting was also the preferred method of despatching cage-trapped squirrels [7]. The same integration of measures was used in Italy for ***M. coypus***, with live-trapping and direct shooting as the two allowed methods of control and animals in cages dispatched shooting them or using gas for euthanasia [3].

2.2. Availability

Species	Availability	Reference(s)
Acridotheres tristis	P	6
Alopochen aegyptiaca	P	6
Callosciurus erythraeus	P	7
Corvus splendens	P	6
Herpestes javanicus	P	8,9
Lepomis gibbosus		
Lithobates catesbeianus		
Muntiacus reevesi	P	
Myocastor coypus	A	3
Nasua nasua	A	8,9
Nyctereutes procyonoides	P	8,9
Ondatra zibethicus	P	3
Oxyura jamaicensis	P	
Perccottus glenii		
Plotosus lineatus		
Procyon lotor	A	8
Pseudorasbora parva		
Sciurus carolinensis	A	7
Sciurus niger	A	7
Tamias sibiricus	P	7

Threskiornis aethiopicus	P	6
Trachemys scripta	A	12

3. Humaneness of the measure			
3.1. Welfare for all measures			
Measure type (if applicable): Shooting to dispatch restrained animals (excludes the prior restraining step, e.g. cage traps, which is assessed in separate assessments)	**Humaneness impact categories**		
Domain	**No impact**	**Mild-Moderate**	**Severe - Extreme**
1: Water deprivation, food deprivation, malnutrition	No intrinsic effect on the animal.		
2: Environmental challenge	No intrinsic effect on the animal.		
3: Injury, disease, functional impairment	Applied correctly ensures a rapid death, without risk of injury or functional impairment.		
4: Behavioural, interactive restriction		There will be short-term physical restraint as the animal is prevented from escaping the shooter.	
5: Anxiety, fear, pain, distress, thirst, hunger etc.		The brief period necessary for approaching and sometimes blocking the animal will almost certainly produce fear, but it will be very short (seconds).	

3.2. Mode of death (if relevant)			
Measure type (if applicable):	**Immediate death (i.e. no suffering)**	**Not immediate death (mild - moderate suffering)**	**Not immediate death (severe - extreme suffering)**
Rationale:	A properly placed gunshot can cause immediate insensibility and humane death.		

3.3. Humaneness summary	Shooting in a cage represents one of the most humane methods of dispatch for restrained animals when applied by trained operatives. The method is normally used also to euthanize severely injured wild animals. A properly placed gunshot can cause immediate insensibility and humane death [1]. Personnel should be trained in the correct and safe use of firearms and the anatomy of the species involved, so to target vital areas. The safety of personnel, the public, and other animals that are nearby should be considered. State and local laws often restrict firearm use and should be checked beforehand.

4. Costs and effectiveness of the measure

4.1. Case studies	
CASE STUDY #1	
Measure type (if relevant):	Shooting on cage traps
Species:	American mink (*Neovison vison*)
Objective:	Containment The Hebridean Mink Project was established with the objective of removing mink from North Uist, Benbecula and South Uist and to reduce mink density from neighbouring South Harris to minimise recolonization of the Uists.
Combined with other measure(s):	The programme relied on traps and dog searches. Captured animals were humanely dispatched with a shot to the brain stem using .22 calibre air pistols.
Country(ies) of application:	Scotland
Geographic scale (km^2) and/or population size measure applied to:	The control site in the Uists comprised approximately 356 islands and skerries totalling 850 km2.
Time period:	2001-2005
Effort:	Uists: 100,824 trap/night, 191 minks trapped, 1.89 mink captured per 1000 trap nights Harris: 41,674 trap/night, 230 minks trapped, 5.51 mink captured per 1000 trap nights
Costs:	Overall costs:

	Personnel costs:
	Equipment and infrastructure:
	Other, including overheads:
Effectiveness:	The method (cage trapping and shooting) allowed to meet the main objective, the removal of minks from the Uists. Dog searches were used as an additional technique for isolated animals (N=55), especially for searching female mink in dens [11]. The last capture in the Uists was in March 2005. After this date, despite a further 7 months of intensive trapping and searching effort, no further signs of mink were found and they were considered likely to have been removed from this region.
Reference(s):	[9]

CASE STUDY #2	
Measure type (if relevant):	Shooting to dispatch restrained animals
Species:	*Myocastor coypus*
Objective:	Control
Combined with other measure(s):	Animals were live-trapped or directly shoot in the field. Trapped animals were dispatched by shooting or using gases.
Country(ies) of application:	Italy
Geographic scale (km^2) and/or population size measure applied to:	41,515 Km2
Time period:	1995-2000
Effort:	220,688 coypus were removed: 118,669 (54%) through trapping and 102,019 (46%) through direct shooting. The proportion of caged animals that were shoot of euthanized with gas is not reported. The number of operators authorized yearly was 1995: 241, 1996: 237, 1997: 464, 1998: 1,139, 1999: 1,176, 2000: 1,479
Costs:	Overall costs:
	Cost for management (i.e. trapping, shooting and carcass disposal) was 2,614,408 €, averaging 435,735/year Cost of removing a single coypu was estimated to be € 13.25/coypu trapped and € 8.21/coypu shot.
	Personnel costs:
	The largest part (60%) of the overall cost of control was related to trapping activities, while direct shooting and carcass disposal accounted for 32 and 8%, respectively.
	Equipment and infrastructure:

	Other, including overheads:
Effectiveness:	The control campaigns did not stop the population expansion nor the increase in damage and economic losses at a national scale. However, the efficacy of local campaigns varied among different ecosystems.
Reference(s):	[3]

4.2. Costs effectiveness summary	The method allows a fast and humane dispatch of trapped animals, but it requires trained personnel. The effectiveness of the projects however, depends more on the technique used to trap the animals. Both for controlling *S. carolinensis* in UK and *M. coypus* in Italy shooting animals in traps is only one of the techniques used to euthanize trapped animals, and live-trapping itself is complemented by direct shooting in the field. Therefore, it is difficult to evaluate cost and effectiveness of a single technique. In Italy, cost of trapping and dispatching coypus was higher than cost of direct shooting, but trapped animals were either gassed or shot.

5. Conclusion	
Overall assessment of the measure (qualitative)	
Shooting cage (or net) trapped animals provides a reliable and humane method for euthanasia. It was used in two mink control projects that were implemented over large areas [9,10] and it is used also for **coypu** [3] and **grey squirrels** [7]. It requires trained staff to ensure the lethality of the first shot. State and local laws may restrict the use of firearm air rifles. The method is applicable to most mammal and bird species, including those listed as IAS of Union Concern.	
Assessor:	Sandro Bertolino
Reviewer 1:	Ana Nunes
Reviewer 2:	Kevin Smith

6. References

[1] AVMA (2020). AVMA Guidelines for the Euthanasia of Animals: 2020 Edition

[2] Rouco, C., Richardson, K. S., & Tompkins, D. M. (2015). Improving animal welfare standards while reducing disease exposure risk during euthanasia of trapped brushtail possums (*Trichosurus vulpecula*). *Animal Welfare (South Mimms, England)*, *24*, 235-239.

[3] Panzacchi, M., Cocchi, R., Genovesi, P., & Bertolino, S. (2007). Population control of coypu *Myocastor coypus* in Italy compared to eradication in UK: a cost-benefit analysis. *Wildlife Biology*, *13*(2), 159-171.

[4] Algar, D., Johnston, M., & Pink, C. (2019). Big island feral cat eradication campaigns: an overview and status update of two significant examples. *Island invasives: scaling up to meet the challenge*, (62), 238-243.

[5] Schiavini, A., Escobar, J., Curto, E., & Jusim, P. (2019). First results from a pilot programme for the eradication of beavers for environmental restoration in Tierra Del Fuego. *Island invasives: scaling up to meet the challenge*, (62), 57-64.

[6] Morrison, S. A., DeNicola, A. J., Walker, K., Dewey, D., Laughrin, L., Wolstenholme, R., & Macdonald, N. (2016). An irruption interrupted: eradication of wild turkeys *Meleagris gallopavo* from Santa Cruz Island, California. *Oryx*, *50*(1), 121-127.

[7] Parrott, D., Quy, R., Van, D., Lurz, P., Rushton, S., Gurnell, J., ... & Reynolds, J. (2009). Review of red squirrel conservation activity in northern England. *Natural England Commissioned Report*.

[8] Garrettson, P. R., & Rohwer, F. C. (2001). Effects of mammalian predator removal on production of upland-nesting ducks in North Dakota. *The Journal of wildlife management*, 398-405.

[9] Roy, S. S., Chauvenet, A. L. M., & Robertson, P. A. (2015). Removal of American mink (*Neovison vison*) from the Uists, outer Hebrides, Scotland. *Biological Invasions*, *17*(10), 2811-2820.

[10] Bryce, R., Oliver, M. K., Davies, L., Gray, H., Urquhart, J., & Lambin, X. (2011). Turning back the tide of American mink invasion at an unprecedented scale through community participation and adaptive management. *Biological conservation*, *144*(1), 575-583.

[11] Roy, S. (2011). Strategies to improve landscape scale management of mink populations in the west coast of Scotland: lessons learned from the Uists 2001–2006. *Island invasives: eradication and management*. IUCN, Gland, 114-117.

[12] Swedish Board of Agriculture´s regulation on slaughter and other killing of animals (SJVFS 2019:8)

Appendix 31. Slaughter with a knife

1. Measure name	
1.1. English:	Slaughter with a knife
1.2. Lethal or non-lethal:	Lethal

1.3. Other languages (if available):

Bulgarian	Прерязване на врата с нож	Italian	Uccisione con un coltello
Croatian	Usmrćivanje nožem	Latvian	Nokaušana ar nazi
Czech	Porážka nožem	Lithuanian	Škerdimas peiliu
Danish	Drab med kniv	Maltese	
Dutch	Slachten met een mes	Polish	Ubój nożem
Estonian	Hukkamine noaga	Portuguese	Abate com faca
Finnish	Lopetus (veitsi)	Romanian	Uciderea animalelor prin secționarea gâtului
French	Abattage avec un couteau	Slovak	Usmrténie/porážka nožom
German	Schlachten mit Messer	Slovenian	Zakol z nožem
Greek	Σφαγή (με μαχαίρι)	Spanish	Matar con cuchillo
Hungarian	Leölés késsel	Swedish	Avlivning med kniv, avblodning, slakt
Irish			

2. Technical details of measure

2.1.a. Measure description

The slaughter of an animal with a knife can be obtained through a transverse cut across the throat, near to the head, causing its exsanguination. This operation is usually accomplished transecting skin, muscle, trachea, oesophagus, carotid arteries, jugular veins, and a multitude of sensory and motor nerves and other vessels [1]. The length of time between the neck cut and loss of consciousness is still controversial [1]. Studies with farmed animals demonstrate a rapid loss of brain activity (measured by EEG) [2,3]. In contrast, direct observation of time to collapse and EEG data indicate that the time from ventral-neck incision to unconsciousness is variable and may be quite prolonged [4,5,6,7,8].

In the same way, opinions on whether the animal feels pain during the neck cut and if the drop in blood pressure causes discomfort or distress are divided [1]. For these reasons, the procedure is not recommended as a sole method of euthanasia; rather it is considered an adjunctive method to ensure death in an unconscious animal [1]. Exsanguination produces a large volume of blood loss and may be disturbing to observe; it also raises biosecurity concerns [1].

In fish, a knife or other sharp instrument is inserted caudally to the skull to sever the spinal cord and cervical vertebrae, followed by pithing to destroy the brain tissue [1]. It is similar to **decapitation** but the head remains physically attached by musculature to the body.

Slaughter with a knife is used in studies that need to collect blood or other tissue samples from wild animals; in these cases, animals are usually anesthetized before exsanguination: e.g. rats (*Rattus norvegicus*) [9], African green monkeys (*Cercopithecus aethiops*) [10], opossum and armadillos [11], wild turkeys (*Meleagris gallopavo*) [15], ducks (*Aythya* spp. and *Anas* spp.) [12], mongooses (*Herpestes javanicus*) [16]. Cardiac exsanguination under carbon dioxide anaesthesia has been practiced in raccoons [13]. Wild turkeys have also been captured with a drop net, transported in boxes into a lab and euthanized by exsanguination [11]. A large number of grey squirrel (***Sciurus carolinensis***, N=180) were live-trapped and then exsanguinated after anaesthesia with ketamine hydrochloride as part of a study into the health and diseases of an urban population [15]. Exsanguination and subsequent hypovolaemic shock and anoxia may not render amphibians and reptiles immediately unconscious, making this an unacceptable method of euthanasia for these groups [17].
2.1.b. Integration with other measures
Slaughter with a knife is used to dispatch animals that have been caught and/or restrained using another method, e.g. **cage traps**. The technique involves the immobilization of the animal, its sedation, a transverse cut across the throat, and its exsanguination. The handling of animals for the extraction from the trap can be fast, but then the animal must be sedate before to proceed to cut the throat and this can take more time, according to the experience of the operator.

2.2. Availability		
Species	**Availability**	**Reference(s)**
Acridotheres tristis	P	11,12
Alopochen aegyptiaca	P	11,12
Callosciurus erythraeus	P	15
Corvus splendens	P	11,12
Herpestes javanicus	P	16
Lepomis gibbosus	P	1
Lithobates catesbeianus	P	
Muntiacus reevesi	P	
Myocastor coypus	P	13,16
Nasua nasua	P	13,16
Nyctereutes procyonoides	P	13,16
Ondatra zibethicus	P	9,15
Oxyura jamaicensis	P	
Perccottus glenii	P	1
Plotosus lineatus	P	1
Procyon lotor	P	13
Pseudorasbora parva	P	1
Sciurus carolinensis	P	15
Sciurus niger	P	15
Tamias sibiricus	P	15
Threskiornis aethiopicus	P	11,12
Trachemys scripta	P	

3. Humaneness of the measure			
3.1. Welfare for all measures			
Measure type (if applicable): Slaughter with a knife (following anesthesia)	**Humaneness impact categories**		
Domain	**No impact**	**Mild-Moderate**	**Severe - Extreme**
1: Water deprivation, food deprivation, malnutrition	No intrinsic effect on the animal.		
2: Environmental challenge	No intrinsic effect on the animal.		
3: Injury, disease, functional impairment	It should be applied to sedated animals; risk of injury or functional impairment are low and in any case they would be produced on anesthetized animals which can then be euthanized with another cut.		
4: Behavioural, interactive restriction		There will be short-term physical restraint while the animal is being handled.	
5: Anxiety, fear, pain, distress, thirst, hunger etc.		The period of handling and restraint necessary to apply the method will almost certainly produce fear. Considering that the method should only be applied to anesthetized animals, the pain should be reduced and mainly due to retraining animals and their anesthesia.	

3.2. Mode of death (if relevant)			
Measure type (if applicable):	**Immediate death (i.e. no suffering)**	**Not immediate death (mild - moderate suffering)**	**Not immediate death (severe - extreme suffering)**
Rationale:	The length of time between the neck cut and loss of consciousness is still controversial. For this reason, animals should first be anesthetized, in which case there is no suffering despite the length of time until death. If the animal is		

	not sedated then the category would increase.		

3.3. Humaneness summary	The slaughter of animals is obtained through a transverse cut across the throat, near to the head, causing its exsanguination. The length of time between the neck cut and loss of consciousness is still controversial. There are different opinions on whether the animal feels pain during the neck cut and if the drop in blood pressure causes discomfort or distress. Therefore, slaughter with a knife should be considered a terminal procedure to be used only in stunned or anesthetized animals. Exsanguination produces a large volume of blood loss and may be disturbing to observe; it also raises biosecurity concerns.

4. Costs and effectiveness of the measure

4.1. Case studies	
CASE STUDY #1	
Measure type (if relevant):	
Species:	
Objective:	
Combined with other measure(s):	
Country(ies) of application:	
Geographic scale (km^2) and/or population size measure applied to:	
Time period:	
Effort:	
Costs:	Overall costs:
	Personnel costs:

	Equipment and infrastructure:
	Other, including overheads:
Effectiveness:	
Reference(s):	

4.2. Costs effectiveness summary	No case studies on the measures application to animals of Union concern could be found. The only study available just reports the number of animals that were live-trapped and then exsanguinated.

5. Conclusion

Overall assessment of the measure (qualitative)

The slaughter of an animal with a knife is obtained through a transverse cut across the throat, near to the head, causing its exsanguination. The length of time between the neck cut and loss of consciousness is variable and may be quite prolonged. Whether the animal feels pain during the neck cut and if the drop in blood pressure causes discomfort or distress is still controversial. Therefore, slaughter with a knife should be considered a terminal procedure to be used only in stunned or anesthetized animals. The method has been used during research involving the dispatch of animals, including ***S. carolinensis***, but not during management projects.

Assessor:	Sandro Bertolino
Reviewer 1:	Ana Nunes
Reviewer 2:	Kevin Smith

6. References

[1] AVMA (2020). AVMA Guidelines for the Euthanasia of Animals: 2020 Edition
[2] Gregory, N. G., & Wotton, S. B. (1984). Time to loss of brain responsiveness following exsanguination in calves. *Research in Veterinary Science*, *37*(2), 141-143.
[3] Schulze, W., Schultze-Petzold, H., Hazem, A. S., & Gross, R. (1978). Experiments on the objective assessment of pain and consciousness in slaughtering sheep and calves by the conventional method (humane killer stunning) and by ritual slaughtering laws (shechita). *Dtsch Tierarztl Wochenschr*, *85*, 62-66.
[4] Blackmore, D. K. (1984). Differences in behaviour between sheep and cattle during slaughter. *Research in veterinary science*, *37*(2), 223-226.

[5] Bager, F., Devine, C. E., & Gilbert, K. V. (1988). Jugular blood flow in calves after head-only electrical stunning and throat-cutting. *Meat science*, *22*(3), 237-243.
[6] Daly, C. C., Kallweit, E., & Ellendorf, F. (1988). Cortical function in cattle during slaughter: conventional captive bolt stunning followed by exsanguination compared with shechita slaughter. *The Veterinary Record*, *122*(14), 325.
[7] Newhook, J. C., & Blackmore, D. K. (1982). Electroencephalographic studies of stunning and slaughter of sheep and calves: part 1—the onset of permanent insensibility in sheep during slaughter. *Meat Science*, *6*(3), 221-233.
[8] Gregory, N. G., Fielding, H. R., von Wenzlawowicz, M., & Von Holleben, K. (2010). Time to collapse following slaughter without stunning in cattle. *Meat Science*, *85*(1), 66-69.
[9] McKiel, J. A., Cousineau, J. G., & Hall, R. R. (1961). Leptospirosis in wild animals in Eastern Canada with particular attention to the disease in rats. *Canadian Journal of Comparative Medicine and Veterinary Science*, *25*(1), 15.
[10] Suleman, M. A., Wango, E., Sapolsky, R. M., Odongo, H., & Hau, J. (2004). Physiologic manifestations of stress from capture and restraint of free-ranging male African green monkeys (Cercopithecus aethiops). *Journal of Zoo and Wildlife Medicine*, *35*(1), 20-24.
[11] Fujita, O., Sanabria, L., Inchaustti, A., De Arias, A. R., Tomizawa, Y., & Oku, Y. (1994). Animal reservoirs for *Trypanosoma cruzi* infection in an endemic area in Paraguay. *Journal of Veterinary Medical Science*, *56*(2), 305-308.
[12] Furuno, K., Lee, K., Itoh, Y., Suzuki, K., Yonemitsu, K., Kuwata, R., ... & Takano, A. (2017). Epidemiological study of relapsing fever borreliae detected in Haemaphysalis ticks and wild animals in the western part of Japan. *PloS one*, *12*(3), e0174727.
[13] Keawcharoen, J., Van Riel, D., van Amerongen, G., Bestebroer, T., Beyer, W. E., Van Lavieren, R., ... & Kuiken, T. (2008). Wild ducks as long-distance vectors of highly pathogenic avian influenza virus (H5N1). *Emerging infectious diseases*, *14*(4), 600.
[14] Spraker, T. R., Adrian, W. J., & Lance, W. R. (1987). Capture myopathy in wild turkeys (*Meleagris gallopavo*) following trapping, handling and transportation in Colorado. *Journal of Wildlife Diseases*, *23*(3), 447-453.
[15] Lewis, E., Hoff, G. L., Bigler, W. J., & Jefferies, M. B. (1975). Public health and the urban gray squirrel: mycology. *Journal of wildlife diseases*, *11*(4), 502-504.
[16] Kusuda, S., Hoson, O., Nakaya, Y., Ogura, G., Oshiro, S., Takara, J., ... & Murata, K. (2010). Induced estrus in female small Asian mongooses (*Herpestes javanicus*) for the purpose of controlling invasive alien species in Okinawa Island. *Mammal study*, *35*(3), 217-219.
[17] Close, B., Banister, K., Baumans, V., Bernoth, E. M., Bromage, N., Bunyan, J., ... & Morton, D. (1997). Recommendations for euthanasia of experimental animals: Part 2. *Laboratory animals*, *31*(1), 1-32.

Appendix 32. Surgical sterilization

1. Measure name	
1.1. English:	Surgical sterilization
1.2. Lethal or non-lethal:	Non-lethal

1.3. Other languages (if available):

Bulgarian	Хирургична стерилизация	Italian	Sterilizzazione chirurgica
Croatian	Kirurška sterilizacija	Latvian	Ķirurģiskā sterilizācija
Czech	Chirurgická sterilizace	Lithuanian	Chirurginė sterilizacija
Danish	Kirugisk sterilisering	Maltese	
Dutch	Chirurgische sterilisatie	Polish	Sterylizacja chirurgiczna
Estonian	Kirurgiline steriliseerimine	Portuguese	Esterilização cirúrgica
Finnish	Kirurginen sterilointi	Romanian	Sterilizare chirurgicală
French	Stérilisation chirurgicale	Slovak	Chirurgická sterilizácia
German	Chirurgische Sterilisation	Slovenian	Kirurška sterilizacija
Greek	Χειρουργική στείρωση	Spanish	Esterilización quirúrgica
Hungarian	Sebészeti sterilizálás	Swedish	Kirurgisk sterilisation
Irish			

2. Technical details of measure

2.1.a. Measure description

Many techniques for surgically sterilizing companion animals have been described, including midline ovariohysterectomy, lateral flank ovariohysterectomy, castration, ovariectomy, laparoscopic ovariohysterectomy, laparoscopic ovariectomy, tubal ligation and vasectomy [1]. Phallectomy has also been used to control *Trachemys scripta* populations [29] and the same species has been submitted to experimental coelioscopic orchiectomy [30].

Surgical sterilization is also commonly used in zoological collections with non-domestic species [2] where rendering an animal permanently infertile is the expected result. By extrapolation, many of these techniques may be applied to free-living wildlife populations; however its application to the management and control of IAS has so far been limited to small enclosed/isolated populations. Whilst surgical sterilization techniques have been developed for some of the mammalian and reptilian IAS which this project deals with [3, 4, 5, 6, 7], application to feral and free-living populations for management and control purposes has so far been limited. Although surgical sterilization may have a perceived greater public acceptance than culling, it is costly because of the use of specialized veterinary staff, drugs and facilities, with potential welfare issues associated with the use of anaesthetics and any surgical complications [8, 9]. Hence, the economic and costs associated with capture, transport and undertaking veterinary surgery are likely to be prohibitive for most wildlife applications, apart from in specific circumstances [10].

Furthermore, surgical sterilization is an invasive technique, and for example in the case of the eradication of the American grey squirrel from Perugia, central Italy, raised concern from some animal welfare organisations that opposed the application of this measure [28].
2.1.b. Integration with other measures
This measure can be part of a Trap, Neuter and Release (TNR) programme, which would require **live trapping** to be a prior step. Additional methods of capture may involve **hand removal** or **nets** (in the case of *Trachemys scripta*) or with **judas animals** [31]. Following surgical sterilization, the animals may also be **kept in captivity**.

2.2. Availability		
Species	**Availability**	**Reference(s)**
Acridotheres tristis	P	
Alopochen aegyptiaca	P	
Callosciurus erythraeus	P	
Corvus splendens	P	
Herpestes javanicus	P	
Lepomis gibbosus		
Lithobates catesbeianus		
Muntiacus reevesi	P	
Myocastor coypus	A	
Nasua nasua	P	
Nyctereutes procyonoides	P	
Ondatra zibethicus	P	
Oxyura jamaicensis	P	
Perccottus glenii		
Plotosus lineatus		
Procyon lotor	A	
Pseudorasbora parva		
Sciurus carolinensis	A	6
Sciurus niger	P	
Tamias sibiricus	P	
Threskiornis aethiopicus	P	
Trachemys scripta	A	29

3. Humaneness of the measure			
3.1. Welfare for all measures			
Measure type (if applicable):	**Humaneness impact categories**		
Domain	**No impact**	**Mild-Moderate**	**Severe - Extreme**
1: Water deprivation, food deprivation, malnutrition		Pre-anaesthetic fasting (withholding food and water) may be indicated in certain species such as Chelonia, waterfowl and Cervidae, but not in others such as rodents [16]. The duration of this fasting period is species dependent and primarily aims to reduce the risk of regurgitation and aspiration under general anaesthesia [17].	
2: Environmental challenge		Consideration should be given to reducing the amount of time between capture and surgical sterilization [6]. Additionally, should there be a need for an animal be fasted for a prolonged period before surgery, or to be kept in captivity during the recovery period, this may involve keeping the animal in a restricted area where it may not have complete control over its environment.	
3: Injury, disease, functional impairment		There is the possibility of injury to both the animal and workers during restraint. Surgical complications (e.g. hemorrhage, infection, wound breakdown, death) can also result which may also compromise animal welfare [18, 19]. As this is an invasive procedure with associated surgical trauma, attention must be paid to the appropriate provision of analgesia.	
4: Behavioural, interactive restriction		The long-term behavioural and social impacts associated with surgical sterilization in non-domestic species have not been subject to extensive research [20].	

5: Anxiety, fear, pain, distress, thirst, hunger etc.		Short terms effects of handling, restraint, prevention of resource access, inability to hide, proximity to humans and pain associated with the procedure would all contribute to varying degrees of anxiety, fear and distress in the captured animals [21]. The species and individual animal's ecology (I.e. if they are prey or predator animals and how close they regularly come to human contact) will affect the level of stress experienced throughout the procedure according to their exposure to humans. There may also be longer lasting effect where the animal now will associate humans with stress and pain, which can cause regular anxiety after release if they are generally exposed to humans in their environment [22]. The length of time that monitoring is required for the procedure and potential duration of stress experienced by the animal will vary according to the species and the methods required (i.e. rodents would not need to be held for an additional fasting period [17]).	

3.2. Mode of death (if relevant)			
Measure type (if applicable):	**Immediate death (i.e. no suffering)**	**Not immediate death (mild - moderate suffering)**	**Not immediate death (severe - extreme suffering)**
Rationale:			

3.3. Humaneness summary	Assuming best practice application of this technique, this measure would rank as having mild-moderate humaneness impact. Despite surgical sterilization being a one-off procedure to render permanent infertility, the long-term welfare impacts of sterilizing on non-domestic species is still lacking research (i.e. behavioural changes, sociability, survival).

4. Costs and effectiveness of the measure

4.1. Case studies	
CASE STUDY #1	
Measure type (if relevant):	Surgical sterilization
Species:	Grey squirrel (*Sciurus carolinensis*)
Objective:	Eradication
Combined with other measure(s):	Combined with live trapping and euthanasia (injection) as part of a wider LIFE funded project, LIFE09 NAT/IT/000095 EC-SQUARE [11]. Surgical sterilization was applied to an enclosed population where culling or other management methods would have potentially jeopardized the public opinion of the project [6].
Country(ies) of application:	Italy
Geographic scale (km^2) and/or population size measure applied to:	The sterilization project focused on an urban population of between 200-300 squirrels living in an area of 12ha, with 324 squirrels being surgically sterilized by the end of the project [6].
Time period:	14 months (between January 2014 and March 2015)
Effort:	
Costs:	Overall costs:
	Surgery costs for the project associated with the sterilization of approx. 300 squirrels was 33,000€ [15].
	Personnel costs:
	Equipment and infrastructure:
	Other, including overheads:
Effectiveness:	The population was completely eradicated from its original location and translocated to a new site once the animals were sterilized. Of the 324 animals captured as part of this project, 3 died following capture but before surgery and 20 animals died post-operatively before release. Long term survival or welfare impacts was not assessed following the project [6].

4.2. Costs effectiveness summary	This measure can be financially expensive depending on the specific method used and requires experienced personnel and facilities to perform these procedures. As a result of these factors the applicability of this measure is very limited to field application. However, in the case of phallectomy in *T. scripta* populations, it has been found to be both economically viable and applicable to large numbers of animals [29]. Surgical sterilization and release of neutered individuals is unlikely to be feasible for the large-scale population management of the three squirrel IAS of EU concern (*Sciurus niger, S. carolinensis* and *Calloscirurus erythraeus*) [10], given that an estimated 80%+ of the female target population would need to be rendered infertile to result in substantial population declines [12, 13, 14]. Surgical sterlisation has also been used on *M. coypu* in Italy for a small group of animals in an urban area, with costs ranging from 100-130 per animal.

5. Conclusion

Overall assessment of the measure (qualitative)

Although surgical sterilization generally has greater public acceptance than culling in the context of IAS management, it remains financially expensive due to the use of veterinary staff, drugs and facilities, with potential welfare issues associated with the use of anaesthetics [8, 9]. However, in the case of phallectomy in *T. scripta* populations, it has been found to be both economically viable and applicable to large numbers of animals [29]. Whilst the advantages of rendering an IAS population permanently infertile are clear, there is a lack of data concerning the welfare of such individuals in the long-term.

Application of this measure on free-living populations of animals has been limited, however examples do include; reducing European rabbit (*Oryctolagus cuniculus*) populations experimentally [23], preventing sheep attacks through coyote (*Canis latrans*) sterilization [24] and reduction in urban Eastern grey kangaroo (*Macropus giganteus*) populations [25]. Due to the economic implications of this approach, this measure has been used in conjunction with other measures to help improve public perception of a wider IAS management programme [6].

Surgical sterilization is not applicable to all situations and requires a complex set of actions, related to the regulation of animals to be removed daily with the availability of veterinarians for the procedure, the containment and care of the animals in the pre and post-operative periods and the necessity to maintain them in captivity for their life or release into controlled areas [26].

The population biology and trapability of the IAS of Union concern may allow the technique to contribute to their management, at least in some local scale contexts. For instance, the raccoon (*Procyon lotor*) is relatively easy to live trap [27]. However, this potential would need to be explored further through an initial species-specific feasibility study [10].

Assessor:	EAZA
Reviewer 1:	Ana Nunes
Reviewer 2:	Ilaria Di Silvestre

6. References

[1] Howe, L. M. (2006). Surgical methods of contraception and sterilization. *Theriogenology*, *66*(3), 500-509.
[2] Patton, M. L., Jöchle, W., & Penfold, L. M. (2007). Review of contraception in ungulate species. *Zoo Biology: Published in affiliation with the American Zoo and Aquarium Association*, *26*(4), 311-326.
[3] Minto, B. W., Nagatsuyu, C. E., Teixeira, C. R., Zanuzzo, F. S., Candido, T. D., Diogo, L. M., & Macedo, A. S. (2017). Minimally invasive hysterectomy in Coatis (Nasua nasua). *Pesquisa Veterinária Brasileira*, *37*(6), 627-629.
[4] Mercedes Cano, A. F. M., & López Esteve, F. G. (2009). Sterilization of a racoon. *Argos-Informativo Veterinario*, (105).
[5] Proença, L. M., & Divers, S. J. (2015). Coelioscopic and endoscope-assisted sterilization of chelonians. *Veterinary Clinics: Exotic Animal Practice*, *18*(3), 555-570.
[6] Scapin, P., Ulbano, M., Ruggiero, C., Balduzzi, A., Marsan, A., Ferrari, N., & Bertolino, S. (2019). Surgical sterilization of male and female grey squirrels (Sciurus carolinensis) of an urban population introduced in Italy. *Journal of Veterinary Medical Science*, 18-0319.
[7] Anta i Vinyals, A. D. (2015). Ovariohysterectomy in a coati. *Argos-Informativo Veterinario*, (172), 60-61.
[8] Levy, J. K., Crawford, P. C., Appel, L. D., & Clifford, E. L. (2008). Comparison of intratesticular injection of zinc gluconate versus surgical castration to sterilize male dogs. *American journal of veterinary research*, *69*(1), 140-143.
[9] Massei, G., Miller, L. A., & Killian, G. J. (2010). Immunocontraception to control rabies in dog populations. *Human-Wildlife Interactions*, *4*(2), 155-157.
[10] IUCN. (2017). Information on non-lethal measures to eradicate or manage vertebrates included on the Union list. Technical note prepared by IUCN for the European Commission.
[11] LIFE09 NAT/IT/000095 EC-SQUARE (2015) https://ec.europa.eu/environment/life/project/Projects/index.cfm?fuseaction=search.dspPage&n_proj_id=3805
[12] Cowan, D. P., Massei, G., & Mellows, R. J. (2006). A modeling approach to evaluating potential applications of emerging fertility control technologies in the UK. In *Proceedings of the Vertebrate Pest Conference* (Vol. 22, No. 22).
[13] Williams, C.K., Davey, C.C., Moore, R.J., Hinds, L.A., Silvers, L.E., Kerr, P.J., French, N., Hood, G.M., Pech, R.P. and Krebs, C.J. (2007). Population responses to sterility imposed on female European rabbits. *Journal of Applied Ecology*, 291-301.
[14] Krause, S. K., Kelt, D. A., Van Vuren, D. H., & Gionfriddo, J. P. (2014). Regulation of tree squirrel populations with immunocontraception: a fox squirrel example. *Human–Wildlife Interactions*, *8*(2), 3.
[15] Balduzzi, A., Marsan, A., Bertolino, S., Martinoli, A., Preatoni, D., Wauters, L. and Carnevale, P., (2015) *Best-practice guidelines to carry out and implement squirrel population eradication through neutering,* Documento prodotto nell'ambito del Progetto LIFE09 NAT/IT/000095 EC-SQUARE, Azione C3: https://ec.europa.eu/environment/life/project/Projects/index.cfm?fuseaction=home.showFile&rep=file&fil=EC_SQUARE_best-practice_guidelines_eradication.pdf
[16] West, G., Heard, D. J., & Caulkett, N. (Eds.). (2014). *Zoo animal and wildlife immobilization and anesthesia*. John Wiley & Sons.
[17] Bednarski, R., Grimm, K., Harvey, R., Lukasik, V. M., Penn, W. S., Sargent, B., & Spelts, K. (2011). AAHA anesthesia guidelines for dogs and cats. *Journal of the American Animal Hospital Association*, *47*(6), 377-385.
[18] Burrow, R., Batchelor, D., & Cripps, P. (2005). Complications observed during and after ovariohysterectomy of 142 bitches at a veterinary teaching hospital. *Veterinary Record*, *157*(26), 829-833.
[19] Palmer, C., Corr, S., & Sandøe, P. (2012). Inconvenient desires: should we routinely neuter companion animals?. *Anthrozoös*, *25*(sup1), s153-s172.
[20] Bertschinger, H. J. (2010). *Controlling wildlife reproduction: reversible suppression of reproductive function or sex-related behaviour in wildlife species*. Utrecht University.
[21] Lindsjö, J., Cvek, K., Spangenberg, E., Olsson, J., & Stéen, M. (2019). The Dividing Line Between Wildlife Research and Management-Implications for Animal Welfare. *Frontiers in veterinary science*, 6, 13. https://doi.org/10.3389/fvets.2019.00013
[22] Hosey, G., & Melfi, V. (Eds.) (2018). Anthrozoology: human-animal interactions in domesticated and wild animals. Oxford University Press.
[23] Twigg, L. E., Lowe, T. J., Martin, G. R., Wheeler, A. G., Gray, G. S., Griffin, S. L., ... & Hubach, P. H. (2000). Effects of surgically imposed sterility on free-ranging rabbit populations. *Journal of Applied Ecology*, *37*(1), 16-39.
[24] Bromley, C., & Gese, E. M. (2001). Surgical sterilization as a method of reducing coyote predation on domestic sheep. *The Journal of wildlife management*, 510-519.
[25] Tribe, A., Hanger, J., McDonald, I. J., Loader, J., Nottidge, B. J., McKee, J. J., & Phillips, C. J. (2014). A reproductive management program for an urban population of eastern grey kangaroos (Macropus giganteus). *Animals*, *4*(3), 562-582.
[26] Bertolino, S., Lurz, P. W., Shuttleworth, C. M., Martinoli, A., & Wauters, L. A. (2016). 25. The management of grey squirrel populations in Europe: evolving best practice.
[27] Hoffmann, C. O., & Gottschang, J. L. (1977). Numbers, distribution, and movements of a raccoon population in a suburban residential community. *Journal of Mammalogy*, *58*(4), 623-636.

[28] LIFE Final Report (2015) for Project LIFE09 NAT/IT/00095 EC-SQUARE https://ec.europa.eu/environment/life/project/Projects/index.cfm?fuseaction=home.showFile&rep=file&fil=LIFE09_NAT_IT_000095_FTR.pdf
[29] Ferri (2019) Piano di controllo e gestione delle specie esotiche di testuggini palustri (Trachemys scripta ssp), https://www.regione.lombardia.it/wps/wcm/connect/85638e14-7506-4107-96d1-52d11f5df1bb/Piano+di+controllo+e+gestione+delle+specie+esotiche+di+testuggini+palustri+-+Azione+A7.pdf?MOD=AJPERES&CACHEID=ROOTWORKSPACE-85638e14-7506-4107-96d1-52d11f5df1bb-mM0b5g0
[30] Innis, Charles & Feinsod, Randon & Hanlon, J & Stahl, S & Oguni, J & Boone, Shaun & Schnellbacher, Rodney & Cavin, Julie & Divers, Stephen. (2013). Coelioscopic orchiectomy can be effectively and safely accomplished in chelonians. The Veterinary record. 172. 10.1136/vr.101475.
[31] Management of the invasive Raccoon Dog (Nyctereutes procyonoides) in the north-European countries (2013) Laymans Report LIFE09 NAT/SE/000344, https://jagareforbundet.se/globalassets/global/mardhundsprojektet/dokument/laymans-report-management-of-the-invasive-raccon-dog-in-the-north-european-countries.pdf

Appendix 33. Humaneness impact categories guidance for measure assessments

The impact categories from Sharp and Saunders (2011) have been used as a guide to provide a framework for the humaneness assessments of the measures.
Taken from: Sharp, T. and Saunders, G. (2011). A model for assessing the relative humaneness of pest animal control methods (Second edition). Australian Government Department of Agriculture, Fisheries and Forestry, Canberra, ACT. Printed by: New Millennium Print.

Impact scales for *3.1 Overall welfare impact*

DOMAIN 1: WATER DEPRIVATION, FOOD DEPRIVATION, MALNUTRITION		
Impact Category	**Description of impact**	**Examples**
NO IMPACT	No effect on food/water intake.	
MILD IMPACT	Short-term water or food restrictions that are within usual tolerance levels for the species.	An animal has a few hours without water, in shade conditions. Short-term deprivation of food.
MODERATE IMPACT	Water or food restrictions which cause serious short-term or moderate long-term effects on physiological state or body condition, but such effects remain within the capacity of the body to respond to nutritional variations and allow spontaneous recovery after restoration of a good quality diet.	An animal has a few hours without water, in hot, sunny conditions. Deprivation of food long enough to bring about mobilisation of body fat stores.
SEVERE IMPACT	Severe restrictions on food/water intake that lead to significant levels of debility.	An animal has many hours without water. Deprivation of food for many days resulting in severe loss of body weight.
EXTREME IMPACT	Extreme restrictions on food/water intake that would likely result in the animal dying from dehydration or starvation.	An animal has many days without water and /or food and dies from severe dehydration and/or starvation.

DOMAIN 2: ENVIRONMENTAL CHALLENGE		
Impact Category	**Description of impact**	**Examples**
NO IMPACT	Exposure to environmental challenge is not a feature of or consequence of the mode of action.	Exposure to ambient conditions that are within an animals’ thermoneutral range.
MILD IMPACT	Short term exposure to environmental conditions which are outside the normal range encountered by the animal but remain within their physiological adaptive capacity.	Exposure to levels of heat or cold which are outside the thermoneutral range, but which do not lead to debility in the long-term.

DOMAIN 2: ENVIRONMENTAL CHALLENGE		
Impact Category	**Description of impact**	**Examples**
MODERATE IMPACT	Marked short-term or moderate long-term environmental challenges that elicit body responses beyond the physiological adaptive capacity of the animal, but where the untoward effects are readily reversed by restoration of normal ambient conditions.	Short-term heat stress caused by exposure to high ambient temperatures combined with exercise.
SEVERE IMPACT	Severe environmental challenges that lead to serious physiological compromise or permanent dysfunction, injury or illness.	An animal is exposed to severe heat or cold which could possibly lead to failure of thermoregulation and collapse.
EXTREME IMPACT	Long-term exposure to extremes of heat or cold that bring about the death of the animal from hyper- or hypothermia.	Animals that are left in leg-hold traps, cage traps or yards in extremes of heat or cold and subsequently die from hyper or hypothermia.

DOMAIN 3: INJURY, DISEASE, FUNCTIONAL IMPAIRMENT		
Impact Category	**Description of impact**	**Examples**
NO IMPACT	Disease, injury or functional impairment is not a feature of or consequence of the mode of action.	
MILD IMPACT	Body responses remain within the homeostatic capacity of the animal to react with no or only minor debility or incapacity.	Minor injuries (e.g. minor skin laceration, oedematous swelling of foot and/or leg, mild mouth injuries). Minor functional impairment (e.g. mild vomiting/retching, diarrhoea).
MODERATE IMPACT	Disease/injury/functional impairment that results in moderately severe debility or incapacity but from which recovery would normally occur spontaneously.	Moderate injuries (e.g. damage to minor tendon or ligament, amputation of a digit, joint haemorrhage, single tooth fracture, major laceration of mouth or tongue, joint dislocation). Moderate or functional impairment (e.g. moderate vomiting/retching, diarrhoea, increased breathing, moderate haemorrhages, convulsions).
SEVERE IMPACT	Injury/disease/functional impairment that result in severe debility or incapacity and serious physiological compromise and would normally cause permanent disability. Includes injuries that are likely to reduce survival if the animal were to be released.	Severe injuries (e.g. deep and wide lacerations, severed tendons, broken foot and leg bones below elbow or stifle, joint dislocations, amputations). Severe or functional impairment (e.g. severe vomiting/retching, diarrhoea, abnormal breathing, severe haemorrhages, convulsions).

DOMAIN 3: INJURY, DISEASE, FUNCTIONAL IMPAIRMENT		
Impact Category	**Description of impact**	**Examples**
EXTREME IMPACT	Injury/disease/functional impairment that result in very severe debility or incapacity due to the effects of traumatic injury, infectious agent or toxin.	Extreme injuries (e.g. death caused by excessive blood loss or shock, spinal chord injury, severe internal bleeding, fractures of more than one limb, severe jaw fracture, fractures of limbs above elbow or stifle). Extreme or functional impairment (e.g. extreme persistent vomiting/retching, diarrhoea, laboured breathing, convulsions, blindness, immobility/ prostration, excessive and prolonged haemorrhaging).

DOMAIN 4: BEHAVIOURAL, INTERACTIVE RESTRICTION		
Impact Category	**Description of impact**	**Examples**
NO IMPACT	No interference with the behavioural needs of an animal (an animal's behavioural needs being those activities which when thwarted produce untoward physiological or psychological effects).	
MILD IMPACT	Mild interference with the behavioural needs of an animal.	Mild and short-term physical restraint resulting in minor behavioural or interactive restriction.
MODERATE IMPACT	Moderate interference with the behavioural needs of an animal resulting in negative physiological or psychological effects which are readily reversed after restoration of normal conditions.	Restraint that results in agitation from not being able to perform natural behaviour that the animal is highly motivated to perform e.g. feeding, moving, resting, grooming, mating, caring for young.
SEVERE IMPACT	Marked interference with the behavioural needs of an animal leading to physiological or psychological compromise that may cause long-term or permanent negative effects.	Severe abnormal self-directed behaviour e.g. chewing/biting of feet and limbs when restrained. Normal defensive and/or escape reactions to visibility of or presence of predators are prevented.
EXTREME IMPACT	Extreme interference with the behavioural needs of individuals or groups of animals leading to psychotic-like behaviour or to agonistic interactions that result in very severe injury or death.	Restraint that results in extreme abnormal self-directed behaviour, excessive aggression, stereotypy (e.g. severe fighting among incompatible social groups, unfamiliar individuals that are in close proximity).

DOMAIN 5: Anxiety, fear, pain , distress , thirst , hunger etc .		
When determining the impact in Domain 5, it is important to remember that this impact is usually a cumulative effect of the other four domains and is generally, but not always, equivalent to the most extreme potential impact.		
Impact Category	**Description of impact**	**Examples**
NO IMPACT	Anxiety, fear, pain, sickness, breathlessness, nausea, lethargy/ weakness, dizziness, greater than normal thirst and/or hunger or other negative affective experiences causing distress are not a feature or consequence of the method.	
MILD IMPACT	Mild anxiety, fear, pain, sickness, breathlessness, nausea, lethargy/weakness, dizziness, unsatisfied thirst and/or hunger or other negative affective experience causing distress.	Limited human contact with no physical handling.
MODERATE IMPACT	Moderate anxiety, fear, pain, sickness, breathlessness, nausea, lethargy/ weakness, dizziness, unsatisfied thirst and/or hunger or other negative affective experience causing distress.	Moderate level of human contact with minimum of physical handling.
SEVERE IMPACT	Severe anxiety, fear, pain, sickness, breathlessness, nausea, lethargy/ weakness, dizziness, unsatisfied thirst and/or hunger or other negative affective experience causing distress.	High level of human contact with a degree of physical handling.
EXTREME IMPACT	Extreme inescapable or unrelieved anxiety, fear, pain, sickness, breathlessness, nausea, lethargy/ weakness, dizziness, unsatisfied thirst and/or hunger or other negative affective experience causing distress which is judged to be at or beyond the limits of reasonable endurance and results in the death of the animal.	Excitement, fear and distress in struggling restrained animals that result in death from capture myopathy.

Impact scales for *3.2. Mode of death*.

Impact category	Description of impact	Examples
NO SUFFERING	No suffering before death. There is immediate death or immediate loss of consciousness lasting until death. Note that components of suffering include (but are not limited to) fear, anxiety, pain, distress, apprehension, sickness, fatigue, thirst, hunger. Aversion refers to the avoidance or attempted avoidance of unpleasant, noxious stimuli and distressing stimuli.	Direct destruction/concussion of brain tissue resulting in rapid unconsciousness e.g. accurate shooting in the head. Inhaled vapour with no irritant effect that induces unconsciousness without pain or discernible discomfort. Does not involve physical handling or restraint.
MILD SUFFERING	Loss of consciousness is not immediate and there is no or only minimal aversion and no or only mild suffering before death.	Inhaled vapour causing mild irritancy and mild pain and/or distress. Mild dyspnoea (breathlessness). Mild degree of sickness e.g. vomiting/retching, diarrhoea, lethargy/weakness etc. Does not involve physical handling or restraint.
MODERATE SUFFERING	Loss of consciousness is not immediate and there is moderate aversion and suffering before death.	Inhaled vapour causing moderate irritancy and moderate pain and/ or distress. Moderate degree of sickness e.g. vomiting/retching, diarrhoea, lethargy/weakness etc. Moderate dyspnoea. May involve physical handling and restraint e.g. to administer an injectable agent via intravenous (IV) or intraperitoneal (IP) route of entry; to apply cervical dislocation; to apply blunt trauma to the head.
SEVERE SUFFERING	Loss of consciousness is not immediate and there is severe suffering before death.	Inhaled vapour causing severe irritancy and severe pain and/or distress. Convulsions occurring during unconsciousness when animal recovers consciousness prior to death (i.e. muscle spasms with periods of relaxation as in clonic convulsions). Severance of major arteries resulting in rapid blood loss, hypovolaemia and shock. Severe degree of sickness e.g. vomiting/retching, diarrhoea, lethargy/weakness etc. Severe dyspnoea. May involve physical handling and restraint e.g. administration of an injectable agent to a non-sedated animal via a difficult-to-access route of entry (e.g. intracardiac, intrahepatic, intrarenal).

Impact category	Description of impact	Examples
EXTREME SUFFERING	Loss of consciousness is not immediate and there is extreme suffering before death.	Inhaled vapour causing extreme irritancy and extreme pain and/or distress. Partial or full paralysis whilst conscious. Convulsions whilst conscious (i.e. prolonged muscle spasm without periods of relaxation as in tonic convulsions). Extreme degree of sickness e.g. vomiting/retching, diarrhoea, lethargy/weakness etc. Extreme dyspnoea. Severe internal haemorrhages causing swelling within confined spaces. May involve physical handling and restraint.