Technical Note on Non-Lethal Measures to Eradicate or Manage vertebrates included on the list of IAS of Union Concern

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Executive Summary

A large number of non-lethal measures are in development, or currently in use to control invasive alien species (IAS). The Regulation (EU) No.1143/2014 *on the prevention and management of the introduction and spread of invasive alien species* (EU 2014), hereafter the IAS Regulation, provides for the eradication and management of invasive alien species of Union concern by lethal or non-lethal measures, but "*shall ensure that, when animals are targeted, they are spared any avoidable pain, distress or suffering*" as far as these do not compromise the effectiveness of the management measures (Article 19(3) of the IAS Regulation). Increasing concerns about the welfare impacts of lethal wildlife management measures, has increased interest in non-lethal control measures. This note provides an annotated inventory of non-lethal techniques useful to eradicate or manage invasive alien vertebrates of Union concern, with a focus on measures to prevent reproduction.

Veterinary fertility control techniques are only a sub-set of the wider methods available (e.g. egg oiling). These non-lethal measures to prevent reproduction include surgical sterilisation, post-fertilisation intervention, immunocontraceptive vaccines and non-vaccine contraceptives. Veterinary fertility control techniques such as surgical sterilisation procedures of free-living animals are economically costly due to the costs associated with capture, transport and the undertaking of surgery. The labour intensive post-fertilisation intervention, e.g. egg removal and reducing egg hatchability, or chemically induced abortion, may not be considered humane or non-lethal, because of the destruction of the embryos or foetuses. Although there are concerns about welfare and non-lethality, these methods are still used and seen as preferable to lethal control of young or adults. Immunocontraception measures, which are still in an experimental phase for many of the species, are becoming more frequently considered, particularly where fertility is inhibited by parenteral injection, with an increasing number of experimental projects of application of this technique in controlled environments. This measure potentially offers less risk of negative consequences of overdosing than would be associated with some hormonal or chemical contraceptive techniques. Non-vaccine contraceptives are widely used in zoo animals and livestock, such as the application of contraceptive implants or via oral delivery. However, the application of the implants to IAS of Union concern is limited due to the size of the animals, field conditions and cost associated with capture, transport and the undertaking of surgery. The oral contraceptive can be delivered in bait or feed, is less costly than methods requiring capture, treatment and release of individual animal, although non-target species could be affected.

An emerging technology for non-lethal control is the use of gene drives (using the CRISPR-Cas9 gene editing tool) to limit the reproduction of IAS, and has the potential to be cost effective for the eradication of IAS, for example of rats and mice on islands, mosquitoes from even large areas, or even fish. However, there are ethical concerns associated with its application on wild populations, and it is important to assess the potential risks before an application is considered.

The inventory also provides information on commonly used non-lethal trapping methods to capture IAS. However, it is important to note that often the aim of capture programmes using non-lethal traps is for euthanasia of the animals, or in the case of small populations the transfer into permanent captivity. A large number of trap designs, adapted to the target species' anatomy, behaviour and habitat, are available. The use of any trap requires professional handling that respects the full set of relevant legislation requirements and animal welfare concerns. Many commonly used traps, e.g. cage traps, pit-fall traps or nets, are not selective and may capture a high number of non-target species. Trapped animals may injure themselves or suffer from stress, which can result in post capture death unless appropriate procedures are followed (i.e. regular inspections of traps, including through remote systems etc.). IAS can also be captured by injecting immobilizing or tranquilising drugs, fired from a dart gun, etc. but also this need to be considered in relation to the full set of relevant legislation requirements, and is only recommended if highly trained personnel, pharmacological preparations and safety is guaranteed. More selective measures are available for some species such as are manual capturing, electrofishing and electro frogging, search with sniffer dogs, etc. These are both time and cost intensive measures, and are only suitable for managing small populations of IAS, or as complementary techniques.

Non-lethal measures to prevent specimens from escaping or entering the wild include the construction of physical and 'virtual' fences (using non suitable habitat, bubbles, light, bioacoustics or electric pulses). Fencing can be very effective but requires regular inspections and often intensive maintenance, in order to ensure the effectiveness and safety of the fence construction. Furthermore, repellents are commonly used for animal control and in the private sector, in agriculture, urban areas or airports to keep animals from entering certain areas. Movement control measures, e.g. wing clipping, pinioning or brailing, are commonly used for bird pet species, however there are increasing welfare concerns regarding these measures.

The application of any measure to control IAS needs to be considered with respect of animal welfare, cost-efficiency in the long term. The unprofessional application of a non-lethal measure can lead to serious injuries and death of the animal. Non-lethal methods offer alternatives to lethal control with reduced concerns for animal welfare and possibly increased public acceptability. Further, complementary measures are often essential for the effectiveness of control measures of IAS managed.

Structure of the document

This document follows a structure that includes three main sections:

(2) Non-lethal measures to prevent reproduction;

(3) Non-lethal measures for capturing;

(4) Non-lethal measures to prevent contained specimens from escaping or entering.

For each measure reported in this note the information on application, effectiveness, common use, related costs and welfare issues was reviewed and discussed. Known uses in relation to other species are described and an evaluation is made whether these could be used in relation to the species on the Union list. All references are listed for each section separately in the bibliography.

Contents

1. Introduction

Invasive alien species (IAS) are one of the major threats to biodiversity worldwide (United Nations, 1992). Control measures are consequently needed to mitigate the impacts of IAS. The control of IAS is often associated with the use of lethal measures, however the use of lethal measures raises concerns for animal welfare and may create negative public perception, affecting the acceptance of invasive species management (Fraser, 2006; Bremner and Park, 2007). Non-lethal methods are often more publically acceptable but can carry increased costs and reduced effectiveness compared to lethal control – in some cases to the extent that these methods are not practical to achieve eradication or management.

The Regulation (EU) No.1143/2014 *on the prevention and management of the introduction and spread of invasive alien species* (EU, 2014), hereafter the IAS Regulation, provides for the adoption of a list of invasive alien species of Union concern ('the Union list'). The IAS Regulation provides that eradication and management of these species may be pursued by lethal or non-lethal measures: The preamble (25) of the IAS Regulation stresses that "*any operator involved in the eradication, control or containment of invasive alien species should take the necessary measures to spare avoidable pain, distress and suffering of animals during the process, taking into account as far as possible the best practices in the field, for example the Guiding Principles on Animal Welfare developed by the World Organisation for Animal Health. Non-lethal methods should be considered and any action taken should minimise the impact on non-targeted species*".

Article 19 of the IAS Regulation states "*the* management *measures shall consist of lethal or non-lethal physical, chemical or biological actions aimed at the eradication, population control or containment of a population of an invasive alien species*", and while applying these measures Member States "*shall ensure that, when animals are targeted, they are spared any avoidable pain, distress or suffering*". Paragraph 3 of the same article states that "*When applying management measures and selecting methods to be used, Member States shall have due regard to human health and the environment, especially non-targeted species and their habitats, and shall ensure that, when animals are targeted, they are spared any avoidable pain, distress or suffering, without compromising the effectiveness of the management measures"*.

The present note is an inventory of non-lethal measures to eradicate or manage the 19 vertebrate species currently included on the list of IAS of Union concern. The listed non-lethal measures include: (a) preventing reproduction, (b) capturing of specimens, and (c) preventing contained specimens from escaping. The following parameters were analysed for each measure:

(1) Application information (e.g. is this measure currently applied? How commonly? In which countries? By which sector (zoos, hobbyists, pet shops, animal shelters, stray animals?)
(2) Effectiveness and common use. For which species has the measure been applied? What was the effectiveness? Information on whether the listed measure has been applied /could be applied for any of the 19 species and which is specifically mentioned.
(3) Information on related costs.
(4) Welfare issues. What is the public perception? How is the measure perceived from the animal welfare point of view?

The note summarises key publications, articles and reviews available in technical and scientific journals, and includes information from internet searches, online databases, grey literature and relevant book chapters, as well as personal communications from scientists, stakeholders, conservation practitioners and governmental bodies. A full bibliography is listed for each section separately.

2. Non-lethal measures to prevent reproduction

Increasing concerns about environmental and welfare impacts of lethal wildlife management techniques is placing increasing constraints on their use (e.g. Fagerstone et al., 2010). The growing reluctance against the use of such methods has led to an increased interest in fertility control as a wildlife management tool. Fertility control generally meets with relatively greater public acceptance than lethal control (e.g. Messmer et al., 1997; Stout, 1997; Barr et al., 2002), although the approach is not without welfare and ethical issues (Hampton et al., 2015). When lethal control is considered unacceptable or unfeasible, for instance for iconic species or in (peri)urban environments, fertility control might be the only option available for managing overabundant wildlife populations

In terms of the time taken to achieve the intended reduction in size of a target wildlife population, culling will always be more efficient than fertility control because reducing population recruitment cannot generate a more rapid population decline than the natural mortality rate allows (Bradford and Hobbs, 2008; McLeod and Saunders, 2014). This also means that compared to culling, the pressure of the animals left in the wild environment is expected to continue in the case that animal are sterilised. However, it is possible that fertility control could be used effectively to maintain population density at a lower level once it has been reduced by culling, particularly where the target species has a relatively low or moderate intrinsic rate of population increase (White, Lewis, and Harris, 1997; Merrill, Cooch, and Curtis, 2006).

Fertility control techniques have been in widespread use by veterinarians for many years, particularly in companion animals and zoological collections (Asa and Porton, 2005; Munson, 2006; Purswell and Kolster, 2006; Levy, 2011; Massei and Miller, 2013). However, only recently have techniques emerged with potential application to managing free-living wildlife populations (Massei and Cowan, 2014).

This section provides an inventory of these non-lethal immunocontraception methods and other birth control measures that are currently applied, or could be applied, to eradicate or manage the vertebrate species included on the list of invasive alien species of Union concern.

The available information is provided here on each assessed measure for prevention of reproduction with respect to the application, its effectiveness and common use, related costs and public perception. Assessment of the current commercial availability of a specific fertility control agent for potential application to wildlife is complex. In some cases, they may be regulated as veterinary medicines but in others as pesticides, either under Plant Protection Products or Biocides Directives or both. However, even if a particular substance/medicine does not currently have an EU approval/authorisation it might still be able to be imported and used in some circumstances, for instance, via a Special Treatment Certificate allowing import of a non-approved veterinary medicine for use inside the EU (this route has been used for fertility control agents but it is not possible to provide specific examples due to commercial sensitivities and associated confidentiality).

Furthermore, even if a particular active substance/ingredient has some form of approval in the EU for a particular application e.g. in domestic or companion animals, this does not necessarily mean that it can be used in the context of wildlife fertility control applications without further regulatory evaluation.

In conclusion, simply because a particular substance/medicine has an approval/authorisation for use in the EU does not necessarily mean that the commercial owner of the product will be willing to make it available for wildlife applications.

2.1. Surgical sterilisation

Surgical castration of male domestic animals has been practised for thousands of years while ovariectomy is quoted in Aristotle's writings as early as 384–322 BC (Bertschinger and Caldwell, 2016). Many techniques for surgically sterilizing companion animals have been described, including midline ovariohysterectomy, lateral flank ovariohysterectomy, castration, early age gonadectomy, ovariectomy, laparoscopic ovariohysterectomy and ovariectomy, and vasectomy (Howe, 2006). These methods are also commonly used in zoological collections (Patton, Jöchle, and Penfold, 2007). A major advantage of surgical sterilisation is that permanent infertility is the expectation. In contrast, the efficacy of many contraceptive techniques is less than 100% and the induced infertility may not be permanent. In some contexts, such as zoological collections, recovery of fertility may be desirable, for instance to maintain genetic diversity. However, in wildlife applications permanent infertility may be desirable although not always essential.

The main application of the approach in free-living animals has been semi-owned but free-roaming populations of domestic cats (*Felis catus*) and domestic dogs (*Canis familiaris*) (Toukhsati et al., 2012). Although surgical sterilization generally has greater public acceptance than culling in this context, it is costly because of the use of veterinary staff, drugs and facilities, with potential welfare issues associated with the use of anaesthetics (Levy et al., 2008; Massei, Miller, and Killian, 2010). Furthermore, this is still an invasive technique, and for example in the case of the eradication of the American grey squirrel from Perugia, central Italy, it still raised concern among the animal welfare organisations that opposed also the application of this option. Nevertheless, because the animals are semi-owned they can be relatively easily caught/trapped, neutered and released/returned (C/T-N-R), as has occurred in the context of campaigns aimed at reducing the reservoir of rabies that free-roaming domestic dog (*C. familiaris*) populations (Carroll et al., 2011; Massei and Miller, 2013). Tubal ligation of females has been used in experimental studies of free-living populations of European rabbit (*Oryctolagus cuniculus*) (Twigg *et al*., 2000; Williams *et al*., 2007). The surgical sterilisation approach has also been explored in the context of free-living wildlife to reduce coyote (*Canis latrans*) predation on sheep (Bromley and Gese, 2001). Furthermore, male vasectomy and distal oviductal transection of females have been used successfully to control fecundity in an invasive population of koala (*Phascolarctos cinereus*) (Tribe et al., 2014). The approach is economically costly. For instance, the estimated cost of surgically sterilising a single free-living white-tailed deer (*Odocoileus virginianus*) was estimated in 2012 to be approximately US$1,000 to cover the costs of capture, veterinary expertise and medicines (Boulanger et al., 2012).

Hence, the economic and costs associated with capture, transport and undertaking veterinary surgery are likely to be prohibitive for most wildlife applications, apart from exceptional circumstances. An example is where small numbers of individuals have become locally iconic, like in Italy, where the eradication of a small population of grey squirrel (*Sciurus carolinensis*) from an urban park of Genova was completed using surgical sterilisation at a cost of around 100 euros per animal (only including the cost of the operation, not of the trapping and transport of animals). In an ongoing eradication of grey squirrels (*S. carolinensis*) from Perugia surgical sterilisation is being used for 10% of the animals (La Morgia et al., 2016). Generally, it is unlikely that any other current contexts involving the IAS of Union concern reflect such exceptional circumstances. However, a large number of invasive *Trachemys scripta* were captured and relocated at the Pistoia Zoo (Italy), where they were sterilised to prevent their reproduction (LIFE09 12NAT/IT/0000395-Lifeemys, 2017). Furthermore, the applicability of this method is clearly context dependant: for example it is unlikely that the capture, surgical sterilisation and release of individuals would be feasible for large-scale population management of the three squirrel IAS of EU concern (eastern fox squirrel (*Sciurus niger*), grey squirrel (*S. carolinensis*), and Pallas's squirrel (*Callosciurus erythraeus*)), given that an estimated 80% or more of the female target populations would need to be rendered infertile to realise substantial reductions in population size (Cowan, Massei, and Mellows, 2006; Cowan and Massei, 2008; Krause, Kelt, Van Vuren, and Gionfriddo, 2014). This consideration is also likely to apply to the Siberian chipmunk (*Tamias sibiricus*), as the other sciurid on the IAS list, and the muskrat (*Ondatra zibethicus*). The population biology and trappability of the other six mammalian 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 (e.g. Hoffmann and Gottschang, 1977). However, this potential would need to be explored further through an initial species-specific feasibility study. Furthermore, even if such a study indicated that the approach could potentially be feasible, it is likely that other methods, such as parenteral injection with appropriate fertility control agents as described below, would be more cost-effective options as they would not incur the veterinary costs associated with surgical sterilisation.

2.2. Post-fertilisation intervention

Methods are available for reducing productivity post-fertilisation for both birds and mammals, although some may not consider destruction of embryos or foetuses to be a non-lethal approach.

2.2.1. Egg removal and reducing egg hatchability

Provided bird nests can be located and are accessible, then eggs can be removed or destroyed to reduce productivity. In some instances, removed eggs can be replaced by dummy eggs to prevent replacement with a new clutch (Baker et al., 1993). Alternatively, eggs can be addled in the nest, by methods such as shaking, pricking or oiling which can effectively reduce their hatchability (Pochop et al., 1998). In the USA, egg oiling with corn oil is permitted by the Environmental Protection Agency (EPA) under the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) exemption for natural products, and is used to reduce reproduction in several avian species. 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.

However, this method is labour intensive and hence costly; thus is probably useful only in small areas (Fagerstone et al., 2010). However, there are several examples where the method has been used successfully to manage local populations of ring-billed gull (*Larus delawarensis*) (Engeman et al., 2012), double-crested cormorant (*Phalacrocorax auritus*) (Ridgway, Middel, and Pollard, 2012), Canada goose (*Branta canadensis*) (Baker et al., 1993) and mute swan (*Cygnus olor*) (Hindman, Harvey, and Conley, 2014), although in the latter case egg oiling did not, on its own, reduce the target adult population. Both dummy eggs and egg oiling have been used on monk parakeet (*Myiopsitta monachus*) in the UK. With respect to welfare, the Humane Society of the United States recommends that egg oiling should be undertaken during early incubation and considers that once an air sac is formed, embryonic development is typically advanced enough such that the approach may no longer be considered humane. In Canada geese (*B. canadensis*), the air sac is formed at around 14 days after laying by which time the eggs begin to float when placed in water; and this can be used as a diagnostic tool with respect to resolving the welfare concern.

This approach may have potential to contribute to the management of some of the avian species on the list of IAS of Union concern. However, the nests must be able to be detected and accessed. Those of the Ruddy duck (*Oxyura jamaicensis*) can usually be located and accessed in their native habitat (Brua, 1999) but their placement on floating vegetation mats may make access difficult; hence, although this approach could potentially be used with this species, detection and accessing nests is likely to be time consuming, and hence costly as was confirmed during feasibility studies in the UK (Henderson and Robertson, 2007). Egyptian goose (*Alopochen aegyptiacus*) nests are sometimes built on the ground including in dense vegetation. However, the species does not generally nest in colonies and regularly uses nest sites in trees, especially in large holes, thus limiting their accessibility. House crows (*Corvus splendens*) nest off the ground in trees or human infrastructure such as pylons. Accessing nests would thus require costly specialist skills and equipment, although the communal nesting habit of this species would make this approach less expensive than for a solitary nesting species. The sacred ibis (*Threskiornis aethiopicus*) nests colonially in trees or in reed beds in wetland areas. These habits will restrict accessibility of nests.

Overall, constraints on the detection and/or accessibility of nests are going to limit the feasibility of this technique for the four avian IAS of Union concern at anything other than limited local scales.

2.2.2. Induced abortion in mammals

Pregnancies in domestic and companion mammals that are considered undesirable or unwanted can be terminated by chemically induced abortion. The approach has also been used in certain wildlife contexts, particularly to terminate unwanted pregnancies in charismatic species. Luteolytic prostaglandins such as tromethamine and cloprostenol have been used in large carnivores, including the African lion (*Panthera leo*) (Bertschinger et al., 2008; Bertschinger and Caldwell, 2016). Three injected doses need to be administered on consecutive or alternate days plus dart administered follow up treatments, which would be unfeasible for most wildlife applications. These drugs can cause side-effects in domestic dogs (*C. familiaris*), thus for African wild dogs (*Lycaon pictus*) the progesterone receptor antagonist aglepristone has been used (Bertschinger and Caldwell, 2016) as a single dose administered by stick syringe while the trapped animal is restrained in a crush. Again, follow-up veterinary treatments may be necessary, which would be unfeasible in most wildlife applications. Furthermore, some may consider this technique not to be non-lethal.

It thus seems unlikely that there are any contexts in which this approach might be suited to the management of any of the IAS of Union concern.

2.3. Immunocontraceptive vaccines

A relatively recent approach to contraception in animals is immunocontraception. This concept targets peptides essential for reproductive processes by incorporating them as antigens in vaccines.

Such a vaccine will stimulate the production of antibodies that compromise the activity of the same endogenous peptides, and thereby inhibit fertility (Miller and Killian, 2002). A key potential advantage of the immunocontraceptive approach, compared to some non-vaccine contraceptives, is the limited secondary hazard posed to predators or scavengers from consuming the bodies of treated animals because any antibody in such bodies would be expected to be destroyed in the gastro-intestinal tracts of the consumers. The immuncontraceptive approach also potentially offers less risk of negative consequences of overdosing that would be associated with some hormonal or chemical contraceptive techniques. For instance, repeat treatments of domestic cats (*Felis catus*) with an injectable immunocontraceptive are not associated with observable negative consequences (Vansandt et al., 2017). The most frequently evaluated immunocontraceptive targets have been mammalian gonadotropin-releasing hormone (GnRH) and mammalian zona pellucida (ZP) proteins.

No substantive research has so far been undertaken towards the development of avian immunocontraceptives. Hence, the approach is not currently applicable to any of the avian IAS of Union concern. In addition to the type of immunological target, the major influence on potential feasibility of the approach for mammals is the method of delivery. An innovative approach to delivery was virally vectored immunocontraception (VVIC) (McLeod et al. 2007). This used genetically modified self-sustaining infectious vectors which potentially offered large-scale application and was the subject of considerable research in Australia and New Zealand. However, several concerns were raised about this approach, including its irreversibility, difficulty of controlling vectors once released and possible genetic mutation of the vectors, hence, this has not progressed beyond studies in captivity (Williams, 2007). The delivery approach used for mammalian immunocontraceptives has thus primarily been parenteral injection, although there is potential for the development of vaccines suitable for oral delivery.

2.3.1. Parenteral delivery (injection and/or darting)

Practical applications have recently begun to emerge that use parenteral delivery of immunocontraceptive vaccines to manage free-living mammal populations. However, these have targeted localised populations that are generally closed, as reviewed by Massei and Cowan (2014). As yet, there are no examples of this technique being used on its own to manage any large-scale, open populations. Nevertheless, the approach has potential to be used synergistically with other management tools.

2.3.1.1. Injectable vaccines targeting mammalian gonadotropin-releasing hormone (GnRH)

Vaccines targeting mammalian GnRH stimulate the immune system to produce antibodies that compromise endogenous GnRH function, and hence suppress downstream endocrine mechanisms controlling reproductive function in both females and males. The GnRH molecule is highly conserved amongst mammals, thus vaccines targeting it are potentially effective in all mammalian species. GnRH targeted immunocontraceptives were originally developed as veterinary medicines to suppress reproductive function in cattle (D'Occhio, 1993) and as an alternative to surgical castration to manage boar taint in pork meat (Dunshea et al., 2001; Zamaratskaia et al., 2008). These vaccines required an initial prime dose, delivered by injection, followed by boosters at regular intervals to maintain sufficient levels of antibody to compromise fertility. These multi-dose GnRH-based immunocontraceptive vaccines have been successfully used for many years in domestic animals without any substantive side-effects being reported (Naz et al., 2005; McLaughlin and Aitken, 2011).

However, these multi-dose vaccines are not appropriate for most wildlife applications because reliable recapture of individuals to administer multiple doses is generally unfeasible.

A single-dose GnRH immunocontraceptive vaccine, known as GonaCon has been developed, consisting of synthetic GnRH molecules coupled to a mollusc protein and formulated in an emulsion with a novel adjuvant (Adjuvac) (Miller et al., 2008). There have been increasing examples of its use in wildlife applications including in white-tailed deer (*O. virginianus*) (Gionfriddo et al., 2009; 2011a), elk (*Cervus elaphus*) (Powers et al., 2011), feral horse (*Equus caballus*) (Killian et al., 2008*),* bison (*Bison bison*) (Miller, Rhyan, and Drew, 2004), feral cattle (Bos taurus/Bos indicus) (Massei et al., 2015), wild boar (Massei et al., 2008, 2012), feral domestic cat (*F. catus*) (Levy, Friary, Miller, Tucker, and Fagerstone, 2011; Benka, and Levy, 2015), black-tailed prairie dog (*Cynomys ludovicianus*) (Yoder and Miller, 2010), California ground squirrel (*Spermophilus beecheyi*) (Nash et al., 2004), grey squirrel (*S. carolinensis*) (Pai et al., 2011), eastern fox squirrel (*S. niger*), (Krause et al., 2014; Krause et al., 2015) and the product has now been registered by the EPA in the USA for use with white-tailed deer (*O. virginianus*), feral horse (*E. caballus*) and feral burro/donkey (*Equus asinus*). It has also been predicted that the availability of this single-dose product, used alongside anti-rabies vaccination, could substantially improve the prospects of eradication of this disease in free-roaming domestic dog (*C. familiaris*) populations, given the potential substantially lower cost compared to surgical sterilisation (Carroll et al., 2011). The longevity of induced infertility varies between species and studies, but is typically reported to last several years after a single-vaccination. Nevertheless, GnRH antibody titres typically decrease with time and fertility may recover (Miller et al., 2008; Massei et al., 2012).

As GonaCon inhibits both testicular and ovarian function, typically neither males nor females exhibit reproductive behaviours post-vaccination with individuals essentially being maintained in their natural state outside of their breeding season. In some species, granulomas or sterile abscesses form at the GonaCon injection site that are sometimes palpable but generally appear to be non-painful. For instance, feral cats (*Felis catus*) treated with GonaCon had palpable, but apparently non-painful, injection-site granulomas (Levy et al., 2011); granulomatous nodules and sterile abscesses at injection sites and in lymph nodes have also been reported in white-tailed deer (*O. virginianus*) (Gionfriddo et al., 2011b), although no evidence of limping or impaired mobility was observed in these animals. Furthermore, Gionfriddo et al. (2009) suggested that "These responses were natural and essential expressions of the strong immune response that is required to induce temporary infertility". However, in some species the injection reactions have been considered to represent welfare concerns. For instance, domestic dogs (*C. familiaris*) vaccinated with GonaCon showed sterile abscesses and draining tracts at the injection site relatively soon after injection (Griffin et al., 2004). Hence a modified form of vaccine has been developed to reduce the reactions in this species (Vargas-Pino et al., 2013).

In a study of the eastern fox squirrel (*S. niger*), open abscesses exuding pus were observed in some GonaCon treated individuals and treated squirrels were more likely than control squirrels to be seen limping or walking stiffly (Krause et al., 2014), although no significant differences in time budgets[1] or social behaviour between vaccinated and untreated individuals were reported (Krause et al., 2015). Injection site abscesses were also reported in a study of the grey squirrel (*S. carolinensis*) (Pai, 2009; Pai et al., 2011). However, it is unclear whether these were anything other than granulomas typical of those expected from an adjuvant vaccine, and hence without any substantive welfare concern. A potential negative welfare effect has been observed in male white-tailed deer (*O. virginianus*) deer which showed abnormal antler development after vaccination with GonaCon (Fagerstone et al., 2008). Treatment of immature male Tammar wallabies (*Macropus eugenii*) with GonaCon

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[1] time budget: sequence and duration of activities engaged in by an individual over a specified period, most typically the 24-hour day

permanently constrains the development of secondary sexual characteristics (Snape, Hinds, & Miller, 2011). Such sex-specific effects could be avoided by only treating females, which are generally regarded as being the more demographically important focus for fertility control.

In general, the disruption of female reproductive hormonal function with GonaCon does not appear to have major effects on female social behaviour in several species, although more research is needed in this area (see review in Massei and Cowan, 2014). Similarly, existing pregnancies are known to have been unaffected by GonaCon vaccination in some species but further species-specific studies are warranted (Miller, Rhyan, and Drew, 2004; Quy et al., 2014).

The economic costs of reducing population densities through use of parenterally delivered GnRH vaccine, such as GonaCon, in a capture, inject and release programme, will inevitably be relatively high compared to other techniques; particularly when this approach will inevitably take longer to achieve a given reduction in target population size than capture and removal. Costs would be reduced for some species, if the vaccine could be delivered remotely by pneumatic syringe dart and this has been shown to be feasible (Evans et al., 2015); although the effectiveness of the method used in this study appeared to reduce vaccine efficacy. The methodology thus needs to be refined before remote delivery of a GnRH vaccine becomes a usable technique.

With respect to the mammalian IAS of Union concern the injection site abscesses of welfare concern in the astern fox squirrel (*S. niger*) suggests that GonaCon is contraindicated for this species. Whilst such severe reactions to GonaCon have not been observed in other *sciurids*, including the grey squirrel (*S. carolinensis*), further species-specific studies would be required to evaluate such reactions before the approach could be recommended for grey squirrel (*S. carolinensis*) or for Pallas's squirrel (*C. erythraeus*). Furthermore, it is unlikely that the capture, vaccination and release of individuals would be feasible for population management of these species, given that an estimated 80% or more of the female target populations would need to be rendered infertile to realise substantial reductions in population size (Cowan, Massei, and Mellows, 2006; Cowan and Massei, 2008; Krause, Kelt, Van Vuren, and Gionfriddo, 2014). This consideration is also likely to apply to the Siberian chipmunk (*T. sibiricus*), as the other sciurid on the IAS list, and also the muskrat (*O. zibethicus*). The population biology and trappability of the other six mammalian IAS of Union concern may allow the technique to contribute to their management, at least in some local scale contexts, but each would require an initial species-specific feasibility study to explore this potential. GonaCon has approvals in the USA for use in wildlife. It may be possible under EU veterinary medicine regulatory processes to obtain approval to import and use GonaCon in free-living animals in Member States.

2.3.1.2. Injectable vaccines targeting mammalian Zona Pellucida (ZP)

The ZP is the layer of glycoproteins that surrounds an ovulated mammalian egg that allows species-specific sperm recognition and binding. Generating antibodies to ZP proteins can thus prevent fertilisation of eggs. The approach will only reduce female fertility. There are four major ZP glycoproteins, known as ZP1, ZP2, ZP3 and ZP4, each with different functions in the oocyte-sperm binding process and with varying degrees of homology among mammalian species (e.g. Kitchener et al., 2009; Gupta and Bhandari, 2011). These differences are partly responsible for the variable results obtained when using a particular ZP vaccine on different species, and make ZP-based vaccines somewhat more target specific than GnRH vaccines (Kitchener et al., 2009; Gupta et al., 2011; Levy 2011).

Porcine ZP (PZP) immunocontraceptive vaccines, derived from ZP isolated from pig ovaries, have been shown to reduce fertility in several ungulate species, including white-tailed deer (*O.*

virginianus) (Rutberg, Naugle, Thiele, and Liu, 2004), feral horse (*E. caballus*) (Turner and Kirkpatrick, 2001), and African elephant (*Loxodonta africana*) (Delsink et al., 2007), but less so in other species such as domestic cat (*F. catus*) (Eade, Roberston, and James, 2009) and rodents (McLaughlin and Aitken, 2011).

Recombinant PZP3 and PZP4 vaccines, delivered in three doses, caused infertility in up to 89% mice, depending on the formulation type (Gupta et al., 2013). Vaccines derived from marsupial ZP have been shown to reduce fertility the eastern grey kangaroo (*Macropus giganteus*) (Kitchener et al., 2009a), koala (*P. cinereus*) (Kitchener et al., 2009b) and brushtail possum (*Trichosurus vulpecula*), (Duckworth et al., 2007). The initial ZP vaccines were delivered as a primer shot, followed by a booster which placed major constraints on field applications with wildlife. Initial vaccine formulations used Freund's complete adjuvant (FCA), which raised safety and welfare issues (Munson et al., 2005), but this has subsequently been replaced with Aduvac (Kirkpatrick, Lyda, and Frank, 2011) or modified FCA (Lyda, Hall, and Kirkpatrick, 2005). Injectable formulations of PZP vaccines, such as the proprietary liposome-containing product SpayVac, with controlled-release properties, have been developed that generate multi-year responses following a single vaccination in grey seal (*Halichoerus grypus*) (Brown *et al*. 1997), feral horse (*E. caballus*) and African elephant (*Loxodonta africana*) (Turner *et al*. 2008), and white-tailed deer (*O. virginanus*) (Rutberg, Naugle, Turner, Fraker, and Flanagan, 2013), although antibody titres do typically decrease with time and fertility may recover. In 2012, a PZP-based injectable vaccine called ZonaStat-H was registered by the Environment Protection Agency (EPA) in the US for the management of feral horse (*E. caballus*) and feral burro/donkey (*E. asinus*) populations.

A possible unwanted outcome of ZP vaccination in females of polyoestrus species is repeated oestrous cycles which could lead to extended breeding seasons and increased movements with, for instance, potential consequences such as increased collisions with vehicles (Curtis et al., 2001; Curtis et al., 2007; Kirkpatrick et al., 2009, 2011; Nunez et al., 2009; Nunez, Adelman, and Rubenstein, 2011). However, several other studies have not reported any effects of ZP vaccines on time budgets, social behaviour or body condition (Miller et al., 2001; Hernandez et al., 2006; Ransom, Cade, and Hobbs, 2010). ZP vaccines have also been known to induce species-specific ovarian pathology, although their incidence is reduced if more highly purified ZP proteins are used (Gupta et al., 2013). PZP vaccines can be administered to pregnant or lactating females without negative effects on embryos or young (Turner, Liu, and Kirkpatrick, 1996; Kirkpatrick and Turner, 2001; Perdok, De Boer, and Stout, (2007; Delsink and Kirkpatrick, 2012). Abscesses at ZP vaccine Injection-sites are rare (~1% in various species) while granulomas, consisting of fluid filled thickened tissue, common (Kirkpatrick et al., 2009; Gray and Cameron, 2010). The survival of female feral horse (*E. caballus*) vaccinated with a ZP vaccine was increased (Turner and Kirkpatrick, 2002; Kirkpatrick and Turner, 2007). This may have reflected infertile animals not accruing the physiological costs of reproduction. This is thus likely to be a generic effect of wildlife fertility control. Indeed, enhanced survival has been reported for tubally ligated European rabbit (*Oryctolagus cuniculus*) (Twigg *et al*., 2000; Williams *et al*., 2007) and GonaCon vaccination improved the body condition of female white-tailed deer (*O. virginianus*) (Gionfriddo *et al*., 2011*b*). However, such effects could potentially lead to increased consequential welfare concerns associated with aging.

As with GnRH injectable immunocontraceptive vaccines, the economic costs of reducing population densities through use of parenterally delivered ZP vaccine in a capture, inject and release programme, will inevitably be relatively high compared to other techniques such as capture and removal. PZP vaccines have been successfully delivered remotely by pneumatic dart, particularly for delivery of booster doses, and this can reduce costs compared to capture and injection by hand.

Nevertheless, Rutberg (2005) estimated that the cost to render infertile a medium to large sized individual mammal varied between US$25 and US$500; while, in 2013, he estimated that each deer treatment with a PZP vaccine cost approximately US$500 for initial capture and vaccination, followed by US$100-US$200 per deer for remote boosting (Rutberg, 2013). In 2005 Delsink et al. (2007) calculated that the average cost of managing elephants through aerial vaccination with a PZP vaccine, cost US$98–110 per animal, inclusive of darts, vaccine, helicopter and veterinary assistance. However, the effort, and hence cost required, to manage a wildlife population with this method will be influenced by the target species population biology, population density, trappability and/or approachability, access to the relevant landscape and efficacy of the contraceptive technique (e.g. Rudolph, Porter, and Underwood, 2000)

With respect to the mammalian species on the list of IAS of Union concern, it is unlikely that capture, vaccinate and release would be feasible for population management of Eastern fox squirrel (*S. niger*), or grey squirrel (*S. carolinensis*), given that it has been estimated that at least 80% of the female target populations would need to be rendered infertile to realise a substantial reduction in population size (Cowan, Massei, and Mellows, 2006; Cowan and Massei, 2008; Krause et al., 2014). This consideration is also likely to apply to Pallas's squirrel (*Callosciurus erythraeus*), Siberian chipmunk (*Tamias sibiricus*) and muskrat (*Ondatra zibethicus*). The population biology and trappability of the other six mammalian IAS of Union concern may allow the technique to contribute to their management, at least in some local scale contexts; but each would require an initial species-specific feasibility study to explore this potential. Such feasibility assessments would need to take into account the potential for extended breeding seasons and increased movements arising from the use of ZP vaccines in polyoestrus species. Injectable formulations contacting ZP vaccines have approvals in the USA for use in wildlife. It may be possible under EU veterinary medicine regulatory processes to obtain approval to import and use injectable ZP vaccines in free-living animals in Member States.

2.3.2. Oral delivery

The injectable GnRH and PZP vaccines offer promise where capture, hand injection and release or injection by dart delivery are viable. However, the injectable approach is only feasible for this relatively limited range of practical applications. Hence, the availability of an immunocontraceptive vaccine that could be delivered orally via baits would potentially greatly increase the scope for the application of immunocontraception as a wildlife management tool.

Development of commercial oral vaccines is challenging, as demonstrated by the fact that although many vaccines currently exist, very few can be orally administered e.g. cholera, polio, rabies and BCG. Typically, live particulate forms appear to be the most successful in producing an immune response. Oral delivery requires 10-100 fold more antigen in the best of conditions to produce an adequate immune response compared to parenteral delivery. Furthermore, successful oral vaccines have been disease related where there is a potential boost of antibody, resulting in extended protection, in response to disease challenge. An effective oral immunocontraceptive vaccine will also need to be protected from the acid and enzymes of the alimentary canal to reach specialized epithelial cells either in the buccal cavity or the intestine.

Against this challenging background an effective immunocontraceptive vaccine for oral delivery has yet to be developed. However, intra-nasal delivery of four doses of mouse ZP3 has been shown to significantly reduce reproductive output in laboratory mice (*Mus domesticus*) (Ma, Li, and Zhang, 2012; Kadir, Ma, Li, and Zhang, 2013). Proof of concept has also been demonstrated for intraocular/intranasal delivery of a vaccine containing bacterial “ghost” cells expressing possum ZP2-

C peptide leading to reduced fertility in captive brushtail possums (*T. vulpecula*) (Duckworth et al., 2010). Several techniques for promoting immune responses to GnRH or ZP targeted vaccines have been explored, such as receptor-specific adjuvants (e.g. Sharma and Hinds, 2012). Once such approach has recently demonstrated proof of concept of reduced fertility in the laboratory Norway rat (*R. norvegicus*) following oral delivery, by lavage, of a formulation containing *Mycobacterium avium* cell wall fragments conjugated to a putative GnRH specific immunogen in the form of a novel GnRH recombinant construct (Defra, 2017). This represents the first evidence of reduced fertility arising from oral dosing with a GnRH targeted immunocontraceptive vaccine. However, there are many further obstacles to overcome before such proof of concept leads to a viable wildlife management tool.

One such obstacle arises from the fact that neither the GnRH nor the ZP vaccine approaches offer species-specificity. Specificity would thus need to be derived from the delivery system. Potential species-specific delivery systems are available for some species including the Wild boar (Massei, Coats, Quy, Storer, and Cowan, 2010) and the Grey squirrel (Pepper and Stocker, 1993) which is an IAS of Union concern. Nevertheless, even a species-specific delivery system is unlikely to be sex-specific so potential negative consequences of vaccinating males as well as females might need to be evaluated and addressed.

An alternative approach to the specificity issue would be to develop a species-specific vaccine. The potential of targeting sperm-surface proteins, which are involved in maintaining species isolation at fertilisation and are thus species-specific by definition, has been explored in the European rabbit (*Oryctolagus cuniculus*) and grey squirrel (*S. carolinensis*) (Moore, Jenkins, and Wong, 1997). However, although oral delivery by lavage of the immunocontraceptive vaccine formulations developed by this research, successfully generated antibodies to sperm-surface proteins in treated females, these were probably insufficient to compromise fertilisation. An investigation of vaccines using a recombinant mouse sperm protein were not found to be useful in terms of generating infertility in either males or females (Hardy et al., 2004). No further work appears to have been undertaken subsequently on the sperm-surface protein approach.

Currently there are no orally deliverable immunocontraceptive vaccines available and thus there are, as yet, no tools to apply this approach to the management of any of the IAS of Union concern.

2.4. Non-vaccine contraceptives

2.4.1. Contraceptive implants

Physical implants, otherwise known as intrauterine devices (IUDs) have been used for many years. Patton et al. (2007) describes their potential use in female camels in the Near East, Arabia, Africa, and Asia which were kept from cycling and/or conceiving by introducing pebbles into the uterus via the cervical canal during oestrus. The pebbles effectively functioned as an intrauterine foreign body preventing conception. The main modern veterinary use of copper IUDs is in domestic cattle (Patton et al., 2007). One wildlife application of IUDs has been in feral horse (*E. caballus*) (Daels and Hughes, 1995; Killian et al., 2008).

Surgical implants containing synthetic hormones are widely used in zoo animals and livestock to impair folliculogenesis, ovulation and egg implantation in females and spermatogenesis in males (Asa and Porton, 2005). Those tested in wildlife include norgestomet, melengestrol acetate, levonorgestrel and quinestrol.

Norgestomet implants inhibited reproduction in female white-tailed deer (*O. virginianus*) and black-tailed deer (*Odocoileus hemionus*) for at least one year (Jacobsen, Jessup, and Kesler, 1995; DeNicola, Kesler, and Swihart, (1997). Melengestrol acetate (MGA) implants, with an estimated effectiveness of two years, have been shown to reduce fertility in a broad range of species such as White-tailed deer (*O. virginianus*) (Plotka and Seal, 1989; Addax (*Addax nasomaculatus*) and Arabian oryx (*Oryx leucoryx*) Hall-Woods et al., 2007), and golden lion tamarin (*Leontopithecus rosalia*) (Wood, Ballou, and Houle, (2001). However, MGA causes uterine pathology in captive coati (*Nasua nasua*) (Chittick et al., 2001), felids and canids (Munson 2006; Moresco, Munson, and Gardner, 2009) and a higher incidence of stillbirth and infant mortality in golden lion tamarins (*L. rosalia*) (Wood et al., 2001). A single administration of an implant containing levonorgestrel has been found to inhibit fertility in some wildlife species for several years, without apparent adverse side effects; including, in the Tammar wallaby (*M. eugenii*), (Nave, Coulson, Short, Poiani, Shaw, and Renfree, 2001), eastern grey kangaroo (*M. giganteus*) (Coulson, Nave, Shaw, and Renfree, 2008), koala (*P. cinereus*) (Middleton, Walters, Menkhorst, and Wright, 2003) and cotton-top tamarins (*Saguinus oedipus*) (Wheaton et al., 2011). Levonorgestrel and quinestrol have also been used to reduce fertility in rodents such as plateau pikas (*Ochotona curzioniae*) (Liu et al., 2012) and Mongolian gerbils (*Meriones unguiculatus*) (Fu et al., 2013). Most implants require capture and handling of individuals for surgical application rendering the technique relatively costly, especially if anaesthesia is required. However, at least one formulation of MGA can be delivered remotely as a ballistic implant (Jacobsen, Jessup, and Kesler, 1995). This biobullet approach would reduce the unit cost of delivery but potentially opens-up the issue of needing to reliably identify, at a distance, previously treated individuals to prevent possible overdosing. Furthermore, the use of hormonal methods on free-ranging wildlife raises ongoing concerns with respect to potential negative welfare effects arising from long-term exposure and possible transfer of biologically active hormones via food chains (Nettles 1997; DeNicola et al., 2000; Asa and Porton, 2005).

The use of surgical implants containing GnRH agonists is another potential approach to inducing long-term contraception. GnRH agonists inhibit oestrus cycling in females and spermatogenesis in males by binding to the gonadotrophs in the pituitary and thus blocking GnRH receptors (Patton et al., 2007). GnRH agonists cause acute, but transient, increases in luteinizing hormone (LH) release, leading to temporarily enhanced endocrine activity and fertility known as “flare”, followed by a decline associated with chronic agonist exposure (Gong et al., 1995; Maclellan et al., 1997; Gobello, 2007). Sustained-release subcutaneous “depo” implants of GnRH agonists, such as deslorelin, have been used to inhibit reproduction for one to two years in several species, including cattle (D’Occhio et al., 2002), Tammar wallaby (*M. eugenii*) (Herbert et al., 2005), and brushtail possum (*T. vulpecula*) (Eymann et al., 2007; Lohr et al., 2009). Deslorelin has also been shown to reduce fertility in domestic cat (*Felis catus*) (Munson et al., 2000), African wild dog (*L. pictus*) (Bertschinger et al., 2000), and red panda (*Ailurus fulgens*), (Koeppel, Barrows, and Visser, 2014). Another GnRH agonist, leuprolide, has been found effective in suppressing reproduction for one breeding season in elk (Wapiti) (*C. elaphus*) (Baker et al., 2001; Conner et al., 2007) and mule deer (*Odocoileus hemionus*; Baker *et al*. 2004). The effectiveness of GnRH agonist use varies with agonist type, slow-release system, dose rate and the duration of treatment (Gobello, 2007; Patton et al., 2007). The side effects of GnRH agonists are similar to those associated with gonad removal, but are reversible with no known effects on lactation (Asa and Porton, 2005). However, because GnRH agonists can cause abortion, they should be administered outside of the breeding season. Surgical implants containing a GnRH agonist are available as veterinary medicines in the EU but further regulatory approval may be required for their use in wildlife.

With regard to potential applications with the IAS of Union concern, there are no contraceptive implants suitable for avian species. Furthermore, none of the mammalian IAS are likely to be suited to this approach in terms of application under field conditions to relatively small species in terms of body size. The use of hormone implants in free-living mammals is likely to raise concerns with respect to potential negative welfare effects arising from long-term exposure and possible transfer of biologically active hormones via food chains. In addition, the approach is specifically contraindicated for the coati (*Nasua nasua*). There is thus little to commend the use of hormone implants relative to the potential use of GnRH agonists. It is unlikely that the capture, implant and release of individuals, required by the GnRH agonist approach, would be feasible for population management of eastern fox squirrel (*S. niger*), or grey squirrel (*S. carolinensis*), given that an estimated 80% or more of female target populations would need to be rendered infertile to realise substantial reductions in population size (Cowan, Massei, and Mellows, 2006; Cowan and Massei, 2008; Krause et al., 2014). This consideration is also likely to apply to Pallas's squirrel (*Callosciurus erythraeus*), Siberian chipmunk (*Tamias sibiricus*), and muskrat (*Ondatra zibethicus*). The population biology and trappability of the other six mammalian 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.

2.4.2. Parenteral delivery (injection and/or darting)

GnRH-toxin conjugates have potential as injectable contraceptives. These are formed by linking synthetic analogues of GnRH to cytotoxins. This enables selective targeting and mortality of cells secreting reproductive hormones, potentially leading to permanent sterility in both males and females. Because of their proteinaceous nature, these conjugates are broken down by digestion and thus do not enter the food chain. Examples include an injectable GnRH-toxin conjugate that suppressed the secretion of LH for up to 6 months in female mule deer (Baker, Nett, Hobbs, Gill, and Miller, 1999), and an injectable GnRH-cytotoxin (pokeweed antiviral protein, PAP) conjugate that disrupted reproduction in adult male domestic dogs (*C. familiaris*), female laboratory Norway rats (*R. norvegicus*) and sheep (*Ovis aries*) for at least six months (Nett, Glode, and Ball, 2003; Ball et al., 2006).

Intratesticular injection of zinc gluconate has been evaluated in domestic dogs (*C. familiaris*) as an alternative to surgical castration (Levy et al., 2008). Relatively low cost, ease of use, and cultural acceptance of a technique that does not require removal of the testes makes this a potential option for use in locations with limited clinical facilities and veterinary skills. However, further investigation is needed to identify risk factors in dogs for adverse reactions to zinc gluconate and to develop strategies for avoidance.

2.4.3. Oral delivery

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 is particularly relevant for those species where high proportions of target populations need to be treated in order to realise substantial reductions in population size (Cowan, Massei, and Mellows, 2006; Cowan and Massei, 2008; Krause et al., 2014). There are a number of possible candidate contraceptive materials that could potentially be delivered orally in bait or feed.

The cholesterol mimic diazacon (20,25-diazacholesterol dihydrochloride) can affect reproduction in both birds and mammals because it competitively blocks cholesterol production, which is a precursor of both male and female reproductive steroids (Fagerstone et al., 2010). Following ingestion of diazacon over a period of one to two weeks, fertility was suppressed for a few months in the black-tailed prairie dog (*Cynomys ludovicionus*) (Nash et al., 2007), monk parakeet (*Myiopsitta monachus*) (Yoder, Avery, Keacher, and Tillman 2007; Avery, Yoder, and Tillman, 2008), and Rose-ringed parakeet (*Psittacula krameri*) (Lambert et al., 2010) under laboratory conditions. Diazacon also reduced cholesterol in the grey squirrel (*Sciurus carolinensis*) (Yoder et al., 2011), although the effect on reproduction was ambiguous (Mayle et al., 2013). Diazacon has a relatively narrow contraceptive window before potential negative side effects occur in terms of welfare (Sachs and Wolfman, 1965; Yoder et al., 2004; Yoder et al., 2007). The efficacy of this compound depends on its bioaccumulation; however, its consequently relatively long elimination half-life represents potential risks to predators and scavengers of treated animals. Therefore, diazacon is more suited for applications with captive wildlife and localised populations experiencing little or no predation where non-target species can be prevented from feeding on diazacon treated baits (Avery et al., 2008; Fagerstone et al., 2010).

Nicarbazin (NCZ) is a complex of two compounds, 4,4'-dinitrocarbanilide (DNC) and 4,6-dimethyl-2-pyrimidinol (HDP). It is an effective and widely used veterinary medicine for preventing intestinal and caecal coccidiosis in poultry. NCZ is also a bird-specific oral contraceptive that disrupts the membrane between the egg albumen and yolk, thus compromising embryo development (Jones, Solis, Hughes, Castaldo, and Toler, 1990). NCZ products are registered in the USA by the EPA for use with Canada geese (*B. canadensis*) (OvoControl G) (Bynum et al., 2007) and feral pigeons (*Columbia livia*) (OvoControl P) Fagerstone et al., 2008). NCZ is also used in Italy to manage urban populations of feral pigeons (*C. livia*) (Ferri et al., 2009), but with unclear effects at the population level. Because NCZ is rapidly cleared from the body once consumption ceases, the effect on fertility is reversible. Furthermore, NCZ thus poses limited risk to any predators or scavengers that consume treated birds. The disadvantage is that NCZ must be fed continuously before and during egg-laying to be effective (Fagerstone et al., 2010). This may underlie the equivocal results reported for population-level effects in the field (Giunchi et al., 2007; Ferri et al., 2009). Economic modelling has demonstrated that NCZ use can potentially be more cost-efficient than egg oiling for the management of the Canada goose (*B. canadensis*) (Caudell, Shwiff, and Slater, 2010). The effect on fertility is not species-specific thus the potential risks to non-target species must be limited by the system use to deliver treated feed or bait; for example, in the use of Nicarbazin on pigeons in Italy, operators must be constantly present at delivery sites to prevent the feeding by non-target species, significantly increasing the costs of the methodology.

MGA can be administered orally as a mammalian contraceptive in feed (Patton et al. 2007). MGA fed daily to cattle suppresses ovulation (Zimbelman et al., 1970)], and oral dosing of White-tailed deer (*O. virginianus*) realised reversible contraception with no subsequent effects on parturition, lactation or offspring survival (Roughton, 1979). However, repeated exposure is required to maintain infertility, which would be challenging with free-living wildlife. Furthermore, MGA is likely to impact on the biology of most mammals thus species-specificity would need to be achieved via the system used to deliver treated feed.

A relatively recent approach to fertility control in mammalian wildlife is to target the mammalian ovary with the aim of inducing premature menopause (Tran and Hinds, 2013). The epoxide 4-vinylcyclohexene diepoxide (VCD) has mammalian ovary specific toxicity and ovarian follicle-depleting properties (Hoyer et al., 2001; Mayer et al., 2002). The administration of VCD by injection or ingestion repeatedly over a period of up to 30 days depletes the ovary of follicles leading to ovarian senescence (Mayer *et al*. 2004; Hu et al., 2006) and permanent sterility. Similarly, repeated oral administration of triptolide, a diterpenoid triepoxide, affects the ovarian function by causing follicular atresia (Xu and Zhao, 2010; Liu et al., 2011). Triptolide can also compromise sperm function in males (Singla, Kaur, Babbar, and Sandhu, 2013). These active compounds are reported to have very short plasma half-lives and are rapidly cleared via the liver. Exposure to these materials thus appears to pose little risk to any predators or scavengers that consume the bodies of exposed individuals. A product (ContraPest), that contains both VCD and triptolide, has now been registered by the EPA in the US for use in the management of the Norway rat (*R. norvegicus*). This product is currently delivered in a liquid formulation (Dyer and Mayer, 2014; Pyzyna et al., 2014) and has been shown to reduce the fertility of wild Norway rats (*R. norvegicus*) after self-administration by individually housed captive individuals (Witmer et al., 2017). Several orders of mammalian species are likely to be affected by this approach; thus species-specificity would need to be realised via the method of delivery and/or limiting availability to areas where only target species can access the formulation, perhaps by using similar approaches to those aimed at reducing non-target species access to approved rodenticide baits.

With regard to potential fertility control tools for the IAS of Union concern diazacon, although potentially offering medium term contraception for both avian and mammalian species, has substantive negative risks regarding welfare and food chain transfer that suggest it is currently unsuited to field application. A product containing diazacon was approved for use against birds in the USA. This registration has now lapsed. There is no current prospect of a commercial formulation being available for any use in animals in the EU.

NCZ has potential application for the four avian IAS of Union concern. The commercially available product aimed at Canada geese (*B. canadensis*) might be accepted by the Egyptian goose (*A. aegyptiacus*) and, perhaps, the ruddy duck (*O. jamaicensis*). However, this species rarely feeds on land thus the approach is unlikely to be feasible for this species. It is also possible that this formulation or the one aimed at the feral pigeon (*C. livia*), will be taken by the house crow (*C. splendens*). It seems probable that a novel formulation would need to be developed for the sacred ibis (*T. aethiopicus*). The disadvantages of the method are firstly, NCZ would have to be applied for the duration of the breeding season of the target species and, secondly, NCZ is not species-specific so measures would need to be taken to prevent, or at least limit, access to baits for non-target species. The approach may well be suited to local populations where other methods are not feasible e.g. due to public opposition to culling. Commercial products containing NCZ are approved for use in free-living bird species in the USA. Commercial products contacting NCZ are approved for use as veterinary medicines in the EU as coccidiostats in domestic poultry. Some additional regulatory approval may be necessary to permit the use of such products with free-living birds in the EU.

Incorporating MGA in baits or feed could potentially reduce the fertility of the mammalian species of IAS concern. However, application would need to be maintained throughout the target species' breeding season and, as it is not species-specific, measures would need to be taken to prevent access for non-target species. Use of MGA is also specifically contraindicated for use in the coati (*Nasua nasua*). These constraints suggest that this approach is currently unsuited to field application.

With regard to the use of VCD plus triptolide this could potential be used for all the mammalian IAS of Union concern, although the available data suggest that it is most likely to be effective in the rodent species Pallas's squirrel (*C. erythraeus*), coypu (*M. coypus*), grey squirrel (*S. carolinensis*), fox squirrel (*S. niger*), Siberian chipmunk (*T. sibiricus*) and muskrat (*O. zibethicus*). Developing a practical application for this approach in any of these species would require substantial species-specific research to establish the duration of required exposure, longevity of response (if permanent sterility was not induced); the development of suitable bait formulations and delivery methods would also need to be species-specific. Nevertheless, if these issues could be successfully addressed, the approach could potentially be a useful tool to contribute to the population management of these species. A commercial product containing VCD plus triptolide has an approval in the USA for use with commensal rodents. Currently the VCD plus triptolide formulation is not licenced for use in the EU.

2.5. Gene drives, in particular using the CRISPR-Cas9 gene editing tool

A ‘gene drive’ is a process of increasing the likelihood that a particular gene is passed on to the next generation (beyond the normal 50% chance found in sexual reproduction) thus quickly spreading its traits through a population. These ‘selfish’ genes occur naturally in the wild, but can be synthetically engineered through gene editing. CRISPR-Cas9 is a new gene editing technology that is more precise than any previously available. It works like scissors, by inserting an enzyme into the target cell to make cuts near the gene that the scientist wants to alter or replace, and inserting the desired gene segment.

CRISPR-Cas9 is a gene editing technique that is cheaper, easier, more precise, and more rapid than ever before, and is thus widely accessible (Piaggio et al., 2016). CRISPR is a fast, straight-forward and low-cost technology accessible almost in any basic laboratory setting (Heidari et al., 2017).

CRISPR gene drives therefore offer unrivalled opportunities in modifying genomes in human and other living organisms (Heidari, Shaw, and Elger, 2017). The potential application is incredibly diverse from human health, including preventative measures for genetic disorders, to wildlife control for human health, conservation or agricultural purposes, for example by reducing pesticide resistance or locally eradicating invasive species (Champer, Buchman, and Akbari, 2016; Esvelt et al., 2014; Harvey-Samuel, Ant, and Alphey, 2017; Mei et al., 2016).

Depending upon the design of these transgenes, they could either supress/eradicate populations (e.g. removing rats from islands) or ‘replace’ populations (e.g. eliminating an undesired trait from a population) (Harvey-Samuel et al., 2017). In addition, there are techniques that would allow for the gene drive to be ‘self-limiting’ so they would disappear from the environment rapidly if releases of individuals carrying the gene drive cease, but there is also the potential for ‘self-sustaining’ gene drives that are designed to persist in the environment and perhaps spread with the target population or even through the entire species (Harvey-Samuel et al., 2017; Revive and Restore, 2015).

The technology has not yet been applied to wild populations of invasive alien species, but it has been applied in laboratory conditions and in field trials.

In relation to managing invasive alien species most of the potential application of CRISPR-Cas9 has been focused on rats and mice on islands, brown tree snake in Guam, mosquitos on Hawai’i as vectors for avian malaria (and as vectors for human diseases), and non-native diseases (Callaway, 2017; Campbell and Long, 2009; Piaggio et al., 2016).

CRISPR-Cas9 gene drives have been successfully applied in the laboratory to the mosquito *Anopheles gambiae* (human malaria vector) that caused an infertility mutation in females to be passed on to all their offspring with transmission rates to progeny between 91.4-99.6%, which is an effective rate for targeting reproduction in an insect population (Hammond et al., 2015).

Experimental work is also underway to use gene drives to create mice that will produce only male offspring (‘SRY’ mice), having the potential to breed a population out of existence (Piaggio et al., 2016). However, the number of modified individuals that would need to be released, time to extinction, the persistence of the gene drive in the face of mutations, and the fertility and fitness of SYR mice and their ability to compete against the wild population remains uncertain, but there are ongoing studies to assess these issues (Gemmell and Tompkins, 2017; Kanavy and Serr, 2017; Piaggio et al., 2016).

Field trials using a self-limiting gene drive that prevents offspring from developing into functional adults (RIDL technique) have successfully suppressed target populations of the mosquito *Aedes aegypti* in the Cayman Islands, Brazil, and Panama (Piaggio et al., 2016).

For self-sustaining gene drives, models suggest that far fewer modified individuals would need to be released, however they would also be harder to control post release, running the risk of dispersal into non-target populations with potential species extinction, or hybridising with other species, and their ability to remain effective in the face of strong evolutionary pressures (resistance), is less clear (Champer et al., 2016; Piaggio et al., 2016; Revive and Restore, 2015).

Populations evolving a resistance to gene drives have been reported (Callaway, 2017). One source of this resistance is the CRISPR system itself which uses an enzyme to cut the DNA and insert the require genetic code. Occasionally the cells sew the incision back together after adding or deleting random DNA letters, resulting in a sequence that the CRISPR system does not recognise halting the spread of the modified code. This form of resistance has been found by the Target Malaria project in Italy (Callaway, 2017). The other source of resistance is natural genetic variation, as populations with high levels of genetic diversity, e.g. *Anopheles gambiae* and *Anopheles coluzzii* across Africa (Miles et al., 2016), would have limited potential gene drive targets as individuals with differences in the selected genetic sequences would be immune to the gene drive (Callaway, 2017). Modelling gene drive application in natural populations has shown that resistance to standard CRISPR-CAs9 gene drive approaches should evolve almost inevitably in most natural populations (Unckless et al., 2017). There is ongoing work to engineer gene drives with lower resistance potential.

There are a number of significant risks to gene drives when used for wildlife control. Mitigation of risks could entail a focus on isolated oceanic islands that have no human inhabitants nor endemic rodents, but to which access can be strictly regulated (Piaggio et al., 2016).

The ethical concerns are significant for the application of CRISPR gene drives to both the human genome and for conservation (‘playing god’ and permanently altering natural systems), particularly for application to germline editing (i.e. passing on to future generations). Due to CRISPR gene drives’ relatively low cost and ease of use, it could allow its application in ‘DIY’ laboratories for biohacking and bio-terrorism (Furrow and Richards, 2017). There is also a current state of uncertainty on safety and security issues and there is no regulatory framework in place (Furrow and Richards, 2017).

There have been calls for greater engagement between the synthetic biology and conservation biology communities (Redford et al., 2014), and in particular to raise the awareness of the potential benefits to conservation (Johnson et al., 2016).

COP 12 Decision XII/24 New and emerging issues: synthetic biology (CBD, 2014) calls for taking a precautionary approach and putting in place effective risk assessment and management procedures and/or regulatory systems to regulate environmental release of any organisms, components or products resulting from synthetic biology techniques. This was reaffirmed in COP 13 Decision XIII/17 Synthetic biology (CBD, 2017).

The Convention on Biological Diversity has also established an Ad Hoc Technical Working Group on Synthetic Biology which will be reviewing developments of synthetic biology, analyse evidence of its benefits and adverse effects and gather information on risk management measures, safe use and best practices (CBD, 2016).

At the IUCN World Conservation Congress in September 2016, Resolution 086 '*Development of IUCN policy on biodiversity conservation and synthetic biology*' was passed (IUCN, 2016), which recognises that synthetic biology is developing rapidly and that some its applications may have the potential to be beneficial to conservation and also to pose risks. The resolution calls for IUCN to examine the beneficial and detrimental impacts of synthetic biology techniques, and with urgency to assess the implications of gene drives and their potential impacts on the conservation and sustainable use of biodiversity.

2.6. Measures to prevent reproduction in use for the control of the 19 vertebrates on the list of IAS of Union concern

Examples of practical applications of fertility control techniques, to manage overabundant wildlife populations, are increasing. Although these have generally targeted closed populations at local scales they have the potential to be used synergistically with other management tools, including culling, or in local contexts where other methods are not feasible. In a number of cases the products discussed are not currently licensed for use in the EU. The following provides a summary and interpretation of the available information on the potential contribution and application of fertility control measures to the eradication or management of each of the 19 IAS of Union concern.

Egyptian goose (*Alopochen aegyptiacus*): Egg oiling could potentially contribute to the population management of this species if sufficient nests can be located and accessed without incurring costs of specialist techniques. A suitable bait formulation containing NCZ, such as the commercial formulation registered in the US for use in the Canada goose, might be contribute to the management of this species, particularly in contexts where other methods are not feasible. However, this would require development of a species-specific delivery system.

House crow (*Corvus splendens*): Egg oiling is unlikely to be suitable for this species due to the costs of locating and accessing nests. A suitable bait formulation containing NCZ, such as the commercial formulation registered in the US for use in the feral pigeon, might contribute to the management of this species, particularly in contexts where other methods are not feasible. However, this would require development of a species-specific delivery system.

Ruddy duck *Oxyura jamaicensis* Egg oiling would be unlikely to meaningfully contribute to the eradication or management of this species except in very limited circumstances. It also could potentially contribute to the population management of this species if sufficient nests can be located and accessed without incurring costs of specialist techniques. A suitable bait formulation containing NCZ, such as the commercial formulation registered in the US for use in the Canada goose, might be contribute to the management of this species, particularly in contexts where other methods are not feasible. However, the species rarely feeds on land so it is very unlikely that this approach would ever be feasible for this species.

Sacred ibis (*Threskiornis aethiopicus*): Egg oiling is unlikely to be suitable for this species due to the costs of locating and accessing nests. A bait formulation containing NCZ might contribute to the management of this species, particularly in contexts where other methods are not feasible.

However, the currently available commercial formulations are unlikely to be suitable for this species, hence a new formulation would need to be developed together with a species-specific delivery system.

Coati (*Nasua nasua*): An approach using either an injectable immunocontraceptive vaccine or a GnRH agonist implant might contribute to the management of this species, provided sufficient numbers of individuals could be captured, treated and released at a suitable cost. This is unlikely to be practical for a widely distributed species, but may play a supporting role in specific, limited circumstances, if other techniques are considered unfeasible. It is possible that oral delivery of a suitable VCD plus triptolide bait formulation could contribute to the population management of this species, provided delivery was species-specific. These potential approaches would require species-specific feasibility studies, probably followed by further technique development.

Coypu (*Myocastor coypus*): An approach using either an injectable immunocontraceptive vaccine or a GnRH agonist implant might contribute to the management of this species, provided sufficient numbers of individuals could be captured, treated and released at a suitable cost. This species is relatively trappable, as was demonstrated during the successful UK eradication campaign (Gosling and Baker, 1989). Nevertheless, this approach is likely to be best suited to local scale contexts if other techniques are considered unfeasible. It is possible that oral delivery of a suitable VCD plus triptolide bait formulation could contribute to the population management of this species provided delivery was species-specific. These potential approaches would require species-specific feasibility studies, probably followed by further technique development.

Fox squirrel (*Sciurus niger*): it is unlikely that measures requiring capture, treatment and release of individuals would be feasible for the population management of this species. It is possible that oral delivery of a suitable VCD plus triptolide bait formulation could contribute to the population management of this species provided delivery was species-specific.

Grey squirrel (*Sciurus carolinensis*): it is unlikely that measures requiring capture, treatment and release of individuals would be feasible for the population management of this species. A specific assessment carried out in Italy (La Morgia, Genovesi, and Masse, 2016) has shown that the technique is not applicable to the eradication of established populations in the wild, and can only be considered for populations present in areas of a size <100 ha. It is possible that oral delivery of a suitable VCD plus triptolide bait formulation could contribute to the population management of this species provided delivery was species-specific.

Pallas's squirrel (*Callosciurus erythraeus*): It is unlikely that measures requiring capture, treatment and release of individuals would be feasible for the population management of this species. It is possible that oral delivery of a suitable VCD plus triptolide bait formulation could contribute to the population management of this species, provided the delivery system was species-specific. Currently the VCD plus triptolide formulation is not licenced for use in the EU.

Muskrat (*Ondatra zibethicus*): it is unlikely that measures requiring capture, treatment and release of individuals would be feasible for the population management of this species. It is possible that oral delivery of a suitable VCD plus triptolide bait formulation could contribute to the population management of this species provided delivery was species-specific.

Raccoon (*Procyon lotor*): This species is relatively easy to live trap (e.g. Hoffmann and Gottschang, 1977). Hence, an approach using either an injectable immunocontraceptive vaccine or a GnRH agonist implant might contribute to the management of this species, provided sufficient numbers of individuals could be captured, treated and released at a suitable cost. This is likely to be at local scale contexts if other techniques are considered unfeasible, for instance in urban settings. It is possible that oral delivery of a suitable VCD plus triptolide bait formulation could contribute to population management, provided delivery was species-specific. Oral rabies vaccine baits can be successfully delivered to this species (Boulanger et al., 2008). These potential approaches would require species-specific feasibility studies, probably followed by further technique development.

Raccoon dog (*Nyctereutes procyonoides*): An approach using either an injectable immunocontraceptive vaccine or a GnRH agonist implant might contribute to the management of this species, provided sufficient numbers of individuals could be captured, treated and released at a suitable cost. This is unlikely to be practical for a widely distributed species, but may play a supporting role in specific, limited circumstances, if other techniques are considered unfeasible. It is possible that oral delivery of a suitable VCD plus triptolide bait formulation could contribute to the population management of this species, provided delivery was species-specific. These potential approaches would require species-specific feasibility studies, probably followed by further technique development.

Reeves' muntjac (*Muntiacus reevesi*): While this species is relatively difficult to capture, an approach using either an injectable immunocontraceptive vaccine or a GnRH agonist implant might contribute to population management, provided sufficient numbers of individuals could be captured, treated and released at a suitable cost. This is unlikely to be practical for a widely distributed species, but may play a supporting role in specific, limited circumstances, if other techniques are considered unfeasible. It is possible that oral delivery of a suitable VCD plus triptolide bait formulation could contribute to the population management of this species, provided delivery was species-specific. These potential approaches would require species-specific feasibility studies, probably followed by further technique development.

Siberian chipmunk (*Tamias sibiricus*): it is unlikely that measures requiring capture, treatment and release of individuals would be feasible for the population management of this species. It is possible that oral delivery of a suitable VCD plus triptolide bait formulation could contribute to the population management of this species provided delivery was species-specific.

Small Indian mongoose (*Herpestes javanicus*): An approach using either an injectable immunocontraceptive vaccine or a GnRH agonist implant might contribute to the management of this species, provided sufficient numbers of individuals could be captured, treated and released at a suitable cost. This is unlikely to be practical for a widely distributed species, but may play a supporting role in specific, limited circumstances, if other techniques are considered unfeasible. It is possible that oral delivery of a suitable VCD plus triptolide bait formulation could contribute to the population management of this species, provided delivery was species-specific. These potential approaches would require species-specific feasibility studies, probably followed by further technique development. Currently the VCD plus triptolide formulation is not licenced for use in the EU.

Amur sleeper *Perccottus glenii*: Research into factors influencing the fertility of fish are primarily focussed on conservation. No reports of measures for the control of fertility in fish have been found.

Topmouth gudgeon *Pseudorasbora parva*: Research into factors influencing the fertility of fish are primarily focussed on conservation. No reports of measures for the control of fertility in fish have been found.

Bullfrog (*Lithobates (Rana) catesbeianus*): No reports of measures for the control of fertility in amphibians have been found.

Red-eared slider (*Trachemys scripta*): Few information for the control of fertility is available for *T. sripta*. A large number of *T. scripta* indinviduals were captured and sterilized within a LIFE project in Italy (LIFE09 12NAT/IT/0000395-Lifeemys, 2017).

An overview of the potential fertility control applications to the 19 vertebrates included currently on the list of IAS of Union concern is presented in Table 1 (see below). These are described with respect to:

- availability, in terms of:
  - **none** (**N**);
  - **under development** (**U**);
  - tools **currently available** (**Y**);

- feasibility, in terms of:
  - **none** (**N**);
  - **low** (**L**) - for instance high unit cost of material and/or delivery, required scale of application unrealistic, and/or unacceptable side effects with respect to welfare;
  - **moderate** (**M**) - for instance constraints due to cost, effectiveness and/or welfare concerns uncertain or greater than alternative methods, potentially suitable for niche or local application only e.g. where lethal methods inappropriate;
  - **high** (**H**) - for instance, costs and effectiveness commensurate with realistic large-scale practical application and no known side effects or unwanted consequences.

Table 1. An overview of the potential fertility control applications to the 19 vertebrates currently on the list of IAS of Union concern. With respect to **availability,** i.e. none (**N**), under development (**U**) or available (**Y**); **feasibility,** i.e. none (**N**), low (**L**), moderate (**M**), or high (**H**).

		Surgical sterilisation		Post-fertilisation intervention				Immuno-contraceptive vaccines				Non-vaccine contraceptives					
				Egg removal and reducing egg hatchability		Induced abortion in mammals		Parenteral delivery		Oral delivery		Contraceptive implants		Parenteral delivery		Oral delivery	
		Availability	Feasibility	Availability	Feasibility	Availability	Feasibility	Availability	Feasibility	Availability	Feasibility	Availability	Feasibility	Availability	Feasibility	Availability	Feasibility
Birds	Egyptian goose *Alopochen aegyptiacus*	Y	N	Y	M	N	N	N	N	N	N	N	N	N	N	Y	M
	House crow *Corvus splendens*	Y	N	Y	L	N	N	N	N	N	N	N	N	N	N	Y	M
	Ruddy duck *Oxyura jamaicensis*	Y	N	Y	L	N	N	N	N	N	N	N	N	N	N	Y	N
	Sacred ibis *Threskiornis aethiopicus*	Y	N	Y	L	N	N	N	N	N	N	N	N	N	N	Y	L
Mammals	Coati *Nasua nasua*	Y	L	N	N	Y	L	Y	M	U	N	Y	M	Y	N	Y	L
	Coypu *Myocastor coypus*	Y	L	N	N	Y	L	Y	M	U	N	Y	M	Y	N	Y	M
	Fox squirrel *Sciurus niger*	Y	N	N	N	Y	N	Y	L	U	N	Y	L	Y	N	Y	M
	Grey squirrel *Sciurus carolinensis*	Y	N	N	N	Y	N	Y	L	U	N	Y	L	Y	N	Y	M
	Pallas's squirrel *Callosciurus erythraeus*	Y	N	N	N	Y	N	Y	L	U	N	Y	L	Y	N	Y	M
	Muskrat *Ondatra zibethicus*	Y	N	N	N	Y	N	Y	L	U	N	Y	L	Y	N	Y	M
	Raccoon *Procyon lotor*	Y	L	N	N	Y	L	Y	M	U	N	Y	M	Y	N	Y	L
	Raccoon dog *Nyctereutes procyonoides*	Y	L	N	N	Y	L	Y	M	U	N	Y	M	Y	N	Y	L
	Reeves' muntjac *Muntiacus reevesi*	Y	L	N	N	Y	L	Y	M	U	N	Y	M	Y	N	Y	L
	Siberian chipmunk *Tamias sibiricus*	Y	N	N	N	Y	N	Y	L	U	N	Y	L	Y	N	Y	M
	Small Indian mongoose *Herpestes javanicus*	Y	L	N	N	Y	L	Y	M	U	N	Y	M	Y	N	Y	L
Fish	Amur sleeper *Perccottus glenii*	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
	Topmouth gudgeon *Pseudorasbora parva*	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Amphi.	Bullfrog *Lith.(Rana) catesbeianus*	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Reptile	Red-eared slider *Trachemys scripta*	U	L	N	N	N	N	N	N	N	N	N	N	N	N	N	N

3. Non-Lethal measures for capturing specimens

This section of the note provides information on applied measures for capturing vertebrates. It covers commonly used trapping tools such as cage and box trap designs, snares, netting techniques, pitfall traps, and floating and submerged traps as well as other capturing techniques including darting, manual capture, electrofishing and electrofrogging. The use of sniffing dogs as a complementary measure is also mentioned. Traps that are harmful to animals are not discussed in this section. Leg-hold traps, for example, which are illegal in the European Union (Council Regulation (EEC) No 3254/91) are not discussed here. Methods with highly possible lethal consequences for the trapped specimen, such as the use of adhesive materials, e.g. glue boards, which are very harmful to target species and rigorously condemned by bird and animal welfare organisations (Charlton, 2014), are likewise excluded from this note, although they were commonly used in non-EU countries, such as the US.

It is important to note the fate of animals caught using non-lethal measures could include lethal dispatch/euthanasia, sterilisation and release into the wild, or transfer into captivity (including zoos and as pets).

3.1. Legislation and standards on traps

Council Regulation (EEC) No 3254/91 prohibits the use of leg hold traps in the Community and the introduction of pelts and manufactured goods of 13 wild animal species (including the raccoon *Procyon lotor*, and muskrat *Ondatra zibethicus*) originating in countries which catch them by means of leg hold traps or trapping methods which do not meet international humane trapping standards. Due to this legislation, leg hold traps have been banned from use in the EU since 1995.

In 1998, the EU agreed two humane trapping agreements, one with Canada and Russia (Council Decision 98/142/EC) and one with USA (Council Decision 98/487/EC), both of which adopted the Agreement on International Humane Trapping Standards (AIHTS) (Council of the EU, 1998). The objectives are to "*establish standards of humane trapping methods, improve communication and cooperation between the parties for the implementation and development of the standards, and facilitate trade between the parties*". The scope of the standards (Article 3) is for wildlife management including pest control, obtaining fur, skin or meat, and for the capture of animals for conservation. They are applicable to killing and restraining traps for the trapping of 19 species (Annex 1 to the AIHTS), including three species that are either currently, or proposed to be, listed as an invasive alien species of Union concern: raccoon *Procyon lotor*, raccoon dog *Nyctereutes procyonoides* and muskrat *Ondatra zibethicus*. In terms of restraining traps, the standards set out requirements for trapping methods, and physical and behavioural indicators of poor welfare (Annex 1 to the AIHTS). Parties to the AIHTS commit to certifying traps in accordance with the standards, ban traps that are not certified, require manufactures to identify certified traps, and ensure that trapping methods are in accordance with the standards (Article 7), however there are possible derogations (Article 10) to these commitments, e.g. in the interests of public health and safety. The agreement entered into force in 2008 following ratification from Russia, and parties had until 2016 to ban the use of traps that were not certified in accordance with the AIHTS (FACE 2017).

3.2. Terrestrial traps

Live traps are usually set up in areas where the target species has been frequently observed, and the target species is attracted using food or scents. A large number of different trap types are in use for capturing various vertebrates, as the technical construction of every trap needs to be adapted to the target species' anatomy, behaviour and habitat (Iossa, Soulsbury, and Harris, 2007). It is important to note that traps used to capture raccoon *Procyon lotor*, raccoon dog *Nyctereutes procyonoides* and muskrat *Ondatra zibethicus* need to be approved under the AIHTS (*Agreement on International humane trapping standards)*, see section 3.1. All capture methods require the regular monitoring of the trap to reduce the possible impacts on animal welfare. Any impacts on animal welfare or survival can be reduced by minimizing the time that the species is held in a capturing device through frequent checking, together with providing food, water and cover. When poorly managed (i.e. infrequent checking, no water or food supply), any live-trap type can become inhumane. A further major conservation and animal welfare concern is the number of non-target species that may be captured. Many traps are not selective and capture a high number of such species, including those that might be protected. The use of baits or adaption of the construction of a trap may help to reduce this risk (Iossa, Soulsbury, and Harris, 2007). In any case regular inspections, event through the support of remote devices, are required to prevent harmful side effects on both target and non-target species.

3.2.1. Terrestrial cage and box traps

Cage or box traps, the most common type of terrestrial trap, consist of a cage appropriate for the size of the target species and a door or gate that closes when a specimen enters the cage. Dimensions of the cage or box vary depending on the size of the target species. The following are approximate dimensions in cm (L x W x H) of commonly used cage traps: Rat — 30 x 10 x 10, Grey squirrel— 45 x 15 x 15, Mink — 60 x 18 x 16, Feral cat — 75 x 30 x25, Rabbit — 70 x 25 x 23, Fox — 150 x 45 x 45 (Charlton, 2014). Checking the traps frequently is required to meet relevant animal welfare standards, which also stipulate the providing of food, water and shelter for the time of capture (Charlton, 2014).

Sherman trap

The Sherman trap is a box trap consisting of a foldable side panel, in the common size of 38x10x11.5cm. When an animal enters, the front door closes. The trap is commonly used to trap small mammals for research purposes (Anthony, Ribic, Bautz, and Garland Jr, 2005). The large Sherman trap is purchased from €48 (54US$) (Wildcare, 2017). The effectiveness depends on the species demography and density in an area. The disadvantages of the Sherman traps are the non-selective approach of the trap's design (Anthony et al., 2005). Furthermore, Anthony et al. (2005) report that 'mortality rates were highest for Longworth traps and small Sherman traps and lowest for large Sherman traps'. Moreover, large Sherman traps are relatively safe for rodents (Anthony et al., 2005).

Longworth trap

The Longworth trap consists of a tunnel which contains the door tripping mechanism and a nest box, which is attached to the back of the tunnel. The commonly used size is 14 x 6.5 x 8.5cm (Anthony et al., 2005). The trap is purchased from €76 (65US$) (Google shopping, 2017). This trap design in commonly used for research on small rodents, mice, voles and shrews (Kock, Jessup, Clark, Franti, and Weaver, 1987; Krebs and Boonstra, 1984). No record of trials with any of the 19 species has been found.

Havahart trap

The Havahart trap consist of a baited cage box, designed to capture squirrels and other small rodents (National Pest Control Agencies, 2015). Havahart traps are purchased from €10 (11US$) (Google shopping, 2017). It is used for research (García, Delgado-Jaramillo, Machado, and Aular, 2012) or pest control, e.g. live trapping of rats (National Pest Control Agencies, 2015).

Tomahawk live trap

Tomahawk live traps (models 201 and 2012, (Tomahawk Live Trap, 2017)) are commonly used to capture invasive squirrel species *Sciurus carolinensis, Tamias sibiricus* and *Callosciurus erythraeus)* in UK, France, Italy and Belgium (Adriaens et al., 2015; Gurnell and Pepper, 2016; Shuttleworth et al., 2015). The trap is purchased from €108 (65US$) (www.livetrap.com, 2017). Larger models are used for capturing larger mammal species e.g. for *Procyon lotor* (in Europe) and *Herpestes javanicus* (e.g. in Japan) or *Nasua nasua*. A population of *Callosciurus erythraeus* was captured using Tomahawk live trap in Belgium (Adriaens et al., 2015).

Multi-catch traps

The traps generally work on a series of baffle doors allowing the squirrels to enter a main chamber (SWCage, 2017). Multi-catch traps are used for invasive squirrels (Gurnell and Pepper, 2016). They are not particularly popular as they rarely catch more than a couple of squirrels at a time and are usually less effective than cage traps for the trigger mechanism (Gurnell and Pepper, 2016).

Funnel trap

A cylindrical cage design with inward-pointing funnelled entrances that make it difficult for animals to exit the trap once inside. Funnel traps can also be made from plastic tubes. They are commonly used to capture reptiles, e.g. snakes, and bird species. A large selection of Funnel traps is available from €25. The effectiveness of funnel traps for capturing birds is low, apart from ground based birds, e.g. duck or gamebirds (Fitch, 1951; Greenberg, Neary, and Harris, 1994), but they work very well for capturing reptiles and are therefore commonly used for reptile monitoring (GAMBLE, 2003; RODDA and SHARP, 1998).

Larsen traps

The Larsen trap is a cage trap for birds consisting of wire with one or more live individuals of the target species inside; other individuals of the same species are thereby attracted to enter through a one-way gate. This trap is primarily used by gamekeepers to capture corvids (Gamekeeper Supplies LTD n.d.), but is also in common use for controlling invasive bird species worldwide (Game and Wildlife Conservation Trust 2017; Suleinam and Taleb 2010). The Larsen trap can be used for the control of crows, magpies, jackdaws and jays (Tsachalidis, Sokos, Birtsas, and Patsikas, 2006). They have also proved effective for catching Egyptian geese (INBO, 2012). A large selection of Larsen traps is available from €60 to €300. Capture rates are particularly high in spring and early summer (BASC, 2016). Legal welfare regulations and licencing can help to mitigate misapplications of the Larsen trap. In Scotland, for example, Larsen traps need to obtain identification tags (RSPB, 2017). Animal welfare organisations criticize the negative long-term effect of stress, that birds caught by a Larsen trap experience (Animal Aid, 2011).

Ladder crow traps (Australian crow trap, MAC trap)

A long, ladder-shaped frame suspended across the top of a wire cage has gaps that allow crows to drop in, but not to fly out. Ladder traps can be purchased for around € 390 (£250) and are very popular among gamekeepers due to their high efficiency. Many online manuals are available to build a ladder trap (Hutton, 2016). They are large scale bird traps specifically modified to capturing crows, but are also effective for trapping starlings, blackbirds, house finches, house sparrows, and white and golden crowned sparrows (Gadd, 1996). Ladder traps are used effectively in the control of invasive bird species, e.g. the house crow *Corvus splendens*(Tsachalidis et al., 2006). The risk of capturing non-target species, e.g. game birds, is lower compared to other cage trap types (Game and Wildlife Conservation Trust, 2017).

Clover trap

The clover trap consists of a collapsible steel-pipe frame surrounded with netting and baited with corn to attract large herbivores. It is commonly used to capture various large herbivores like white tailed deer or elk, e.g. for relocation (Beringer et al., 2002; Haulton, Porter, and Rudolph, 2001; Thompson et al., 1989). Clover traps are commonly used because they are portable, relatively inexpensive to build, and can be used in closed forest condition (D'eon, Pavan, and Lindgren, 2003). On the other hand, their effectiveness has been questioned for the high rate of non-target species captures and the relatively high escape rate of captured individuals (D'eon, Pavan, and Lindgren, 2003). Animals captured in clover traps are not protected from predators and may be easily excited by outside disturbances, and the loose netting can cause injuries and stress resulting in shock or trauma (Haulton et al., 2001).

Stephenson box traps

The Stephenson box trap consists of two doors and a wooden box that is 2.7 m high x 1.30 m wide x 3.20 m long. It was developed for restocking programs and capturing and marking ungulates and is used, for example, for capturing white-tailed deer (Anderson, Nielsen, and Nielsen, 2002) or mountain goats (Alpine Ungulates Research, 2017). Detailed cost information has not been found. Installation of such traps requires extensive manpower or machinery due to their average weight of 260 kg (Anderson, Nielsen, and Nielsen, 2002).

Corral trap

Corral traps are generally panels made for sheep or goats with square mesh and steel T-posts, and are used with bait. They are effective at capturing feral hogs (Texas Agri Life Extension Service, 2010). In addition to their use by the hunting sector, corral traps are also used for wildlife management and research, and are commonly employed to capture large groups of wild boars, deer and feral pigs (Campbell and Long, 2009; Finnegan and Stone, 1993; Toïgo et al., 2008). Coral traps are in use for capturing large numbers of geese or swans (Whitworth, et al., 2007). Corral traps can be purchased from € 3,000 for each unit, but can also be built from readily available materials (Track Side Trapping, 2017). Animals, especially deer, that try to escape the corral trap may injure themselves, often seriously (Drummond, 1995) and therefore corrals should be carefully planned.

Chardonneret traps (Potter traps)

Potter traps are used for capturing living birds, which are attracted by bait placed in a two-celled wire cage, for the purpose of bird ringing (Weaver, 1981). Potter traps can be purchased from €55 (Moudry-traps, 2017; Third Wheel Ringing Supplies, 2017). With a view to the stress that wild birds are exposed to while captive in a Potter trap, the length of time of their entrapment should be kept as short as possible (Micol and Jouventin, 2002). The stress level of the captured bird in a Potter trap can be ameliorated by providing enough space to move, sufficient water and food (Romero and Romero, 2002).

Nest box trap

Many types of nest box traps have been developed for birds of all sizes. In principle, individual birds are trapped in a box entered for the purpose of nesting (Friedman, Brasso, and Condon 2008; Lombardo And Kemly, 1983). (Weaver, 1981). Nest box traps are used for ornithological research, bird monitoring and pest control, and are common in the USA for capturing the European starling (Knittle and Guarino, 1976). Nest box traps are purchased from €27 (32US$) (https://www.vanerttraps.com/, 2017) and commonly available in the USA to trap House Sparrows and European Starlings (Place, 2017). Nest box traps are effective when large bird populations need to be monitored (Friedman et al., 2008). Nest box traps have also been developed for many mammals, such as dormice, squirrels and martens (Hämäläinen et al., 2016).

3.2.2. Neck hold traps (non-lethal snares, noosing)

Non-lethal stop-snares (neck hold traps) consist of wire loops that tightens around the neck of the target species but does not close beyond a certain diameter so as not to kill the animal (Iossa et al., 2007; Short, Weldon, Richardson, and Reynolds, 2012). These are inexpensive to buy, e.g. 10 fox snares can be purchased for around €20 (£17) (Collins Nets Ltd, 2017). Today, stop - snares are mostly used for pest control and fur trapping, although also for wildlife research purposes (Iossa, Soulsbury, and Harris, 2007). Reptiles are commonly captured with nooses for research purposes (Department for Environment UK, 2017; Krysko, Seitz, Townsend, and Enge, 2006).

The welfare impact of non-lethal snare traps is difficult to assess, as individuals often sustain injuries from the wire ligature, which can in turn lead to tissue necrosis (pressure necrosis) and ultimately death in the days following release (Stocker, 2007). Another major concern is that snares capture and stress a large number of individuals of non-target species and can cause them serious injuries. In general, snares have a negative post-release impact on affected animals (Witmer, 2005), and the use of non-lethal snares is faced with largely negative public perception (Charlton, 2014). Snare design, location and operational procedure are fundamental to reduce injury or deaths (Short et al., 2012).

3.2.3. Modified leg-hold traps

Leg-hold snares

Leg-hold snares are made of a wire loop that tightens around the leg of an animal to capture it, and are attached to the ground or an anchor by a chain or cable. They are used for research, animal control and species management programs (Blundell, Kern, Bowyer, and Duffy, 1999). The most common leg-hold traps, mainly used in the USA, are the Belisle Footsnare® and WCS Collarum® (Vantassel and Groepper, 2016). The WCS Collarum was designed to capture carnivores, e.g. the red fox or feral dogs and it is purchased from €112 (124 US$) (Contact Magnum Trap Co, 2017). Similar to neck-hold snares (section 3.1.2.), injuries from the pressure of the wire ligature are common and can affect the long-term survivability of released individuals, although the overall mortality rate of vertebrates trapped by leg-hold snares is relatively low (Blundell et al. 1999; Iossa, Soulsbury, and Harris, 2007). Due to the high rate of injuries caused by (humane) leg-hold snares, welfare concerns nevertheless support the negative public perception of this method (Iossa, Soulsbury, and Harris, 2007).

Raccoon egg traps (Egg trap)

The egg trap 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. It is designed especially for the humane capturing of raccoons, widely used in Canada and the USA, and relatively inexpensive at around €14 per trap (14US$)[2]. Egg traps are highly efficient compared to cage traps, and their use for research purposes is strongly recommended (Proulx et al., 1993). 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 (Proulx et al., 1993).

Noose carpets

Noose carpets are pieces of poultry fencing laid on the ground, with monofilament nooses tied to the fencing wires which effectively capture large birds by the legs (Anderson and Hamerstrom, 1967; Engel and Young, 1989). Detailed cost information is not available, because they are generally self-made (Research Gate, 2017). Noose carpets are particularly effective when a bird species returns to

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[2] http://www.theeggtrapcompany.com/ordering_info.html

a known spot (Anderson and Hamerstrom, 1967). Noose carpets are inefficient to capture ravens, because they tend to manoeuvre around the trap (Engel and Young, 1989).

Bal-chatri traps

The principle of the Bal-chatri trap is similar to that of the noose carpet. Bal-chaltri traps were designed to catch raptors in East India, and are commonly used for research on birds like eagles and owls (Smith and Walsh, 1981; Thorstrom, 1996). They consist of a portable wire mesh cage with slip-nooses made of nylon monofilaments. Often a rodent is placed inside the cage to bait the target bird species, and the attacking target bird is ensnared when its feet contact the nooses (Thorstrom, 1996). The trap is purchased from €150 (167US$) (Northwoods Falconry, 2014). The Bal-chatri trap must be properly assembled to prevent larger birds from flying off with it, and must be constantly monitored, which may be difficult and time-consuming in hard-to-access areas. Trapped raptors need to be attended immediately to avoid injury and stress. Further, the welfare of the bait needs to be considered.

3.2.4. Terrestrial nets

Drop net (clap-net, whoosh-net)

Various drop nets have been developed to capture different target species. In general, a piece of netting is spread out horizontally above the ground and dropped rapidly onto the target animal, attracted with bait. Drop nets can cover anywhere between 2 and 10 m. and are effective at capturing small and medium-sized bird species, e.g. starlings (Proulx et al., 1993) and ungulates (Jedrzejewski and Kamler, 2004). There are serious welfare aspects to be considered regarding the use of drop nets, however: the stress levels of large herbivores, e.g. white-tailed deer (*Odocoileus virginianus*), increase greatly when they are captured in a drop net, and their intensive struggling can result in death or serious long-term injuries (Denicola and Swihart, 2012).

Drive nets

Drive nets are used to capture ungulates (especially deer species) and lagomorph (e.g. hares) for research and restocking programs. For ungulates, a single line of net 2.5-3.0 m high is set joining 50 m sections to attain the desired final length. For lagomorphs, nets are lower. Nets are hung from poles and trees and placed on the side of the poles opposite to the direction where the animals came from. The net is attached loosely to poles, allowing the net to fall down easily once the animal ran into it, forming a bag over the animal. The morning of capture, people set up the net and remain hidden near, on the side where animals came from. A line of beaters, drive the animals towards the net. Once entering the net, animals should be immediately restrained and immobilised. The most important problem associated with capture in ungulates are injuries and stress, which often results in post capture death. Mortality in roe deer is 1-4% (Boutin, Angibault, Van Laere, and Delorme, 1993; Jean-Michel et al., 1993; Sullivan et al., 1991). Drive nets have also been used to capture muntjac (*Muntiacus reevesi*) (Mountford and Chapman, 1993).

Dho-gaza nets

The Dho-gaza net consists of netting attached to the ground and a frame at its four corners. A string attached to one end to the pole frame is run through the outer mesh squares of the netting along all four sides and then reattached to the frame, causing the netting to be drawn shut behind any bird that hits it. Live bait can be placed near the trap to attract raptors (PSBO, 2014). Dho-gaza nets are very effective at capturing raptors (Zuberogoitia et al., 2008) and owls for breeding programs, bird monitoring, hunting purposes and ornithological research (Rosenfield and Bielefeldt, 1993; Zuberogoitia et al., 2008).

Mist nets
Traditionally mist nets were used in Japan, Southern Europe and North America for hunting (Mena, Stallings, Regalado, and Cueva, 2000; Riley, 2002) Today ornithologist use this technique to monitor small bird species populations (Spotswood et al., 2012). It can also be used for pest control (Broom, 1999) and bats monitoring (Francis, 1989; Moreno and Halffter, 2000). In order to minimise capture-related injuries and suffering the mist bet needs to be set up by trained staff only and constantly monitored (Wilson and McMahon, 2006) hence it requires high costs for personnel. Mist netting techniques have a very low mortality and injury rate (Spotswood et al., 2012) if regularly inspected. The most common fatal injuries are wing injuries, stress-relates disorders and cuts (Veltri and Klem, 2005). Predation is another mortality risk. Many manuals and guidelines are published, which are helping to reduce the risks by training staff (De Beer et al., 2001; Geupel, Pyle, and DeSante, 1993; Renner et al., 2013).

Net-gun captures (rocket nets, cannon nets)
The net gun is a device designed to fire a net to capture a wide variety of species, from birds to other small, medium and large vertebrates (Kock et al., 1987; Macdonald and Baker, 2004). The size and type of the net depends on the target species' size. In remote areas helicopters can be used to capture large vertebrates (helicopter net gun captures). A rocket guns or Cannon nets work in a similar way. Net guns are used to capture livestock, for wild life monitoring, breeding programs and wildlife research (Krausman, Hervert, and Ordway, 1985). The advantage of helicopter net gun capturing is the accessibility of remote area and the highly selective approach. Nevertheless, injuries of herbivores, e.g. broken legs, are frequent (Webb, Lewis, Hewitt, Hellickson, and Bryant, 2008). Herbivores captured by net guns have relatively low risk of mortality compared to other measures, e.g. darting or drop-nets (Kock et al., 1987; Krausman et al., 1985). If the measure is used with inexperienced operators to capture wild birds the risk of injury or mortality increases (Macdonald and Baker, 2004).

3.2.5. Pit-fall traps (drop box trapping)

A pit-fall trap consists of a container with smooth sides, buried at the ground level. Some pit fall traps use bait to attract target species (Iossa, Soulsbury, and Harris, 2007). Drop boxes follow a similar principle, using a box that has been buried into the ground. Pitfall traps are mainly used to capture small terrestrial vertebrates (Iossa, Soulsbury, and Harris, 2007). The use of drift fencing (Fisher el., 2008) increases the efficiency driving animals to the pit-fall from large areas. Drift fences in combination with pit fall traps are often used to determine the species richness of an area to monitor the abundance of species (Crosswhite, 1999). Drift fences are used also for small mammals and other vertebrates (Braun, 2005). Pit-fall traps could inexpensively be built from plastic bottle or other containers. Pit-fall traps for professional hunting are purchased from €99 (110US$). Pit-falls are effective in capturing small vertebrates and invertebrates in isolated locations (Animal Ethics, 2017). All pit-fall traps are adapted to the species size and habitat preference. For example, the rabbit drops traps (a modified pit-fall design). Rabbits are caught when they enter a tunnel and fall through a hinged flap into the trap. Pit- fall traps have been used in the eradication of invasive alien species, e.g. rats or rabbits (Micol and Jouventin, 2002). Greenlees et al. (2006) successfully used pitfalls (plastic vials 50 mm in diameter and 120 mm deep) to capture cane toads (*Bufo marinus*) in difficult accessible floodplain areas (Greenlees, Brown, Webb, Phillips, and Shine, 2006). Pit-fall traps are non-selective (Gamble, 2003). In general pit fall trapping is effective and inexpensive as "*there are no moving parts that could injure animals during operation, it is a safe for staff to operate, and it can collect large numbers of animals*", (Richter, 2009). Eventually they can cause serious injuries to the trapped animal when the traps are not checked frequently by trained stuff (Richter, 2009). For example trapped animals may suffer from dehydration and hyperthermia in hot weather (Animal Ethics, 2017) and vice versa in cold climates.

3.3. Floating and submerged traps

Hancock trap and Bailey trap

Hancock traps or Bailey traps are constructed of a heavy duty aluminium frame and chain link mesh (Rosell and Hovde, 2001). The Hancock trap and Bailey trap are used in aquatic or semiaquatic habitats. Both are very commonly used to capture beavers, e.g. the Eurasian beaver (*Castor fiber*) (Buech, 1983; Rosell and Hovde, 2001). The Hancock trap is an efficient measure to trap aquatic rodents (McNew Jr, Nielson, and Bloomquist, 2007). It is commonly in use for the humane trapping of beavers and otters in the USA (Breck, Wilson, and Andersen, 2001; McNew Jr et al., 2007; Serfass, Brooks, Swimley, Rymon, and Hayden, 1996). It is important that the trap be set up correctly so that it can't slide out into deep water and drown the trapped individual (Rosell and Kvinlaug, 1998). The Hancock trap is purchased from €465 (519US$) (Northern Sport Co., 2017). The most common non-lethal method of capturing *O. zibethicus* are Hancock traps (Kadlec, Pries, and Mustard, 2007), with apples, carrots, potatoes and sweet potatoes making effective bait (Rosell and Hovde, 2001).

Sundeck trap

Sundeck traps are used for freshwater turtles. A floating wire cage is submerged in a water body and anchored (Vogt, 1980). The target turtle is attracted by a bait in the centre of the cage which can reach climbing a mesh vinyl coated wired ramp. Sundeck traps are purchased from €152 (169 US$). The Sundeck trap is considered to be a highly effective trap to capture freshwater turtle species (Vogt, 1980). The Solarium Turtle Trap is a larger variation of the Sundeck trap using entrance ramp surface (Heinson's Store, 2017).

Modified "Bolue" trap

This floating trap consists of a round platform, made of wood or cork, to be used in a water body. The freshwater turtle become caught in the net as they attempt to climb over the platform. The trap is used to capture turtles for monitoring, research and invasive alien species management (Valdeón, Crespo-Diaz, Egaña-Callejo, and Gosá, 2010b). Baits can also be used to attack specific target terrapins. The "Bolue" trap was developed for capturing individuals for research, but is also used to control the invasive alien turtle *Trachemys scripta* Schoepff, 1792 (Red-eared slider), for example in Spain since 2010 (LIFE09 NAT/ES/000529, 2013; Valdeón, Crespo-Diaz, Egaña-Callejo, and Gosá, 2010a). However, the efficiency of the "Bolue" trap is limited, as many individuals escape from the platform before they are captured (Sarat, 2014).

"Aranzadi" turtle trap (ATT)

The Aranzadi turtle trap is a floating cage to be used in a water body with a slippery inside frame that freshwater turtles cannot climb. The frame is made of PVC tubes on top of which cork is fixed. The ATT trap is used for population monitoring and other scientific purposes (Valdeón et al., 2010a). The trap is more effective when it is places in a sunny spot. It is one of the most effective traps to capture the invasive alien turtle *Trachemys scripta* (Red-eared slider) (Valdeón et al., 2010b). The ATT trap was successfully tested within the LIFE project (LIFE09 NAT/ES/000529, 2013). According to Valdéon et. al. (2010b), the ATT trap is the most effective trap for capturing *Trachemys scripta*.

Hoop net

A large number of baited and un-baited hoop nets are available (Heinson's Store, 2017; Jgermano, 2012). For commercial fisheries, double-ended hoop nets set within 20 m of the shore and submerged at 2–3 m depths are used. Each hoop net comprises steel or wooden hoops (Colotelo et al., 2013). Hoop net traps are purchased from €5 (5.5US$) (Amazon UK, 2017). They are partially submerged, and can be adapted depending upon the target species. As the hoop net is a non-selective trap, a large amount of bycatch consisting of gamefish, turtles and mammals is caught

(Larocque et al., 2012). According to Larocque et al. (2012) hoop nets are likely to have lethal consequences to mammals and turtles. It is common tool to monitor and trap freshwater invertebrates, e.g. crabs and crayfish (Fulton et al., 2012), and vertebrates, e.g. fish, turtles (Fratto et al., 2008; Mali, Brown, Ferrato, and Forstner, 2014), ,

Drift nets

Drift nets are a commercial fishing technique. The mesh-size of the netting is usually adapted to the size of the target species (Richards, 1994). Drift nets are used for capturing fish, but they were also in use for research, e.g. in the field of invasion biology (Pintor, Sih, and Kerby, 2009). It can also be used to capture aquatic bird and mammals and other vertebrates. Welfare is very poor, since the drift net can injure or kill mammals by drowning them (Broom, 1999).

Larval Fish Light Traps

Larval Fish Light Traps attract positively phototaxic organisms by a floating light source (Jones, 2006; Ponton, 1994). Detailed cost information is not available. They are used for fishing, to control fish or for research purposes, when larval live specimens are required (Doherty, 1987; Jones, 2006) or for monitoring aquatic species population (Sigurdsson, Morse, and Rochette, 2014). This traps are an inexpensive way to trap a large number of individuals in a short time (Doherty, 1987).

3.4. Darting

The target species is captured by injecting immobilizing or tranquilising drugs, which are fired from a dart gun (DEFRA, 2017). A blowgun has been used for darting ungulates easy to approach, e.g. Alpine ibex (*Capra ibex*) and red deer (*Cervus elaphus*) in the Alps (Marco and Lavin, 1999). Alternatively, jab sticks can be used to administer immobilizing drugs (Landriault, Hall, Hamr, and Mallory, 2006). The specific type of equipment used is dependent upon distance from which the target animal will be darted, and the target species' anatomy (incl. skin thickness) (Arnemo et al., 2006; Barret, Bøe, Lydersen, and Swenson, 2013). The dart gun can be fired from the ground, car or helicopter, requiring extremely skilled and well trained experts (Barret et al., 2013). Dart guns can be purchased from €3,410 (3,800US$) (Barret et al., 2013). Jab sticks are available from €707 (795US$). The mishandling of the dart-gun, or providing an overdose, can be lethal to the target animal. In addition the use of immobilizing or tranquilising drugs involves risks of mortality and can affect the target species' health post release (Arnemo et al., 2006; Kock et al., 1987). Once darted the animal needs to be reached as soon as possible to reduce risks to the animal's welfare, for example many large vertebrates suffer from overheating and exhaustion after being hunted (Barret et al., 2013; DEFRA, 2017). Darting is only recommended if highly trained personnel, pharmacological preparations and safety is guaranteed and authorised (DEFRA, 2017). In EU member states tranquillising equipment such as dart guns are normally considered weapons (GB Home Office, 2013). Therefore, national and EU law needs to be respected.

3.5. Manual capturing

Manual capture, capturing species 'by hand', is a highly selective mechanical method used to control species populations such as invasive reptiles and amphibians in aquatic and terrestrial habitats (Pitt, Vice, and Pitzler, 2005) and for research (Snow and Witmer, 2010). Manual capturing often requires the use of capturing tools, e.g. hand dip nets (hand nets) or fishing rods with a noose instead of a hook. Hand dip nets are used for the manual capturing of small vertebrates, e.g. small birds (Bloom, Clark, Kidd, Bird, and Bildstein, 2007), reptiles, amphibians or fish (CEM, 2015). Hand dip nets are purchased for around €10 (11US$) (Google shopping, 2017).The use of hand dip nets is ethically acceptable (Bloom et al., 2007). On the one hand manual captures contain almost no risk of capturing non-target species, but on the other hand manual capturing is man power intensive and time consuming. For example, hand capturing was not effective to capture large populations of invasive American bullfrog (Snow and Witmer, 2010). Single adult bullfrogs are best captured at night by hand capture or using a floating, multi-catch wired cage trap (Snow and Witmer, 2011). No concerns regarding animal welfare are known for this measure.

3.6. Electrofishing

Electrofishing involves the submerging of a cathode and anode to create an electric field in the water which forces the fish from swimming by immobilization (Kennard et al., 2011). Electrofishing is a common tool for freshwater field studies and the removal of invasive alien species, as the target fish species can be removed selectively from an aquatic habitat, while non-target species may be released (Platts, Megahan, and Minshall, 1983; Schramm, Grado, and Pugh, 2002). The measure was not primarily developed as a non-lethal measures. But the measure can be adapted for the non-lethal capture of fish. For example, the Life+ Trout project used electrofishing to capture the invasive Atlantic trout (*Salmo trutta*) in river basins of central Apennine. After capturing the alien specimens were transferred to small lakes for fishing isolated from the river networks (LIFE+Trota, 2017). The measure requires significant logistics, e.g. coordination of the fisher, who handle the cathode and anode, and the catcher, who captures the paralyzed fish with a net, or the handling of the fish after catching. This measure is manpower intensive. 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 (Schulz, 1995; Perrow, Jowitt, and Zambrano González, 1996). In addition, poor netting practices and the overheating of the water holding buckets, in which the captured fish is placed, can harm the fish (Fisheries, 1997). However, when personnel are properly trained the risks to the fishes welfare are significantly reduced (Reynolds and Harlan, 2011).

3.7. Electrofrogging

Electrofrogging is similar in principle to electrofishing. After the target frog is localized, a submerging of a cathode and anode is used to create an electric field in the water (Allen and Riley, 2012). An electrical field of <50 cm diameter near is created near the visually localized target frog. Juvenile and adult frogs can be paralysed for 30 seconds to one minute, allowing them to be collected by hand (Orchard, 2011). Detailed information on costs is not available. It requires trained staff that are familiar with the vocalisations of the target species (e.g. male adult male bullfrogs), and the use of the equipment. This measure is commonly applied in amphibian research in wetlands. Electro frogging is also used to control invasive alien amphibians, especially to control populations of the American bullfrog (O’Connor, 2013; Orchard, 2011). Few studies show that electro frogging can have long term stress-related effects on growth and survival. Especially large individuals are more likely to get injured (Allen and Riley, 2012).

3.8. Sniffer (detection) dogs as a complementary measure

Trained dogs can locate and pursue animals. Besides the common use of sniffer dogs for hunting, border control or drug crime detection, sniffer dogs are used for IAS management, and can be trained to detect vertebrates, e.g. raccoon dogs, foxes, minks. The detection rate of dogs for carnivores is high (Against Corvid Traps, 2017), and they are very effective in use for finding reptiles, for example, in Florida (USA) sniffer dogs are trained to detect pythons (Staletovich, 2017). Sniffer dogs were in use to find both individuals and eggs of the Common slider (*Trachemys scripta*) (Scalera 2006). Although the training is time intensive the use of sniffer dogs is cost-effective in many sectors, especially when the last few animals have to be discovered in eradication projects.

3.9 Non-lethal measures in use to capture the 19 vertebrates on the list of IAS of Union concern

The following section provides a summary of the available information on the non-lethal measures to capture specimens for each of the 19 IAS of Union concern for their eradication or management.

The results of this discussion are also presented in Table 2 (see below) in the following categories:

- **Not effective - evidence based** (**N**) - The measure was tested or applied for the vertebrate of the list of IAS of Union concern and considered to be *not effective*. The information is based on evidence e.g. publication or technical note;
- **Effective - evidence based** (**Y**) - The measure was tested or applied for the vertebrate of the list of IAS of Union concern and considered *to be effective*. The information is based on evidence e.g. publication or technical note;
- **Possibly feasible** (**P**) - the measure is currently not applied for the vertebrate of the list of IAS of Union concern, but it is commonly in use to prevent the escape of species of similar taxonomical, morphological or ecological group, e.g. aquatic rodents. The feasibility of the particular measure is based on strong evidence e.g. publication or technical note.

The individual measures commercial availability are presented in Table 3 (see below). The information is categorized in the following categories:

- **Commercially available within the EU** (**Y**) - The supplies are commercially available within the EU (Including e-commerce). The average price and at least one provider is known;
- **Commercially not available within the EU** (**X**) - Based on a desk survey, the supplies are not commercially available in the EU (Including e-commerce);
- **Limited or partially available** (**L**) - The supplies are only available for non-commercial purposes, e.g. research, or the use of the trap is legally restricted in EU Member States.

Egyptian goose (*Alopochen aegyptiacus*): The Larsen traps are effectively used to remove small emerging breeding groups of Egyptian geese, which are known to be highly territorial (INBO, 2012, Huysentruyt et al., 2014). Trapping efforts generally focus on moulting flightless birds, and while moult trapping has been successful for Canada geese, Egyptian geese are, due to their excellent diving capacities, not susceptible to the current moult trapping systems (Huysentruyt et al., 2014). The following terrestrial traps are used to capture large numbers of geese: Coral trap, Chardonneret traps (Potter traps) and nest box trap. Further mist nets and net-gun captures (e.g. cannon netting) are available measure for capturing bird species, but face the high risk of injury or mortality.

House crow (*Corvus splendens*): The Larsen trap and ladder crow trap are used for capturing the House crow. Chardonneret traps (Potter traps) are also commercially available to trap bird crow species. The modified leg-hold traps, the noose carpets have been shown to be not effective (Engel and Young, 1989). The terrestrial Dho-gaza net is also used for large bird species, e.g. the Eurasian Eagle-Owl (*Bubo bubo*) (Zuberogoitia et al., 2008).

Ruddy duck (*Oxyura jamaicensis*): According to the UK Ruddy Duck Control Trial Final Report (2002) it may be possible to catch small numbers of Ruddy ducks using traps (funnel traps in the winter, coral traps during breeding and post-breeding), however, trapping is unlikely to succeed in catching large numbers of ruddy ducks, and trapping success may be site specific (U.K. Ruddy Duck Control Trial, 2008). Further mist nets and net-gun captures (e.g. cannon netting) are available measure for capturing bird species, but face the high risk of injury or mortality.

Sacred ibis (*Threskiornis aethiopicus*): No species specific information on trap effectiveness was found for this species. Chardonneret traps (Potter traps) and nest box trap are used to capture bird species. These potential approaches would require species-specific feasibility studies, probably followed by further technique development.

Coati (*Nasua nasua*): Raccoon sized Tomahawk live trap are used to capture the coati (Hirsch, 2006; CABI, 2017). Complementary sniffer dogs are also being used to locate individuals.

Coypu (*Myocastor coypus*): Cage traps have been successfully used to capture *M. coypus* in the EU and North America. They were used to capture over 40,000 Coypu in the UK leading to their eradication in the 1980s (Baker, 2006), and to control populations cost-effectively in Italy (Bertolino & Viterbi, 2010). Double-entrance live cage traps, placed on the ground and on floating rafts, and Tomahawk live traps have been effectively used to trap individuals (Cocchi & Riga, 2008). In the UK Baker & Clarke (1988a) used welded wire mesh cages baited with carrot that measured 850 x 250 x 250 mm, and in a different study Baker & Clarke (1998b) showed that floating traps are four times more effectve than land traps. In the Loire Atlantique department in France cages traps were attached to a raft made of resin-coated cellular polycarbonate and anchored to trees or vegetation on the banks (Mazaubert, 2016). In the USA, the USDA APHIS have designed multiple capture traps, and tested different lures: coypu urine, fur extract, synthetic anal gland secretion and commercially available apple based lure, with the coypu fur extract being the most effective (see Burkey et al., 2008 for details).

Fox squirrel (*Sciurus niger*): Box traps with a dimension of 60 x 20 x 20cm, the Tomahawk or the Live Trap Multi-catch traps, are all used for the population management of this species. In particular the Tomahawk live traps are used to capture invasive squirrels.

Grey squirrel (*Sciurus carolinensis*): Single-capture traps, e.g. Tomahawk live traps, and multi-capture traps are commonly used to capture *S. carolinensis.* The populations of *S. carolinensis* on an island of the North Wales (UK) was effectively controlled by using commercially produced 079 Albi Squirrel Traps. ”*Each was 7” wide 9” high x24” long, and manufactured from 100 9 100 9 14 g heavy duty galvanised welded mesh*” (Schuchert et al., 2014). Live trapping of the grey squirrel involves attracting squirrels to a trap with baits, and according to Mayle et al. (2007) “*Yellow whole maize has proved to be the best all round bait*”; nuts and hazelnuts are also used, but more expensive

Pallas's squirrel (*Callosciurus erythraeus*): Box traps of the dimension of 45 x 15 x 15 cm, e.g. the Tomahawk, or the multi-catch traps, are feasible for the population management of squirrels. In broadleaf woodlands in Lombardy (Italy) single capture traps of two dimensions and multi capture traps were effectively used to trap the Pallas’s squirrel (Mazzamuto, 2015). The species was eradicated in Belgium using mesh wire traps (Adriens et al. 2015)

Muskrat (*Ondatra zibethicus*): Muskrat can be trapped using live catching bait-traps, bait-traps with body-gripping, cages with body-gripping traps (FACE, 2014). In the USA the Hancock/Bailey trap and ‘family’ multi-capture traps are used to capture the species, with apples, carrots, potatoes and sweet potatoes all being effective bait (Kadlec et al., 2007).

Raccoon (*Procyon lotor*): This species is relatively easy to live trap (e.g. Hoffmann and Gottschang, 1977). “*Cage traps, body-gripping, and foothold traps are very effective, especially in conjunction* *with exclusion and/or habitat modification*" (Boggess, 1994). Traps should be at least 25.4 x 30.5 x 81.3 cm. Modified Leg-hold snares and neck-hold snares are not recommended to manage populations due to welfare concerns. The Raccoon EGG^{TM} traps, designed especially for the humane capturing of raccoons, are widely used and have been found to be more effective than wire cage traps (Austin et al. 2004).

Raccoon dog (*Nyctereutes procyonoides*): Many different traps can be used to capture Raccoon dogs, but one of the most efficient is a Finnish cage trap called the KANU-trap (https://riista.fi/sv/jakt/direktiv-for-jagare/metsastystavat/fangst-med-falla-och-sax) (Dahl & Åhlén, in prep). The effectiveness of box and cage traps is the highest when the trap is located "*alongside streams, moist areas, dry islands in the wetlands and marshes, and places with human activities such as garbage"* (FACE, 2014b). Sniffer dogs were in use to find individuals.

Reeves' muntjac (*Muntiacus reevesi*): The species is relatively difficult to capture. Clover traps, long nets, drop nets and rocket nets are used for the management of invasive deer species in the USA, and the species has also been caught in corral traps set for wild boar in the UK (Ward and Leeds, 2011). Darting could be a feasible approach to capture individuals if highly trained personnel, pharmacological preparations and safety is guaranteed. Potential approaches, developed for capturing deer species, would require species-specific feasibility studies, probably followed by further technique development.

Siberian chipmunk (*Tamias sibiricus*): In France the species has been trapped for research purposes using Sherman traps (8 x 8x 26cm) baited with peanut butter and sunflower seeds (Marmet et al. 2011; Marsot et al. 2013). Further potential approaches require species-specific feasibility studies, probably followed by further technique development. The species has also be captured for research purposes in its native range in Korea, using one-door Tomahawk live trap (Model 201, 40 × 13 × 13 cm), baited traps with peanut butter and shelled peanuts (Jo et al., 2014).

Small Indian mongoose (*Herpestes javanicus*): Based on control efforts in Mauritius, which primarily use wooden box traps (live drop traps) baited with salted fish to reduce mongoose populations over relatively small areas, trapping programmes need to be run almost constantly as mongooses re-colonise trapped areas very quickly and long-term control over larger areas cannot be achieved by trapping alone (Roy et al., 2002).

Amur sleeper (*Perccottus glenii)*: Landing nets, fish (funnel net) traps, electrofishing, and fishing with rod and line are used to confirm presence of the species rather than as a control method (e.g. Nehrig & Steinhof, 2015; Pupina et al. 2015), intensive trapping is not seen as an option to control or eradicate the species (De Vries et al. 2012).

Topmouth gudgeon *Pseudorasbora parva*: According to the RAFTS Invasive Species and Biosecurity Programme in Scotland, there are no physical control measures available for the species (http://www.invasivespeciesscotland.org.uk/asian-topmouth-gudgeon-pseudorasbora-parva/). Britton and Brazier (2006) state that the species small size (12-70 mm) make conventional measures, such as netting and electrofishing, not feasible.

Bullfrog (*Lithobates (Rana) catesbeianus*): An effective and often used measure is manual capturing. Hand dip nets are used to support manual capturing of Bullfrogs. Snow & Witmer (2011) used multiple capture trap (l 69 cm × 69 cm × 25 cm) constructed with 1.3cm × 1.3 cm wire mesh, which were placed near the waterline of an invaded pond or floating in the water. Louette et al. (2013) used double fyke net (height and width of the first hoop 80 and 90 cm, respectively, three narrowing funnels in each fyke, leader net 7 m, and mesh size 8 mm) to capture Bullfrog tadpoles, metamorphs and adults in shallow water bodies. Pit falls in combination with drift fences are another effective but non-selective approach to capture a large number of Bullfrogs, that requires constant checking of the trap. Electrofrogging was used to control large populations of the American bullfrog.

Red-eared slider (*Trachemys scripta*): The following floating and submerged traps are in use to capture the red-eared slider: The sundeck trap, the modified “Bolue” trap, the “Aranzadi” turtle trap (ATT) and the hoop net. A case study in Spain found that modified Aranzadi turtle traps caught an average of 70% of observed terrapins (Valdeón et al., 2010b). Small populations, in shallow and accessible aquatic habitats, are managed using manual capturing. Further “sniffer dogs can be used to detect and remove both turtles and their eggs. Eggs can also be found and removed by following females at nesting areas” (Scalera, 2009). Sniffer dogs were in use to find both individuals and eggs of the common slider (Scalera, 2006).

Table 2. An overview of the non-lethal measures in use to capture the 19 vertebrates on the list of IAS of Union concern in respect to their effectiveness. Y = YES, evidence to show the measure is effective to capture the species; P = POSSIBLE, the measure may be effective based on its use for similar species; N = NO, there is evidence to show that the measure is not effective to capture the species.

		Terrestrial Traps												
		Terrestrial Cage and Box Traps												
		Sherman trap	Longworth trap	Havahart trap	Tomahawk Live Trap	Multi-catch traps	Funnel trap	Larsen traps	Ladder crow traps	Clover trap	Stephenson box traps	Corral trap	Chardonneret traps (Potter traps)	Nest box trap
BIRDS	**Egyptian goose** *Alopochen aegyptiacus*	-	-	-	-	-	-	Y	-	-	-	P	P	P
	House crow *Corvus splendens*	-	-	-	-	-	-	Y	Y	-	-	-	Y	-
	Ruddy duck *Oxyura jamaicensis*	-	-	-	-	-	Y	-	-	-	-	Y	P	-
	Sacred ibis *Threskiornis aethiopicus*	-	-	-	-	-	-	-	-	-	-	-	P	P
Mammals	**Coati** *Nasua nasua*	-	-	-	Y	-	-	-	-	-	-	-	-	-
	Coypu *Myocastor coypus*	-	-	-	Y	-	-	-	-	-	-	-	-	-
	Fox squirrel *Sciurus niger*	Y	-	-	Y	Y	P	-	-	-	-	-	-	P
	Grey squirrel *Sciurus carolinensis*	P	-	-	Y	Y	Y	-	-	-	-	-	-	P
	Pallas's squirrel *Callosciurus erythraeus*	P	-	-	Y	Y	P	-	-	-	-	-	-	P
	Muskrat *Ondatra zibethicus*	-	-	-	Y	-	-	-	-	-	-	-	-	-
	Raccoon *Procyon lotor*	-	-	-	Y	-	-	-	-	-	-	-	-	-
	Raccoon dog *Nyctereutes procyonoides*	-	-	-	P	-	-	-	-	-	-	-	-	-
	Reeves' muntjac *Muntiacus reevesi*	-	-	-	-	-	-	-	-	P	-	Y	-	-
	Siberian chipmunk *Tamias sibiricus*	Y	-	-	-	-	-	-	-	-	-	-	-	-
	Small Indian mongoose *Herpestes javanicus*	Y	P	-	P	P	-	-	-	-	-	-	-	-
Fishes	**Amur sleeper** *Perccottus glenii*	-	-	-	-	-	-	-	-	-	-	-	-	-
	Topmouth gudgeon *Pseudorasbora parva*	-	-	-	-	-	-	-	-	-	-	-	-	-
Amp.	**Bullfrog** *Lithobates (Rana) catesbeianus*	-	-	-	-	Y	-	-	-	-	-	-	-	-
Rept.	**Red-eared slider** *Trachemys scripta*	-	-	-	-	-	-	-	-	-	-	-	-	-

Table 2. Continued

		Terrestrial Traps										
			Modified leg-hold traps				Terrestrial nets					
		Neck hold traps	Leg-hold snares	Raccoon egg traps	Noose carpets	Bal-chatri traps	Drop net	Drive nets	Dho-gaza nets	Mist nets	Net-gun captures	Pit-fall traps
BIRDS	**Egyptian goose** *Alopochen aegyptiacus*	-	-	-	P	P	-	-	-	P	P	-
	House crow *Corvus splendens*	-	-	-	X	-	-	-	P	-	-	-
	Ruddy duck *Oxyura jamaicensis*	-	-	-	-	-	-	-	-	-	-	-
	Sacred ibis *Threskiornis aethiopicus*	-	-	-	P	P	-	-	-	P	P	-
Mammals	**Coati** *Nasua nasua*	-	-	-	-	-	-	-	-	-	-	-
	Coypu *Myocastor coypus*	-	-	-	-	-	-	-	-	-	-	-
	Fox squirrel *Sciurus niger*	-	-	-	-	-	-	-	-	-	-	-
	Grey squirrel *Sciurus carolinensis*	-	-	-	-	-	-	-	-	-	-	-
	Pallas's squirrel *Callosciurus erythraeus*	-	-	-	-	-	-	-	-	-	-	-
	Muskrat *Ondatra zibethicus*	-	-	-	-	-	-	-	-	-	-	-
	Raccoon *Procyon lotor*	Y	Y	Y	-	-	-	-	-	-	-	-
	Raccoon dog *Nyctereutes procyonoides*	P	P	-	-	-	-	-	-	-	-	-
	Reeves' muntjac *Muntiacus reevesi*	-	-	-	-	-	P	P	-	-	P	-
	Siberian chipmunk *Tamias sibiricus*	-	-	-	-	-	-	-	-	-	-	-
	Small Indian mongoose *Herpestes javanicus*	-	-	-	-	-	-	-	-	-	-	-
Fishes	**Amur sleeper** *Perccottus glenii*	-	-	-	-	-	-	-	-	-	-	-
	Topmouth gudgeon *Pseudorasbora parva*	-	-	-	-	-	-	-	-	-	-	-
Amp.	**Bullfrog** *Lithobates (Rana) catesbeianus*	-	-	-	-	-	-	-	-	-	-	Y
Rept.	**Red-eared slider** *Trachemys scripta*	-	-	-	-	-	-	-	-	-	-	-

Table 2. Continued

		Floating and submerged traps												
		Aquatic rodents trapping					Fish traps							
		Hancock trap and Bailey trap	Sundeck trap	Modified "Bolue" trap	"Aranzadi" turtle trap	Hoop net	Drift net	Double Fyke net	Larval Fish Light Traps	Darting	Manual capturing	Electrofishing	Electrofrogging	Sniffer dogs – complementary measure
BIRDS	**Egyptian goose** *Alopochen aegyptiacus*	-	-	-	-	-	-	-	-	-	-	-	-	-
	House crow *Corvus splendens*	-	-	-	-	-	-	-	-	-	-	-	-	-
	Ruddy duck *Oxyura jamaicensis*	-	-	-	-	-	-	-	-	-	-	-	-	-
	Sacred ibis *Threskiornis aethiopicus*	-	-	-	-	-	-	-	-	-	-	-	-	-
Mammals	**Coati** *Nasua nasua*	-	-	-	-	-	-	-	-	-	-	-	-	P
	Coypu *Myocastor coypus*	-	-	-	-	-	-	-	-	-	-	-	-	-
	Fox squirrel *Sciurus niger*	-	-	-	-	-	-	-	-	-	-	-	-	-
	Grey squirrel *Sciurus carolinensis*	-	-	-	-	-	-	-	-	-	-	-	-	-
	Pallas's squirrel *Callosciurus erythraeus*	-	-	-	-	-	-	-	-	-	-	-	-	-
	Muskrat *Ondatra zibethicus*	P	Y	-	-	-	-	-	-	-	-	-	-	-
	Raccoon *Procyon lotor*	-	-	-	-	-	-	-	-	-	-	-	-	-
	Raccoon dog *Nyctereutes procyonoides*	-	-	-	-	-	-	-	-	-	-	-	-	Y
	Reeves' muntjac *Muntiacus reevesi*	-	-	-	-	-	-	-	-	P	-	-	-	-
	Siberian chipmunk *Tamias sibiricus*	-	-	-	-	-	-	-	-	-	-	-	-	-
	Small Indian mongoose *Herpestes javanicus*	-	-	-	-	-	-	-	-	-	-	-	-	-
Fishes	**Amur sleeper** *Perccottus glenii*	-	-	-	-	-	P	-	P	-	-	P	-	-
	Topmouth gudgeon *Pseudorasbora parva*	-	-	-	-	-	P	-	P	-	-	P	-	-
Amp.	**Bullfrog** *Lithobates (Rana) catesbeianus*	-	-	-	-	Y	-	Y	-	-	Y	-	Y	-
Rept.	**Red-eared slider** *Trachemys scripta*	-	Y	Y	Y	Y	-	-	-	-	Y	-	-	Y

Table 3. An overview of the commercial availability of the non-lethal measures to capture the 19 vertebrates currently on the list of IAS of Union concern. With respect to availability*:* Commercially available in the EU (Y*),* commercially not available in the EU (X*), l*imited or partially available (L*).*

			Availability in the EU
Terrestrial traps	Terrestrial Cage and Box Traps	Sherman trap	Y
		Longworth trap	Y
		Havahart trap	Y
		Tomahawk Live Trap	Y
		Multi-catch traps	-
		Funnel trap	Y
		Larsen traps	Y
		Ladder crow traps	Y
		Clover trap	-
		Stephenson box traps	-
		Corral trap	Y
		Chardonneret traps (Potter	Y
		Nest box trap	Y
		Neck hold traps	Y
	Modified leg-hold traps	Leg-hold snares	Y
		Raccoon egg traps	Y
		Noose carpets	X
		Bal-chatri traps	Y
	Terrestrial nets	Drop net	-
		Drive nets	-
		Dho-gaza nets	-
		Mist nets	-
		Net-gun captures	-
		Pit-fall traps	Y
Floating and submerged traps	Aquatic rodents trapping	Hancock trap and Bailey trap	Y
		Sundeck trap	Y
		Modified “Bolue” trap	X
		“Aranzadi” turtle trap	X
		Hoop net	Y
	Fish traps	Drift nets	-
		Larval fish light traps	-
		Darting	Y
		Manual capturing	-
		Electrofishing	L
		Electrofrogging	L
		Sniffer dog – complementary measure	Y

4. Non-lethal measures to prevent contained specimens from escaping or entering

4.1. Fencing

Fences are physical barriers that prevent animals escaping from or entering into a certain area (Kotchemidova, 2008). They are primarily used to keep livestock from escaping or to protect forest plantations from large herbivores (Goddard et al. 2001; Hidinger, 2009). Fence designs for preventing the escape of farmed or captive vertebrates are commercially available for almost all terrestrial vertebrates.

Fences are also used for the purpose of invasive alien species management, e.g. to exclude them from protected areas (Long and Robley 2004) or to minimize the impact of invasive alien predators to threatened species (Somers and Hayward 2012), and as a method to support large island eradications as 'interior fences' (Bode et al. 2013; Parkes et al., 2017). Exclusion fences are currently in common use to mitigate the invasive impacts of stoats (*Mustela erminea*), European red foxes (*Vulpes vulpes*), feral cats (*Felis catus*), feral goats (*Capra hircus*), feral pigs (*Sus scrofa*) and feral rabbits (*Oryctolagus cuniculus*) in Australia and New Zealand (Clapperton and Day 2001; Long and Robley 2004; Scofield, Cullen, and Wang 2011). Fencing is a complementary measure for conservation strategies aiming to control invasion by bullfrogs, and is used to direct bullfrog movement away from infested habitats (Devisscher et al. 2012). Outside of nature conservation, fencing is used worldwide to keep vertebrates off roads to reduce road accidents (Clevenger, Chruszcz, and Gunson 2001; McCollister and van Manen, 2010).

Forest fences are commonly used in Europe, and consist of wooden posts, which are buried at least 60cm in the ground, and wire lines, that support a mesh wire netting or a rectangular wire mesh netting (Trout and Pepper, 2006). The purpose of forest fences is to protect forest regeneration from deer (Gill ,1992; Horsley, Stout, and DeCalesta, 2003; Kuiters and Slim, 2002). The material for a forest fence can be purchased from €58 for 50m (excl. manpower and machinery).

The exclusion of IAS using fences is considered to be effective as long as intensive maintenance is provided (Long and Robley, 2004). Electric fences in particular are effective at excluding any non-flying vertebrates, and are often combined with conventional fences to increase efficacy. However, gates and crossings are known to be weak points that can be exploited by vertebrates to escape or enter (Long and Robley 2004). A cost-benefit analysis on exclusion fences in New Zealand concludes that "*for every million dollars invested on predator-proof fencing, 297 ha of habitat has been protected*" (Scofield, Cullen, and Wang, 2011). The cost-efficiency of fencing as a method to exclude invasive alien species ranges from NZ$ 11 to 155 per hectare and year depending on the targeted taxon, geographical location and accompanying management measures (Cullen, Fairburn, and Hughey, 2001; Scofield, Cullen, and Wang, 2011).

Fence design differs according to the target species (Thomson Group ,2017) and also in relation to environmental conditions, e.g. in case of flooding, snow storms etc. potentially occurring in the area. The construction of fences to prevent the migration of amphibians and reptiles, for example, depends on the target species' climbing ability. In general, amphibian and reptile fencing is used for nature conservation purposes, e.g. pest control (Burns et al., 2012). 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 (Thomson Group, 2017). Temporary amphibian and reptile fencing is made of polythene sheets (1200 mm high, clear Polythene) affixed to wooden fence stakes and last up to two years. Amphibian and reptile fencing is commonly used to protect species from ongoing construction work, to mitigate road mortality or to support wildlife management actions and for population studies. In-situ research that tested the efficiency of electric and two types of wire mesh fences (with varying size holes) against 16 different vertebrate pest species, found that it is possible to design a completely effective multi-species exclusion fence (Day and MacGibbon, 2007). They identified that 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 and that ongoing maintenance, precise construction and exceptional product quality are required for the fences to be fully effective (Day and MacGibbon, 2007). Risks need to be managed, such as the use of double-door pedestrian and vehicle gates, remote monitoring, and the use of gate alarms if gates are left open is also needed along with the required staffing to immediately respond (Day and MacGibbon, 2007).

The construction of protective barriers for species bred in fur farms, e.g. the American mink, is legally regulated in some countries. In Newfoundland and Labrador (Canada), for example, the fence mesh must extend 1.83 metres (6 ft.) in height above and 30.48 centimetres (1 ft.) below ground level for a total fence mesh height of 2.13 metres (7 ft.) (Newfoundland and Labrador Regulation 38 2012). Within the EU some countries, e.g. Finland, Poland, Spain and Denmark adopted regulation on securing fur farms with fences (Clapperton and Day, 2001). The Danish legislative framework on fur farming requires that fur farms need to be enclosed by a 150cm high fence, which must to be buried at least 50cm in the ground. The top 50cm of the fence must be covered with a material on the inside, that the mink cannot climb (Animal Keeping Act, Denmark 2017).

Fences can have lethal consequences for animals. Animals can injure themselves by trying to pass the fence (Woodroffe, Hedges, and Durant, 2014). Common fence injuries are laceration, ischaemic and crush injury, dislocation of the hip, myopathy (Austen, 2008). Regular inspection and maintenance can mitigate the number of injured or killed vertebrates. Concerning exclusion fences, environmental and landscape considerations need to be included in the early stages of planning and fence design (Hayward and Somers, 2012).

In the aquatic realm, physical and 'virtual' fences (which can use bubbles, light, bioacustics or electric pulses) can be used to prevent the spread of invasive alien fish species. While physical barriers are used, e.g. to block invasive alien sea lamprey (*Petromyzon marinus*) from their spawning habitat as part of their management programme in the Great Lakes, they can interrupt natural stream flow and block non-target species (Miehls, Johnson, and Hrodey 2017). Electrical dispersal barriers are being used on the Chicago Sanitary and Shipping Canal to prevent Asian carps, Eurasian ruffe (*Gymnocephalus cernuus*) and other non-native fishes from entering the Great Lakes System in North America, though it is not 100% effective (Cudmore et al. 2017; Dawson, Reinhardt, and Savino 2006). Bioacoustic barriers are also being evaluated in controlled experiments, which have found that they are >90% effective (Murchy 2016). Research on bioacoustics, light and bubble barriers in preventing upstream movement of sea lamprey (*Petromyzon marinus*) found that they had little effect (Miehls, Johnson, and Hrodey, 2017).

4.2. Repellents and deterrents

Repellents are any kind of substance or device that keeps an animal from entering a certain area. The varieties are numerous and very diverse. Repellents include natural substances (predator-odour–based repellents) (Cox et al., 2010), e.g. predator's urine, chemical substances (chemical defences), acoustic and ultrasonic devices (Mason, 1998). Repellents are used for animal control and in the private sector, in agriculture, urban areas or airports (Giunchi, Gaggini, and Baldaccini, 2007; Hile et al., 2004). Chemical repellents are considered as an effective alternative to lethal pest control measures. Predator-odour–based repellents, e.g. tiger-fecal-odour, are claimed to be efficient for excluding large herbivores (Cox et al., 2010). Visual repellents, e.g. eyespots or predator effigies, are designed to affect birds or mammals (Mason, 1998). Chemical repellents against rabbits, rodents, pigeons and pets are available from €17 (19US$) (Google shopping, 2017). Acoustic repellents include distress calls, or sirens (Mason 1998, Bishop et al., 2003). Acoustic ground rodent mole repellents can be purchased from €7 (8US$). Many repellents offer only short-term benefits, the animals rapidly becoming habituated to their effects. This has led to many claims for 'effectiveness' when the method may have no long-term benefit. The rotation of different repellents or deterrents can improve their effectiveness, as can reinforcing their effects through scaring or limited lethal control (Cook et al., 2008). Our understanding of the effectiveness of many nonlethal repellent measures is limited due to a lack in knowledge of animal behaviour (Shivik et al., 2003) and further controlled experiments are needed to better understand their short and long-term effects.

4.3. Access management

Access management can limit the number of escaping individuals: zoos, fur farms and fish farms, for instance, apply access management measures for the purpose of biosecurity and escape prevention. Security fencing or enclosed sheds mitigate the risk of escape and prevent other domestic animals, people, and wildlife from coming into contact with the captive species, and simple measures to prevent escapes, such as keeping doors closed, can help to minimize the risk of farm animals escaping (CMBA, 2013). Pen constructions used for fur farming need to meet certain criteria in order to prevent animals from escaping and conform to animal welfare regulations (Grandin, 1990; Korhonen and Niemelä, 1997). The proper handling and maintenance of such pens is essential to prevent escapes. The access control security systems play a crucial role in preventing the escape of specimens.

In marine aquacultures, escape risks are posed by structural failures such as progressive mooring failure, breakdown and sinking of steel fish farms, or abrasion and tearing of nets, as well as by operating errors on the part of the personnel. Technical regulations for the design, dimensioning, installation and operation of sea-cage farms, as well as regular training of the fish farm staff, are required to mitigate the risk of fish escaping (Jensen et al., 2010). Electronic gates and controlled access by PIN or proximity readers, enable control of both vehicle and pedestrian movement.

4.4. Movement control

4.4.1. Wing clipping

The technique of wing clipping varies between bird species and their flying ability, but generally the first 5 to 10 outermost flight feathers (primaries) on both wings are clipped (McMillan 2011). The number of feathers clipped and the degree of cutting will determine the ability to fly, however; under certain conditions, wing-clipped birds may be capable of enough flight to escape enclosures.

Unprofessional handling may injure the bird. One wing clipping is the standard used by UK gamekeepers to restrict flight of pheasants during early life or when in breeding pens and does not cause the problem of disorientation. As bird feathers are periodically moulted, clipped wings will regrow and the animal will regain its ability to fly. The timing and frequency of wing moult varies widely between different species. The procedure should therefore be undertaken by trained staff or avian veterinarians. Furthermore, the ability to fly is considered an important part of a bird's quality of life, and wing-clipped birds may lethally injure themselves while trying to fly (Devisscher et al., 2012; McMillan, 2011). Wing clipping is nevertheless recommended for pet birds.

4.4.2. Pinioning

Pinioning is a surgical procedure consisting of the removal of one pinion joint. The operation typically involves amputation of the part of the wingtip from which the flight feathers (primaries) grow by severing the second and third metacarpal bones. An alternative method is a tendonectomy involving the removal of part of a tendon from the wing, which results in reduced flying ability but leaves the bird fully feathered. Pinioning permanently prevents bird from flying. In several countries, e.g. Australia and Germany, use of this measure is legally restricted to certain bird species (Dollinger et al., 2013; NSW, 2017). To guarantee the health of affected birds and because they need to be anesthetised for the procedure, pinioning must be performed by a registered veterinarian. Birds exhibited in zoos were often pinioned in the past, but due to the negative public perception of this measure and for animal welfare reasons, pinioning is now used less in zoos (Klausen, 2014).

4.4.3. Brailing (bird straps)

Brailing is a measure of preventing a bird from flying by tying its wings, usually with plastic strips, making it is impossible for the animal to open them fully. It is a traditional method used to keep large birds from escaping (Curton, 2001).

4.5. Measures in use to prevent contained specimens from escaping or entering for the control of the 19 vertebrates on the list of IAS of Union concern

The following section provides a summary and interpretation of the available information on the potential contribution and application of non-lethal measures to prevent contained specimens from escaping or entering for the management of each of the 19 IAS of Union concern. The classification is based on evidence from peer reviewed papers, technical reports and notes or expert opinions. Table 4 summarizes the feasibility of each measure to capture specimens in four categories:

- **Not effective - evidence based** (**N**) - The measure was tested or applied for the vertebrate of the list of IAS of Union concern and considered to be **not** effective. The information is based on strong evidence e.g. publication or technical note;
- **Effective - evidence based** (**Y**) - The measure was tested or applied for the vertebrate of the list of IAS of Union concern and considered **to be** effective. The information is based on strong evidence e.g. publication or technical note;
- **Possibly feasible** (**P**) - the measure is currently not applied for the vertebrate of the list of IAS of Union concern, but it is commonly in use to prevent the escape of species of similar taxonomical, morphological or ecological group, e.g. aquatic rodents. The feasibility of the particular measure is based on strong evidence e.g. publication or technical note.

The availability of each measure is also summarized in Table 4, in the following categories:

- **Commercially available within the EU** (Y) - The supplies are commercially available within the EU (Including e-commerce). The average price and at least one provider is known;
- **Commercially not available within the EU** (X) - Based on a desk survey, the supplies are not commercially available in the EU (Including e-commerce);
- **Limited or partially available** (L) - The supplies are only available for non-commercial purposes, e.g. research, or the use of the trap is legally restricted in EU Member States.

Egyptian goose (*Alopochen aegyptiacus*): All techniques of movement control can be applied for the management of large and medium size bird species. However, pinioning is not feasible for the management of large populations in the wild due to animal welfare concerns. Wing clipping and pinioning is widely in use to prevent birds to escape from zoos or among game keepers.

House crow (*Corvus splendens*): All techniques of movement control can be applied for the management the House crow. However, pining is not recommendable for the management of large populations in the wild due to animal welfare concerns.

Ruddy duck (*Oxyura jamaicensis):* All techniques of movement control can be applied for the management of large and medium size bird species. However, pinioning is not feasible for the management of large populations in the wild due to animal welfare concerns.

Sacred ibis (*Threskiornis aethiopicus*): All techniques of movement control can be applied for the management of large and medium size bird species. However, pinioning is not feasible for the management of large populations in the wild due to animal welfare concerns.

Coati (*Nasua nasua*): Fences are applied for zoos and fur farming to prevent the coati from escaping. Exclusion fences are currently in common use to mitigate the invasive impacts of carnivores. Further it would be feasible to adopt exclusion fences to exclude the species from protected areas, as is it in use for other carnivores. This approaches would require species-specific feasibility studies.

Coypu (*Myocastor coypus*): In Germany the landscape park Branitz war protected from the invasion of coypus by fencing of channels (Walther et al., 2011). Exclusion fences are used in the USA to prevent re-introduction of coypus after eradication actions (Carter & Leonard, 2002).

Fox squirrel (*Sciurus niger*): Chemical repellents such as Squirrel-Away™ are commercially available. Squirrel repellent spray are available from €6 (Google shopping, 2017). Chemical repellents are in use to prevent terrestrial rodents from entering. The feasibility is limited, because currently available repellents offer only short-term benefits.

Grey squirrel (*Sciurus carolinensis*): Chemical repellents use predator odours, e.g. of the red fox (*Vulpes vulpes*), the raccoon (*Procyon lotor*), to deter grey squirrels from eating butternuts (*Juglans cinerea*) (Rosell, 2001). The effectiveness is limited, because currently available repellents offer only short-term benefits. It is unlikely that repellents would be feasible for the population management of this species.

Pallas's squirrel (*Callosciurus erythraeus*): Chemical repellents are under development for the control of *C. erythraueus* in Japanese frosts (Tamura & Ohara, 2005) are in use to prevent rodents from entering. The feasibility is limited, because currently available repellents offer only short-term benefits.

Muskrat (*Ondatra zibethicus*): Accesses management was effectively used to prevent muskrat damage to water courses (Campbell-Palmer *et al.*, 2016). "*In this scenario flood walls are protected against muskrat by 1) enlarging them (over-dimensioning), 2) inserting mesh wire or steel walls or 3)* *outside reinforcement with a strong layer of large stones, concrete, or bitumen*" (Bos, 2017). In North America**,** some sites have had great success in excluding the species with fencing, where wire mesh is used above and below ground. An example, 'Ducks Unlimited' in Canada uses 5 cm × 5 cm galvanized stucco wire mesh (hardware cloth), with about 0.30m of the mesh vertically buried, about 0.5m upright, and another 0.3m set to a 45^{o} angle sloped away from the wetland (Kadlec et al., 2007). However, it is important to note that muskrats did burrow under a fence (buried to 0.45m) at one site, and Kadlec et al. (2007) recommend the fencing should likely be increased to 0.6m or more. In addition, berm slopes surface protection using chain link fencing and rip rap can be effective (Kadlec et al., 2007).

Raccoon (*Procyon lotor*): "*Exclusion, if feasible, is usually the best method of coping with raccoon damage*" (Boggess, 1994). Fencing and access management are used in fur farms to prevent the species from escaping or entering. In the USA nest-protector fences (5.1cm mesh chicken fence, 61cm high) were effectively used to protect bird nesting from the raccoon (Sargeant et al., 1974). Electric fences, which should be turned on in the evening before dusk, and turned off after daybreak, increase the effectiveness (Boggess, 1994). Fencing designs require species specific adaptations. Exclusion fences are only feasible when it is a complementary measure for further conservation strategies.

Raccoon dog (*Nyctereutes procyonoides*): Fencing and access management are used in fur farms to prevent the species from escaping or entering. "*A one metre high fence is enough to contain raccoon dogs"* (Mulder, 2013). According to legal requirements, e.g. in Poland and Finland, a two meters high fence is required. As mostly raccoon dogs escape through the negligence of their pet owner, further actions to raise the public awareness are needed (Mulder, 2013). Exclusion fences are only feasible when it is a complementary measure for further conservation strategies.

Reeves' muntjac (*Muntiacus reevesi*): Exclusion fencing using common deer fences is not effective to exclude the muntjac. Muntjacs can either go through the fence or go under the fence (Smith-Jones, 2004). Mesh wire fences "*Electric fences, chestnut paling and dead hedging are often used, but are less reliable than wire fences at excluding muntjac*" (Cooke, 2004).

Siberian chipmunk (*Tamias sibiricus*): Chemical repellents are in use to prevent rodents from entering. The Siberian chipmunk can be effectively excluded using electronic ultrasonic repellents, which are commercially available from 39 EUR (The good life, 2017, Bird-X, 2017). Chemical repellents are in use to prevent rodents from entering.

Small Indian mongoose (*Herpestes javanicus*): Repellents and deterrents could be feasible for the prevention of the entering of the small Indian mongoose. The application would require species-specific feasibility studies. Sensitive areas and nests can be protected by placing fencing and metal barriers on trees as the species does not climb very well (Roy et al. 2002).

Amur sleeper *Perccottus glenii*: In aquacultures access management measures are in use that could be feasible for the management of the Amur sleeper. Physical and 'virtual' fences are applied to prevent the spread of invasive alien fish species. Nehring and Steinhof (2015) recommend to the installation of migration barriers (electrical deterrent systems, air bubble curtains, chloride or pH-altered locks, facilities for ship hulls cleaning and ballast water exchange etc.) in the Main-Danube Canal and other key canals in Europe, to prevent the further spread of the Amur sleeper.

Topmouth gudgeon *Pseudorasbora parva*: In aquacultures access management measures are in use that could be feasible for the management of the topmouth gudgeon. Physical and 'virtual' fences are applied to prevent the spread of invasive alien fish species. Further species-specific research is required.

Bullfrog (*Lithobates (Rana) catesbeianus*): Permanent (made of 2 to 8 mm x 1200 mm HDPE (polypropylene) with a top return and has an average lifespan of around 15 years) and temporary amphibian fencing (made of polythene sheets) are used to prevent the bullfrog from spreading. Various designs of fences to prevent the migration of amphibians are commonly in use for temporal purposes.

Red-eared slider (*Trachemys scripta*): Various designs of fences to prevent the migration of reptiles are commonly in use for temporal management purposes. Cadi and Joly (2004a) used a 1 cm mesh grating (50cm high) to prevent the Red-eared slider escape from invaded ponds for research purposes.

<u>Table 4.</u> An overview of the potential non-lethal measures to prevent contained specimens from escaping or entering to the 19 vertebrates currently on the list of IAS of Union concern. With respect to **availability,** i.e. Commercially available in the EU (Y), limited or partially available (L), commercially available in the EU (N), feasibility**,** i.e. not effective - evidence based (N), possibly feasible (P) or effective -evidence based (Y).

		Fencing		Repellents and deterrents		Access management		Movement control					
								Wing clipping		Pinioning		Brailing	
		Availability	Feasibility	Availability	Feasibility	Availability	Feasibility	Availability	Feasibility	Availability	Feasibility	Availability	Feasibility
BIRDS	**Egyptian goose** *Alopochen aegyptiacus*	-	-	-	-	-	-	Y	P	L	P	-	-
	House crow *Corvus splendens*	-	-	-	-	-	-	Y	P	L	P	-	-
	Ruddy duck *Oxyura jamaicensis*	-	-	-	-	-	-	Y	P	L	P	-	-
	Sacred ibis *Threskiornis aethiopicus*	-	-	-	-	-	-	Y	P	L	P	-	-
Mammals	**Coati** *Nasua nasua*	Y	Y	-	-	-	-	-	-	-	-	-	-
	Coypu *Myocastor coypus*	Y	P	-	-	-	-	-	-	-	-	-	-
	Fox squirrel *Sciurus niger*	-	-	Y	P	-	-	-	-	-	-	-	-
	Grey squirrel *Sciurus carolinensis*	-	-	Y	Y	-	-	-	-	-	-	-	-
	Pallas's squirrel *Callosciurus erythraeus*	-	-	L	Y	-	-	-	-	-	-	-	-
	Muskrat *Ondatra zibethicus*	Y	Y	-	-	Y	L	-	-	-	-	-	-
	Raccoon *Procyon lotor*	Y	Y	Y	Y	-	-	-	-	-	-	-	-
	Raccoon dog *Nyctereutes procyonoides*	Y	Y	Y	Y	-	-	-	-	-	-	-	-
	Reeves' muntjac *Muntiacus reevesi*	Y	Y	-	-	-	-	-	-	-	-	-	-
	Siberian chipmunk *Tamias sibiricus*	-	-	Y	Y	-	-	-	-	-	-	-	-
	Small Indian mongoose *Herpestes javanicus*	-	-	-	P	-	-	-	-	-	-	-	-
Fishes	**Amur sleeper** *Perccottus glenii*	L	-	-	-	-	-	-	-	-	-	-	-
	Topmouth gudgeon *Pseudorasbora parva*	L	-	-	-	-	-	-	-	-	-	-	-
Amp.	**Bullfrog** *Lithobates (Rana) catesbeianus*	Y	Y	-	-	Y	P	-	-	-	-	-	-
Rep	**Red-eared slider** *Trachemys scripta*	Y	Y	-	-	-	-	-	-	-	-	-	-

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