Article:

Soulsbury, C, Gray, H, Smith, L orcid.org/0000-0002-2966-3559 et al. (5 more authors) (2020) The welfare and ethics of research involving wild animals: A primer. Methods in Ecology and Evolution, 11 (10). pp. 1164-1181. ISSN 2041-210X

https://doi.org/10.1111/2041-210x.13435

Methods in Ecology and Evolution

THE WELFARE AND ETHICS OF RESEARCH INVOLVING WILD ANIMALS: A PRIMER

Carl Soulsbury[1*], Helen Gray[2*], Lauren Smith[2*], Victoria Braithwaite[3†], Sheena Cotter[1], Robert W. Elwood[5], Anna Wilkinson[1] & Lisa M. Collins[2*]

*Joint lead authors

[1] School of Life Sciences, University of Lincoln, UK

[2] Faculty of Biological Sciences, University of Leeds, UK

[3] Department of Biology, Penn State University, USA

[5] School of Biological Sciences, Queen's University Belfast, UK

† 30th September 2019

*Corresponding author: L.Collins@leeds.ac.uk

ABSTRACT

1. Wild animals are used in scientific research in a wide variety of contexts both *in situ* and *ex situ*. Guidelines for best practice, where they exist, are not always clearly linked to animal welfare and may instead have their origins in practicality. This is complicated by a lack of clarity about indicators of welfare for wild animals, and to what extent a researcher should intervene in cases of compromised welfare.

2. This *Primer* highlights and discusses the broad topic of wild animal welfare and the ethics of using wild animals in scientific research, both in the wild and in controlled conditions. Throughout, we discuss issues associated with the capture, handling, housing and experimental approaches for species occupying varied habitats, in both vertebrates and invertebrates (principally insects, crustaceans and molluscs).

3. We highlight where data on the impacts of wild animal research are lacking and provide suggestive guidance to help direct, prepare and mitigate potential welfare issues, including the consideration of end-points and the ethical framework around euthanasia.

4. We conclude with a series of recommendations for researchers to implement from the design stage of any study that uses animals, right through to publication, and discuss the role of journals in promoting better reporting of wild animal studies, ultimately to the benefit of wild animal welfare.

Key words: capture-mark-recapture, animal ecology, ethics, 3Rs, 9Rs, animal welfare, legislation

1. INTRODUCTION

Research involving wild animals covers a wide range of species using different techniques and impacts individual animals, groups, up to the level of whole ecosystems (Sikes & Paul 2013). Fieldwork may often be conducted in less than ideal conditions—in poor weather, non-sterile environments, areas exposed to climate extremes—and has the potential to harm the study animals during capture and handling (Chinnadurai *et al.* 2016). Despite the complexities of these situations, ensuring animal welfare should be a critical part of wild animal study design.

In this paper, we use the World Organisation for Animal Health (OIE 2017) definition of animal welfare, which states that welfare is, '*how an animal is coping with the conditions in which it lives...Animal welfare refers to the state of the animal; the treatment that an animal receives is covered by other terms such as animal care, animal husbandry, and humane treatment.*' Current ethical considerations surrounding the use of wild animals in research are grounded principally in the 3Rs (reduce, refine, replace: Russell, Burch & Hume 1959). The 3Rs were originally designed for laboratory animal research, in which the animals are used as human models, and where the impact of manipulations or procedures is limited to animals participating in the study (Russell *et al.* 1959; Lindsjö, Fahlman & Törnqvist 2016). There are specific issues in the wider application of the 3Rs to wild animal research (Box 1), which led to new proposed variations (9Rs: Curzer *et al.* 2013). Even so, a broad synthesis on working with wild animals in research is lacking. In this paper, we outline the critical welfare-related considerations associated with carrying out wild animal research. These include the welfare implications of capturing, handling and housing; the welfare implications of ecological manipulations and experimental approaches; the consideration of end-points for the study: release, rehoming and euthanasia; and finally, the ethical considerations for publishing research conducted on wild animals. It is not our goal to provide explicit instructions but rather to provide a launch-point for discussions when planning experiments, and encourage the researcher to consider both focal and non-focal animal welfare when designing and implementing experiments. We provide a framework to aid that goal.

2. WELFARE CONSIDERATIONS IN CAPTURING, HANDLING AND HOUSING OF WILD ANIMALS

Any form of intervention on a wild animal will have some impact on that individual, directly or indirectly. A standard ethical approach to the justification of research is to balance research gains against the costs or harm to all involved, and attempt to minimise the negative effects wherever possible (Graham & Prescott 2015; Brønstad *et al.* 2016). In this section, we discuss some of the most common types of intervention in wild animal studies.

2.1 Capturing wild animals

Capturing events are stressful for wild animals (Wilson & McMahon 2006). The impact on the individual ranges from minor to severe; short to long-term; and may be physical, physiological and/or psychological (see Table 1 in: Kukalová, Gazárková & Adamík 2013). The primary consideration of any field researcher must be to minimise these impacts, both to the individual and population.

There are many ways to capture wild animals (see Schemnitz *et al.* 2009), but they generally follow the same rules and techniques (Box 2). Selection of a context- and species-appropriate method is of critical importance and should minimise the number of injuries, mortalities and by-catch. Across studies (Table 1), it is clear that there is considerable taxon-specificity in accepted welfare levels. For example, within vertebrate research, avian studies report much lower injury and mortality rates than all other taxa (Table 1). A key part of reducing any form of injury is continual review and refinement of techniques. Sources of injury or mortality can be predicted by the technique chosen (Vedhuizen *et al*. 2018), timing—e.g. cold or hot weather (Clewley *et al.* 2018; Read *et al.* 2018), or because the target animal has certain risk factors such as size, age, or species (Schonfield *et al.* 2013; Clewley *et al.* 2018; Veldhuizen *et al.* 2018). These risks should be appropriately identified before commencing (see suggested refinement below).

How can we improve capture techniques? There needs to be a universal maximum level of acceptable injury and mortality. Rather than restricting methods of capture, such thresholds would serve to identify problematic techniques that need urgent refinement. Such rates should continue to be debated, but thresholds of <2% mortality are suggested (Arnemo *et al*. 2007). Injury rates are harder to characterise since injuries could range from minor (e.g. superficial abrasion) to serious (e.g. broken bone) (Iossa, Soulsbury & Harris 2007). Studies have used injury scoring (e.g. mammals: Powell & Proulx 2003; Iossa *et al.* 2007), but these typically focus on probability of survival and not pain or long--term effects on fitness (Iossa *et al*. 2007). There is no accepted threshold for injury levels; we suggest that: (a) researchers actively report whole body injury scores (e.g. Table 4 in Iossa *et al.* 2007), and (b) the following maximum injury thresholds as acceptable for capture techniques: <2% serious injuries, <5% moderate injuries, <10% mild injuries only.

A second way we can improve capture techniques is through more thorough risk assessment processes identifying the potential consequences for both target species as well as affected non-target species. This provides an opportunity to consider the entire process—including handling and processing— and identify suitable areas for refinement.

Thirdly, there should be standard reporting in journal methods of injury and mortality rates; such data would then available for future review, analyses and further refinement.

Regardless of method used, there is always the likelihood that non-target species are caught. Selectivity of method is an important consideration in method choice, and many non-target species may be at greater risk of injury and mortality than target species (Iossa *et al*. 2007). Again, clear reporting of selectivity rates (% of total captures) anf injury rate of non-target species should be part of methods sections.

Finally, physical injury and pain are only one facet of the distress associated with capture methods. Anxiety, stress and escape behaviour will also negatively impact animal welfare (Marks *et al.* 2004). When prolonged, distress having deleterious effects on animal health and subsequent survival (Moberg 1999). Trap type (Cattet *et al.* 2003) and coverings (Bosson, Islam & Boonstra 2012) can impact capture stress levels.

In contrast to vertebrates, invertebrates have received little attention in terms of efficacy and mortality rates of capture techniques, with no comparative studies available. Evidence from commercial fishing of crustaceans suggests injury and mortality rates can be high during capture (Table 1). For insects, mortality is often an expected outcome of sampling, unless the aim is the mark and recapture of individuals, live experimentation, or husbandry in the laboratory. Mortality is not always necessary for sampling and many techniques exist that minimise mortality and allow safe release of captured insects – methods are often designed for convenience of sampling, rather than a specific purpose. Drinkwater, Robinson and Hart (2019) provide important insights into the shifting public opinion and laws to protect invertebrate welfare during scientific studies. Their recommendations very much align with the principles of the 3Rs: to use appropriate power analyses; reduce by-catch by refining trapping methods and retain by-catch for further studies; and minimise suffering (Drinkwater *et al*., 2019).

2.2 Handling wild animals

Handling wild animals should be avoided whenever but, if necessary, should be minimal. Total processing time from capture to release should be minimised: faster total processing time can reduce stress, injury and mortality (Langkilde & Shine 2006; Ponjoan *et al.* 2008; Deguchi, Suryan & Ozaki 2014). During the interval between capture and release, many species benefit from being kept in the dark, either completely or at least by covering the eyes (e.g. Mantor, Krause & Hart 2014).

2.3 Physical sampling

The welfare implications of specific procedures used during handling have received little attention, despite the importance of handling methods being recognised in laboratory settings (Cloutier *et al.* 2015, Gouveia & Hurst 2017). A handful of studies have compared broad outcomes, such as survival between groups undergoing different procedures (Douglass *et al.* 2000; Wimsatt *et al.* 2005). However few studies have compared the stress of specific procedures during handling: for example the stress of microchipping versus toe-clipping in lizards (Langkilde & Shine 2006); or the additive stress of blood sampling that after capture in snakes (Bonnet, Billy & Lakušić 2020). For most species and handling procedures, the extent that procedures themselves cause additive stress and the duration over which they compromise welfare is unclear. This component of wild animal studies needs to be addressed.

The impact repeated exposure to procedures have on an animal, cumulatively, over their lifetime is less clear. Existing evidence indicates repeated captures have either no effect (Rode *et al.* 2014), or deleterious effects (Cattet *et al.* 2008; Sharpe *et al.* 2009). This depends on the species, methods, and parameters measured. Research into cumulative impacts of repeated procedures has also received little attention and again, needs urgent research attention.

2.3.1 Anaesthesia and surgery

Anaesthesia can be used during the capture and/or handling process. Field wildlife anaesthesia can improve safety for both researchers and animals, and is often necessary for both invasive (e.g. surgical, blood collection) and non-invasive (e.g. morphometric, collaring) research. The use of anaesthesia in wild animals is challenging as there are little information available on procedures, difficult environmental conditions, and mixed welfare outcomes (reviewed by Chinnadurai *et al.* 2016). Anaesthesia comes with its own increased risk of mortality, even with well-established protocols (0.2-2.2% mortality: Arnemo et al. 2006; 9% mortality (Chirife & Millan 2014). It requires a high level of training and skill and may engage specific national legislation or regulation. It is particularly challenging in smaller animals as there are smaller margins of error with dosage. In particular, continuous monitoring of stress levels and degree of unconsciousness is essential , in order to avoid over or under-dosing record-keeping of anaesthetic events (Chinnadurai *et al.* 2016). Whilst most widely used in vertebrates, anaesthesia can also be used for invertebrates (see Lewbart *et al*. 2012), some of which are suitable for field use (e.g. Venarsky & Wilhelm 2006; Loru *et al.* 2010). However, in most scenarios anaesthesia is unnecessary and in general has been poorly studied in invertebrates.

Anaesthesia can reduce stress during handling (e.g. Mentaberre *et al*. 2010), but can also lead to behavioural changes post-anaesthesia (e.g. fish: Caudill *et al*. 2014; nest abandonment in birds: Machin & Caulkett 2000). Handling without anaesthesia can potentially return animals to their social groups more quickly and allow release without danger of predation. When anaesthesia is used and recovery is slower, trapped animals may need food, water, help to maintain thermoregulation, and other resources, as well as protection from predation, conspecifics or weather until they can be returned to the wild. Given the level of complexity involved in the use of anaesthesia and post-anaesthetic care, it is essential that researchers and veterinarians evaluate all aspects of the protocol, prior to commencing work, in an effort to minimize animal risk. All available options should be considered before researchers choose to use anaesthesia.

Regardless of species, any form of surgery is significant and alternatives should be considered. This is especially true when carrying out surgery in the field, given the additional challenges of administering anaesthesia, maintaining aseptic techniques, and potentially introducing antibiotics to wild animals and the environment (Mulcahy 2013; Fiorello *et al.* 2016). Guidance on the considerations for field surgery are detailed in Chinnadurai *et al.* (2016) and Fiorello *et al.* (2016), including the provision of analgesia.

2.3.2 Blood and haemolymph sampling

Blood sampling is invasive and should be justified in any study protocol. Many of the key considerations in blood sampling are species- and study-specific. For vertebrates, these include site of blood sampling (e.g. caudal, brachial, facial or pinnal veins), blood volume, and the temporal pattern of sampling. In particular, no more than 10% of blood volume should be taken at once, equating to approximately 1% body mass, or if sampled multiple times, no more than 1% blood volume every 24 hours (Diehl *et al.* 2001). Little consideration has been given to sampling from invertebrates. The small size of many invertebrates makes it difficult to take haemolymph samples, and often small volumes must be collected. With the exception of cephalopods, sampling of haemolymph from invertebrates operates with little guidance. Cephalopods lack superficial blood vessels making blood sampling difficult (Fiorito *et al.* 2015); additionally, their haemolymph is pale blue (oxygenated) or colourless (deoxygenated), meaning haemorrhage can be difficult to detect (Fiorito *et al*. 2015). For other invertebrates, it is recommended that a minimum volume for analysis is taken if the animal is to be released or live afterwards. Techniques for microsampling small invertebrates exist (e.g. Piyankarage, Featherstone & Shippy 2012). The presence of an open haemocoel simplifies sampling, however, the hydrostatic skeleton of many insects means that the haemolymph can be under pressure and too large a puncture can result in excessive bleeding (SCC personal observation). To ensure the insect survives the procedure, it is critical the cuticle is punctured at a shallow angle to avoid piercing the gut. Moderate volumes of haemolymph (2-50ul) can be sampled without adverse effects on survival by using a narrow gauge needle for larger insects (e.g. >0.15g), or a pulled glass capillary tube for smaller insects. If large or whole body volumes must be taken, researchers must consider welfare and plan for potential euthanasia.

2.3.3 Marking and tagging

Animals can be marked using external marks—colouring, tattooing, branding or appendage clipping (reviewed by Silvy, Lopez & Peterson 2005); external tags or devices—radiotransmitters, leg rings, ear tags, collars, harnesses; or internal tags or markers—PIT tags, chemical markers. The relative merit of each technique varies based on the species and the study purpose (Figure 1 & Box 3).

Marking, even with small physical marks (such as leg rings or nail varnish), can have negative effects on an individual's health and behaviour (Table 2). Marks made by ear, toe, exoskeleton or fin clipping, skin punches, or permanent marks such as tattooing and branding are considered controversial (Murray & Fuller 2000; Hagler & Jackson 2001). Ethically, the question remains whether these types of marking methods should be permitted and contradictory findings regarding their impacts only muddy the water. For example, when compared with other techniques, toe clipping has been reported as both more (Narayan *et al.* 2011) and less stressful than PIT tagging (Langkilde & Shine 2006; Guimaraes *et al.* 2014). Exoskeleton—or sometimes leg or wing— clipping in invertebrates is only applicable to a handful of species (Hagler & Jackson 2001), but may also impact reproduction (e.g. Hall *et al*. 2015). In many cases, alternative methods of marking are available (visible and UV-visible tattooing: Petit *et al.* 2012; McGregor & Jones 2016), and studies need to make compelling justification for using more invasive methods of marking, including a specific cost-benefit analysis.

Some forms of identification are relatively lightweight (e.g. British Trust for Ornithology, AA bird ring = 0.04g), but devices such as geolocators, radiotransmitters and GPS transmitters are considerably heavier. Evidence suggests that behaviour and fitness can be impacted by device weight (Bodey *et al.* 2017) and researchers follow a rule of thumb that devices should weigh no more than 3-5% of an animal's body mass. These thresholds are somewhat arbitrary (Gessaman & Nagy 1988) and based on limited data. For example, the 3% rule appears to be extrapolated from studies of albatross and petrel device load and behaviour (Phillips, Xavier & Croxall 2003). Although there are studies demonstrating negative effects of devices at or greater than 5% of body mass, this has also been shown to be the case with devices less than 3% of body mass (Table 2; Bodey *et al.* 2017). Exceeding the 5% and 3% thresholds in vertebrate studies is more commonplace for specific groups, for example bats (O'Mara, Wikelski & Dechmann 2014) and chelonia (Fordham *et al.* 2006).

Threshold rules are often not considered invertebrates, with insect biologgers weighing anything from 2 to 100% of the insect's body mass (Kissling, Pattemore & Hagen 2014). Few studies have examined the impacts on insect welfare, particularly regarding the energetic costs of carrying such loads and impacts on social behaviour and survival (12% studies quantified impact: Batsleer *et al*. 2020). Tagged individuals are often the largest in the population and have better inherent survival (Le Gouar *et al.* 2015), but further research is needed to fill the knowledge gap and inform best practice (Batsleer *et al*. 2020). Additionally, for all species, it is important to consider the standard fluctuations in body mass that individuals may experience even within relatively short timescales (e.g. Blackburn *et al.* 2016). Despite technological advancement leading to ever-smaller devices, this has not decreased the percentage device weight being carried but instead, devices are being deployed on smaller species (Portugal & White 2018). Researchers must minimise the weight of the transmitter, rather than to maximise the load carried.

In addition to the weight of any biologging device, researchers must consider the mode of attachment to the animal's body. Broadly, there are two main methods: internal implantation or external attachment. The effects of such attachments have been previously reviewed in birds (see Barron *et al*. 2010; Costantini & Moller, 2013) and marine mammals (Walker *et al*. 2012). Wide ranging effects of device attachment have been reported, from seemingly no response, to negative impacts on behaviour, health, reproduction and survival (key examples given in Table 2). Long term behavioural and physiological measures outside of the focus of a given study are often not recorded and as such, the true impact of devices is likely unknown. The choice and placement of biologging devices needs careful consideration for the ecology, lifestyle, morphology and physiology of the study species (Casper 2009). The impacts should be considered beforehand (Todd Jones *et al.* 2013) and reported as standard in subsequent publications, including, metrics of impacts (Wilson *et al*. 2019).

Before deciding on a device and attachment, consideration of data recovery is required. Some devices capture, store, and send data remotely, whereas others use timed or biodegradable drop-offs, thereby removing the need for a second capture event and additional stress. Remote drop-off and download technology are not always feasible as they can add significant weight to devices (Thomas, Holland & Minot 2012). Additionally, using biodegradable material or weak links may limit long-term device attachment and function—for example, the collection of physiological data may not allow remote downloads or drop-offs. Though not always possible, attempts should be made to detach or remove devices. Where devices are left on long-term post-study, this should be accounted for in the cost-benefit analysis.

2.3.4 Capturing and killing

Field researchers may be faced with the choice whether animals need to be killed as part of the study design. For some studies, the collection of samples by killing is almost routine (e.g. collecting voucher specimens for museums: Russo *et al*. 2017; sampling for many invertebrates: Hohbein & Conway 2018). At the opposite extreme, there is considerable debate centred on whether it is ethical to ever kill an animal (Hayward *et al*. 2019). A number of journals have published guidance on this issue—there will be scenarios where killing of wild animals is justifiable, but that that justification needs to be provided and prior exploration of alternatives evidenced (Vucetich & Nelson 2007; Costello *et al.* 2016; ASAB 2020; Table 3), and reported in the ensuing publication. Journals editors and reviewers ultimately play a key role in shaping this by rejecting studies that do not adequately justify their choice, or where suitable available alternatives have not been used. Where researchers hide their methods deliberately this should be viewed as research misconduct.

2.3.5 Holding and keeping wild animals in captivity

Animals taken from the wild should only be held in captivity where completely necessary and, if the aim is not form a captive population, for a duration that allows their safe release. The process of bringing animals into captivity, e.g. transportation (Box 4), exposes individuals to multiple stressors that can lead to significant initial stress and extended changes to the stress-coping mechanisms that can allow adjustment to captivity (Adams *et al.* 2011; Angelier *et al.* 2016). Researchers should not underestimate the difficulty of designing sets of captive conditions for different species (Schmidt 2010; Box 5). There are arguments for keeping the housing, diet and social conditions ecologically relevant (Beaulieu 2016), however, using standard conditions allows greater reproducibility between studies (Griffith *et al.* 2017). Where some studies include holding animals temporarily in captivity (<24 hours; (Quinn *et al.* 2009) to ~60 days: (Mellish *et al.* 2006), even short periods of confinement may impact an individual's physiology and behaviour post-release (Cooper 2011). For invertebrates, it is possible to hold and breed many species in captivity in large numbers. When obtaining breeding stocks, it is advisable to do so from established captive colonies where these exist (Harvey-Clark 2011).

3. WELFARE CONSIDERATIONS IN ECOLOGICAL MANIPULATIONS AND EXPERIMENTAL APPROACHES

There is widespread use of ecological and environmental manipulations on wild animals in the field. These studies are undoubtedly important in disentangling complex processes, yet few studies properly consider the resulting welfare impact (Cuthill 1991). There is real diversity in the type and nature of experiments and manipulations carried out in the wild (Table 4). Many of these studies directly aim to induce some sort of change that impacts fitness, but it is important to consider longer term and lifelong impacts on individuals. Where studies are likely to have foreseeable direct harm, it is important to consider the balance of risk and reward (Emlen 1993) and utilize frameworks such as the 3Rs in study design (Cuthill 2007) with evidence-based justification of samples sizes, e.g. power analysis. Since manipulation studies can, and do, impact individual animals as part of their aims, it is important that journals and referees interrogate the study's design thoroughly, ensuring full justification of the method.

Researchers should also generally consider the unintended consequences of any work in the field. Researchers may change the environment (see Fedigan 2010) either by direct action or through the presence of the researcher, e.g. impacting predation rates (Isbell & Young 1993). Similarly, studies that manipulate the environment can have ecosystem-wide effects, such as changing species assemblages (Thompson 1982).

4. THE WELFARE IMPLICATIONS OF THE COGNITIVE ABILITIES OF THE STUDY SPECIES

Our understanding of animal sentience, the ability of an animal to experience positive and negative affective states (Duncan 2006), is inextricable to our perception of the cognitive abilities of that particular species. Researchers must consider the cognition of their study species and the implications of their research on the animal as a result of this. Unfortunately, there are still vast gaps in our knowledge of cognition across the animal kingdom and our general perception of a species' cognition is not necessarily reflective of their actual cognitive abilities. Recent research has found remarkable cognitive abilities in species that are traditionally considered unintelligent (e.g. Matsubara, Deeming & Wilkinson, 2017). This presents a challenge to our knowledge of animal sentience.

Researchers should familiarise themselves with information regarding the cognitive abilities of their study species and, where there is uncertainty around their cognitive abilities, they should be treated as though they have the capacity for both positive and negative affective state (Chan, 2011).

5. END-POINTS: THE CONSIDERATION OF RELEASE, REHOMING AND EUTHANASIA FOR WILD ANIMALS

During work involving wild animals, researchers will be faced with a choice of how to proceed at the end of any capture event or study. The available options are normally limited to keeping the animal in captivity temporarily or indefinitely, releasing it back into the wild, or euthanasia, depending on local or national regulations. We note that use of the term euthanasia (as opposed to killing, which we have used more generally throughout the paper) is reserved for those situations where killing is not only carried out humanely, but also to the benefit of the animal (Broom 2007).

5.1 Release of wild animals

Where capture, handling, and processing durations are rapid, animals should—wherever practically, legally and ecologically feasible—be released back at the site of capture when they have fully recovered from procedures (Box 6). For animals held for long time periods, their absence from the social group, territory, or home range can cause changes in status with knock-on impacts for resource retention (Krebs 1982). If animals are released after being held in captivity, as small a number as possible should be used, based upon sample size calculations. In addition, if kept for extended periods in captivity, reintroduction is needs to be carefully managed. Unless animals are bred specifically for release, i.e. research surrounding reintroduction programmes for conservation or restocking of wild populations, wild animals bred in captivity are generally unsuitable for release into the wild.

5.2 Injured or sick wild animals

It is inevitable that researchers will encounter, or unintentionally cause, sickness or injury to wild animals. When faced with a sick or injured wild animal there are three possible courses of action: no intervention; treatment; or euthanasia (Kirkwood, Sainsbury & Bennett 1994). From a purely welfare perspective, there are circumstances under which each of these is justifiable. Treatment is justifiable if an animal is likely to recover without treatment but its welfare will be improved by treatment (e.g. by reducing the time to recovery), or if the animal is unlikely to recover without treatment and treatment—with subsequent management and release—can be accomplished with relatively little stress to the animal. Treatment can involve minor procedures such as cleaning wounds and administering antibiotics (Elbroch *et al.* 2013) to minor stitching (Melton 1980). In most countries, such treatment must be conducted by, or under the guidance of a veterinarian. From the perspective of wildlife research, rapid *in situ* treatment is preferable. Choosing to treat a wild animal is therefore an important part of contingency planning during the design stage (Box 2).

In rare cases, injured wildlife may be brought into captivity for rehabilitation, but this should only be considered in extreme cases. For most researchers, there is insufficient capacity for the housing and treatment of wild animals for extended periods of time. If a wild animal requires such a significant degree of rehabilitation, then dedicated rehabilitation centres or euthanasia should be considered as the only options. If animals are to be released from rehabilitatation centres, careful consideration needs to be given to the impact of release on host populations (Mullineaux 2014).

5.3 Euthanasia

Inevitably, there will be circumstances when wild animals will need to be euthanised. This is performed when an animal's pain and/or distress is substantial and/or giving treatment is not possible (Figure 2), or where post-study release is not feasible (e.g. many invertebrate studies). Once the decision to euthanise has been made, it is the researcher's responsibility to ensure that it is conducted in a way that minimises pain, distress, and time to clinical death. In evaluating methods of euthanasia, researchers should consider the following key factors: (1) their ability to induce loss of consciousness and death with minimal pain and distress; (2) time required to induce loss of consciousness; (3) reliability of method; (4) safety of personnel; (5) irreversibility of method; (6) compatibility with intended animal use and purpose; (7) documented emotional effect on observers or operators; (8) compatibility with subsequent evaluation, examination, or use of tissue; (9) drug availability and human abuse potential; (10) compatibility with species, age, and health status; (11) ability to maintain equipment in proper working order; (12) safety for predators or scavengers should the animal's remains be consumed; (13) legal requirements; and (14) environmental impacts of the method of disposal of the animal's remains (AVMA 2013).

Methods of euthanasia are exceptionally varied, and it is beyond the scope of this review to cover them all (but see Leary *et al.* 2013). Preparation beforehand is critical, especially knowing the identity and availability of the responsible person with the appropriate level of training and experience. Species that are less commonly used should have appropriate methods and guidance drawn up in advance of the work (e.g. cephalopods: Andrews *et al.* 2013). There is continued debate about the use of certain methods (e.g. for reptiles and amphibians: Lillywhite *et al.* 2017), so it is important to check current, up-to-date guidance and periodically check for refinements in euthanasia protocols. Appropriate methods for euthanasia of invertebrates, including cephalopods, requires further study, but there is existing taxa-specific guidance available (see Murray 2006; Andrews *et al.* 2013).

Death must be confirmed before disposal of animal remains. A combination of criteria is most reliable in confirming death. In mammals and birds these include a lack of central pulse, breathing, corneal reflex and response to firm toe pinch, inability to hear respiratory sounds and heartbeat through a stethoscope, greying of the mucous membranes, and *rigor mortis*. None of these signs alone, except rigor mortis, confirms death. For other taxa, death must be verified carefully using taxa-specific criteria (Andrews *et al.* 2013; Lillywhite *et al*. 2017). Animal remains must be handled appropriately and in accordance with local or national legislation. Regulations apply not only to the disposal of remains, but also the management of chemical residues (e.g. medicines, euthanasia agents) that have the potential to cause secondary poisoning.

6. KEY RECOMMENDATIONS TO RESEARCHERS AND PUBLISHERS

Throughout this paper, it has been clear that there needs to be greater emphasis on the ethical standards of studies conducted on wild animals. Journals often require varying amounts of details about the welfare precautions taken, state of the animals, and the procedures undertaken with justification; many published papers have neglected to include such key information (Field *et al.,* 2019). Journals must take a more active role in protecting animal welfare as a 'critical control point' for publications.

To move forward, we have three key recommendations:

1. Any research proposal involving the use of animals—including invertebrates—should embed the 3Rs (Box 2) or 9Rs (Curzer *et al.* 2013) firmly within the design phase of the study and, where possible, include and report post-study or post-experimental monitoring.

2. The research proposal should be subject to ethical review prior to study commencement. The ethics committee, and reference number, should be identified in the publication's methods or ethics section to allow reviewers and editors to query the ethical review independently. Retrospective applications to an ethics committee should be clearly identified as such within the manuscript and should only be approved if replication of the work would result in significant further harm, and the original work would have otherwise been approved using standardised approaches.
3. There needs to be standardised reporting of key information in methods and results for all studies using wild animals. For some time, these have been used or advocated in laboratory animal work (Kilkenny *et al.* 2010), a similar standard for wild animals is critical (ARROW: Field et al. 2019). Within this, details of the impacts of experiments should be included even if they are not part of the study, e.g. injury and mortality rates. A key future aim should be to use the availability of data in publications to inform future welfare guidance in areas that have currently little research or information.

7. CONCLUSIONS

Wildlife research is an exceptionally broad subject that incorporates a wide variety of study types on many different species and in wildly differing locations. In all areas of research on wild animals, the concept of welfare remains the same. Consideration of welfare should be paramount when studies are designed and conducted to safeguard the welfare of the study animals and improve the quality of science. Whilst this paper is not meant to be the definitive guide to wild animal welfare, it represents a condensed information source that crystallises key areas of ethical and welfare concern and highlights specific areas that need future study. We stress the need for clear reporting and minimum requirements with regard to research practice (Bodey *et al.* 2017; Field *et al.* 2019). Clear reporting in published articles will allow the research community to benefit from collective information to enhance and refine research techniques for wild animals.

Acknowledgements

We'd like to thank two anonymous reviewers and the Associate Editor for extensive comments that have improved this paper.

Dedication

Since writing this paper, our colleague Professor Victoria Braithwaite has sadly passed away. Victoria was an inspirational scientist and hugely influential in the field of animal behaviour and welfare. The authors wish to acknowledge Victoria's contributions both to this paper and to scientific thinking in this area. Thank you Victoria, you are very much missed.

Author contributions

CDS, HG, LS drafted the main text, with all authors (LC, RE, AW, VB, SC, CDS, LS, HG) contributing to sections and to revisions.

8. REFERENCES

Adams, N.J., Farnworth, M.J., Rickett, J., Parker, K.A. & Cockrem, J.F. (2011) Behavioural and corticosterone responses to capture and confinement of wild blackbirds (*Turdus merula*). *Applied Animal Behaviour Science,* **134,** 246-255.

Albin, D. & Karpov, K.A. (1998) Mortality of lingcod, *Ophiodon elongatus*, related to capture by hook and line. *Marine Fisheries Review,* **60,** 29-34.

Aldridge, H. & Brigham, R.M. (1988) Load carrying and maneuverability in an insectivorous bat - a test of the 5-percent rule of radio-telemetry. *Journal of Mammalogy,* **69,** 379-382.

Andrews, P.L.R., Darmaillacq, A.-S., Dennison, N., Gleadall, I.G., Hawkins, P., Messenger, J.B., Osorio, D., Smith, V.J. & Smith, J.A. (2013) The identification and management of pain, suffering and distress in cephalopods, including anaesthesia, analgesia and humane killing. *Journal of Experimental Marine Biology and Ecology,* **447,** 46-64.

Angelier, F., Parenteau, C., Trouve, C. & Angelier, N. (2016) Does the stress response predict the ability of wild birds to adjust to short-term captivity? A study of the rock pigeon (*Columbia livia*). *Royal Society Open Science,* **3**.

Animal Behaviour, (2020) Guidelines for the treatment of animals in behavioural research and teaching. *Animal Behaviour,* **159,** I-X.

Anthony, N.M., Ribic, C.A., Bautz, R. & Garland Jr, T. (2005) Comparative effectiveness of Longworth and Sherman live traps. *Wildlife Society Bulletin,* **33,** 1018-1026.

Barron, D.G., Brawn, J.D. & Weatherhead, P.J. (2010) Meta-analysis of transmitter effects on avian behaviour and ecology. *Methods in Ecology and Evolution,* **1,** 180-187.

Batsleer, F., Bonte, D., Dekeukeleire, D., Goossens, S., Poelmans, W., Van der Cruyssen, E., Maes, D. & Vandegehuchte, M.L. (2020) The neglected impact of tracking devices on terrestrial arthropods. *Methods in Ecology and Evolution*, **11**, 350-361.

Beaulieu, M. (2016) A bird in the house: the challenge of being ecologically relevant in captivity. *Frontiers in Ecology and Evolution,* **4,** 141.

Bennitt, E., Bartlam-Brooks, H.L., Hubel, T.Y. & Wilson, A.M. (2019) Terrestrial mammalian wildlife responses to Unmanned Aerial Systems approaches. *Scientific Reports*, **9**,1-10.

Berteaux, D., Duhamel, R. & Bergeron, J.M. (1994) Can radio collars affect dominance relationships in *Microtus*. *Canadian Journal of Zoology-Revue Canadienne De Zoologie,* **72,** 785-789.

Berzins, L.L. & Dawson, R.D. (2018) Experimentally altered plumage brightness of female Tree Swallows (*Tachycineta bicolor*) influences nest site retention and reproductive success. *Canadian Journal of Zoology*, **96**, 600-607.

Blackburn, E., Burgess, M., Freeman, B., Risely, A., Izang, A., Ivande, S., Hewson, C. & Cresswell, W. (2016) An experimental evaluation of the effects of geolocator design and attachment method on between-year survival on whinchats *Saxicola rubetra*. *Journal of Avian Biology,* **47,** 530-539.

Blackburn, J. &Schmidt, D. (1988) Injury and apparent mortality rates from incidental trawl catches of halibut, king crab, and Tanner crab in the Kodiak area, 1977-81. *Alaska Department of Fish and Game, Division of Commercial Fisheries, Regional Information Report 4K88-21.*

Bodey, T.W., Cleasby, I.R., Bell, F., Parr, N., Schultz, A., Votier, S.C. & Bearhop, S. (2017) A phylogenetically controlled meta-analysis of biologging device effects on birds: Deleterious effects and a call for more standardized reporting of study data. *Methods in Ecology and Evolution*.

Bonnet, X., Billy, G. & Lakušić, M. (2020) Puncture versus capture: which stresses animals the most? *Journal of Comparative Physiology B*, Online Early

Borremans, B., Sluydts, V., Makundi, R.H. & Leirs, H. (2015) Evaluation of short-, mid- and long-term effects of toe clipping on a wild rodent. *Wildlife Research,* **42,** 143-148.

Bosson, C.O., Islam, Z. & Boonstra, R. (2012) The impact of live trapping and trap model on the stress profiles of North American red squirrels. *Journal Of Zoology,* **288,** 159-169.

Bowlin, M.S., Henningsson, P., Muijres, F.T., Vleugels, R.H.E., Liechti, F. & Hedenstrom, A. (2010) The effects of geolocator drag and weight on the flight ranges of small migrants. *Methods in Ecology and Evolution,* **1,** 398-402.

Brønstad, A., Newcomer, C.E., Decelle, T., Everitt, J.I., Guillen, J. & Laber, K. (2016) Current concepts of harm–benefit analysis of animal experiments–report from the AALAS–FELASA working group on harm–benefit analysis–part 1. *Laboratory animals,* **50,** 1-20.

Brooks, C., Bonyongo, C. & Harris, S. (2008) Effects of global positioning system collar weight on zebra behavior and location error. *Journal of Wildlife Management,* **72,** 527-534.

Broom, D. (2007) Quality of life means welfare: how is it related to other concepts and assessed? *Animal Welfare,* **16,** 45-53.

Brydges, N.M., Boulcott, P., Ellis, T. & Braithwaite, V.A. (2009) Quantifying stress responses induced by different handling methods in three species of fish. *Applied Animal Behaviour Science,* **116,** 295-301.

Burgdorf, S.J., Rudolph, D.C., Conner, R.N., Saenz, D. & Schaefer, R.R. (2005) A successful trap design for capturing large terrestrial snakes. *Herpetological Review,* **36,** 421-424.

Burley, N., Krantzberg, G. & Radman, P. (1982) Influence of colour-banding on the conspecific preferences of zebra finches. *Animal Behaviour,* **30,** 444-455.

Burton, A.C., Neilson, E., Moreira, D., Ladle, A., Steenweg, R., Fisher, J.T., Bayne, E. & Boutin, S. (2015) Wildlife camera trapping: a review and recommendations for linking surveys to ecological processes. *Journal of Applied Ecology*, **52**, 675-685.

Caccamise, D.F. & Hedin, R.S. (1985) An aerodynamic basis for selecting transmitter loads in birds. *Wilson Bulletin,* **97,** 306-318.

Casper, R.M. (2009) Guidelines for the instrumentation of wild birds and mammals. *Animal Behaviour,* **78,** 1477-1483.

Cattet, M., Boulanger, J., Stenhouse, G., Powell, R.A. & Reynolds-Hogland, M.L. (2008) An evaluation of long-term capture effects in ursids: Implications for wildlife welfare and research. *Journal of Mammalogy,* **89,** 973-990.

Cattet, M.R., Christison, K., Caulkett, N.A. & Stenhouse, G.B. (2003) Physiologic responses of grizzly bears to different methods of capture. *Journal of Wildlife Diseases,* **39,** 649-654.

Cattet, M.R.L. (2013) Falling through the cracks: Shortcomings in the collaboration between biologists and veterinarians and their consequences for wildlife. *ILAR Journal,* **54,** 33-40.

Chan, K. (2011) Ethical extensionism under uncertainty of sentience: duties to non-human organisms without drawing a line. *Environmental Values*, **20**, 323-346.

Chopin, F.S., Arimoto, T. & Inoue, Y. (1996) A comparison of the stress response and mortality of sea bream *Pagrus major* captured by hook and line and trammel net. *Fisheries Research,* **28,** 277-289.

Clewley, G.D., Robinson, R.A. & Clark, J.A. (2018) Estimating mortality rates among passerines caught for ringing with mist nets using data from previously ringed birds. *Ecology and Evolution,* **8,** 5164-5172.

Cloutier, S., Wahl, K.L., Panksepp, J. & Newberry, R.C. (2015) Playful handling of laboratory rats is more beneficial when applied before than after routine injections. *Applied Animal Behaviour Science,,* **164,** 81-90.

Cooper, C.E. (2011) Southern brown bandicoots can be successfully returned to the wild after physiological experiments. *Wildlife Research,* **38,** 30-33.

Costantini, D. & Moller, A.P. (2013) A meta-analysis of the effects of geolocator application on birds. *Current Zoology,* **59,** 697-706.

Costello, M.J., Beard, K.H., Corlett, R.T., Cumming, G.S., Devictor, V., Loyola, R., Maas, B., Miller-Rushing, A.J., Pakeman, R. & Primack, R.B. (2016) Field work ethics in biological research. *Biological Conservation*, **203**, 268-271.

Coughlin, C.E. & van Heezik, Y. (2014) Weighed down by science: do collar-mounted devices affect domestic cat behaviour and movement? *Wildlife Research,* **41,** 606-614.

Culver, E.F. & Chick, J.H. (2015) Shocking results: Assessing the rates of fish injury from pulsed-dc electrofishing. *North American Journal of Fisheries Management,* **35,** 1055-1063.

Curzer, H.J., Wallace, M.C., Perry, G., Muhlberger, P.J. & Perry, D. (2013) The ethics of wildlife research: A nine R theory. *ILAR Journal,* **54,** 52-57.

Cuthill, I. (1991) Field experiments in animal behavior - methods and ethics. *Animal Behaviour,* **42,** 1007-1014.

Cuthill, I.C. (2007) Ethical regulation and animal science: why animal behaviour is not so special. *Animal Behaviour,* **74,** 15-22.

Deguchi, T., Suryan, R.M. & Ozaki, K. (2014) Muscle damage and behavioral consequences from prolonged handling of albatross chicks for transmitter attachment. *Journal of Wildlife Management,* **78,** 1302-1309.

Diehl, K.H., Hull, R., Morton, D., Pfister, R., Rabemampianina, Y., Smith, D., Vidal, J.M. & Vorstenbosch, C.V.D. (2001) A good practice guide to the administration of substances and removal of blood, including routes and volumes. *Journal of Applied Toxicology: An International Journal,* **21,** 15-23.

Douglass, R.J., Kuenzi, A.J., Wilson, T. & Van Horne, R.C. (2000) Effects of bleeding nonanesthetized wild rodents on handling mortality and subsequent recapture. *Journal of Wildlife Diseases,* **36,** 700-704.

Drahokoupilová, T. & Tuf, I.H. (2012) The effect of external marking on the behaviour of the common pill woodlouse *Armadillidium vulgare*. *ZooKeys*, **176**, 145-154.

Drinkwater, E., Robinson, E.J. & Hart, A.G. (2019) Keeping invertebrate research ethical in a landscape of shifting public opinion. *Methods in Ecology and Evolution*.

Duncan, I.J. (2006) The changing concept of animal sentience. *Applied Animal Behaviour Science*, *100*, 11-19.

Eggers, S., Griesser, M., Nystrand, M. & Ekman, J. (2006) Predation risk induces changes in nest-site selection and clutch size in the Siberian jay. *Proceedings Of The Royal Society B-Biological Sciences,* **273,** 701-706.

Elbroch, L.M., Jansen, B.D., Grigione, M.M., Sarno, R.J. & Wittmer, H.U. (2013) Trailing hounds vs foot snares: comparing injuries to pumas *Puma concolor* captured in Chilean Patagonia. *Wildlife Biology,* **19,** 210-216.

Ellers, O. & Johnson, A.S. (2009) Polyfluorochrome marking slows growth only during the marking month in the green sea urchin *Strongylocentrotus droebachiensis*. *Invertebrate Biology*, **128,** 126-144.

Emlen, S.T. (1993) Ethics and experimentation - hard choices for the field ornithologist. *Auk,* **110,** 406-409.

Emlen, S.T., Demong, N.J. & Emlen, D.J. (1989) Experimental induction of infanticide in female wattled jacanas. *Auk,* **106,** 1-7.

Enge, K.M. (2001) The pitfalls of pitfall traps. *Journal of Herpetology,* **35,** 467-478.

European Convention for the Protection of Animals during International Transport (2006) Available: https://www.coe.int/en/web/conventions/full-list/-/conventions/treaty/193 Accessed May 2018

Fedigan, L.M. (2010) Ethical issues faced by field primatologists: Asking the relevant questions. *American Journal of Primatology,* **72,** 754-771.

Field, K.A., Paquet, P.C., Artelle, K., Proulx, G., Brook, R.K. & Darimont, C.T. (2019) Publication reform to safeguard wildlife from researcher harm. *PLoS Biology*, **17**, e3000193.

Fiorello, C., Harms, C.A., Chinnadurai, S.K. & Strahl-Heldreth, D. (2016) Best-practice guidelines for field-based surgery and anesthesia on free-ranging wildlife. II. Surgery. *Journal of Wildlife Diseases,* **52,** S28-S39.

Fiorito, G., Affuso, A., Basil, J., Cole, A., De Girolamo, P., D'angelo, L., Dickel, L., Gestal, C., Grasso, F. & Kuba, M. (2015) Guidelines for the care and welfare of cephalopods in research–A consensus based on an initiative by CephRes, FELASA and the Boyd Group. *Laboratory animals,* **49,** 1-90.

Fisher, D.O. & Blomberg, S.P. (2009) Toe-bud clipping of juvenile small marsupials for ecological field research: No detectable negative effects on growth or survival. *Austral Ecology,* **34,** 858-865.

Fordham, D., Georges, A., Corey, B. & Brook, B.W. (2006) Feral pig predation threatens the local persistence of snake-necked indigenous harvest and turtles in northern Australia. *Biological Conservation,* **133,** 379-388.

Fox, S.F. & McCoy, J.K. (2000) The effects of tail loss on survival, growth, reproduction, and sex ratio of offspring in the lizard *Uta stansburiana* in the field. *Oecologia*, **122**,327-334.

Gauthier–Clerc, M., Gendner, J. -., Ribic, C. A., Fraser, W. R., Woehler, E. J., Descamps, S., Gilly, C., Le Bohec, C. & Maho, Y. L. (2004) Long–term effects of flipper bands on penguins. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, **271(Suppl 6)**, S423-S426

Gardner, C.J., Deeming, D.C., Wellby, I., Soulsbury, C.D. & Eady, P.E. (2015) Effects of surgically implanted tags and translocation on the movements of common bream *Abramis brama* (L.). *Fisheries Research,* **167,** 252-259.

Gessaman, J.A. & Nagy, K.A. (1988) Transmitter loads affect the flight speed and metabolism of homing pigeons. *Condor,* **90,** 662-668.

Gibb, R., Browning, E., Glover-Kapfer, P. & Jones, K.E. (2019) Emerging opportunities and challenges for passive acoustics in ecological assessment and monitoring. *Methods in Ecology and Evolution*, **10**, 169-185.

Gouveia, K. & Hurst, J. (2017) Optimising reliability of mouse performance in behavioural testing: the major role of non-aversive handling. *Scientific* Reports, **7**, 44999.

Graham, M.L. & Prescott, M.J. (2015) The multifactorial role of the 3Rs in shifting the harm-benefit analysis in animal models of disease. *European Journal of Pharmacology,* **759,** 19-29.

Griffith, S.C. et al. (2017) Variation in reproductive success across captive populations: Methodological differences, potential biases and opportunities. *Ethology,* **123,** 1-29.

Guimaraes, M., Correa, D.T., Filho, S.S., Oliveira, T.A.L., Doherty, P.F. & Sawaya, R.J. (2014) One step forward: contrasting the effects of Toe clipping and PIT tagging on frog survival and recapture probability. *Ecology and Evolution,* **4,** 1480-1490.

Harvey-Clark, C. (2011) IACUC challenges in invertebrate research. *Ilar Journal*, **52**, 213-220.

Haulton, S.M., Porter, W.F. & Rudolph, B.A. (2001) Evaluating 4 methods to capture white-tailed deer. *Wildlife Society Bulletin,* **29,** 255-264.

Henry, P.Y. & Jarne, P. (2007) Marking hard-shelled gastropods: tag loss, impact on life-history traits, and perspectives in biology. *Invertebrate Biology*, **126,** 138-153.

Hohbein, R.R. & Conway, C.J. (2018) Pitfall traps: A review of methods for estimating arthropod abundance. *Wildlife Society Bulletin*, **42**, 597-606.

Hooijmeijer, J., Gill, R.E., Mulcahy, D.M., Tibbitts, T.L., Kentie, R., Gerritsen, G.J., Bruinzeel, L.W., Tijssen, D.C., Harwood, C.M. & Piersma, T. (2014) Abdominally implanted satellite transmitters affect reproduction and survival rather than migration of large shorebirds. *Journal of Ornithology,* **155,** 447-457.

Ibanez-Alamo, J.D. & Soler, M. (2012) Predator-induced female behavior in the absence of male incubation feeding: an experimental study. *Behavioral Ecology And Sociobiology,* **66,** 1067-1073.

Invertebrate Link, (2002) A code of conduct for collecting insects and other invertebrates. *British Journal of Entomology and Natural History,* **15,** 1-6.

Iossa, G., Soulsbury, C.D. & Harris, S. (2007) Mammal trapping: a review of animal welfare standards of killing and restraining traps. *Animal Welfare,* **16,** 335-352.

Isbell, L.A. & Young, T.P. (1993) Human presence reduces predation in a free-ranging vervet monkey population in Kenya. *Animal Behaviour,* **45,** 1233-1235.

Jacob, J., Ylonen, H. & Hodkinson, C.G. (2002) Trapping efficiency of Ugglan traps and Longworth traps for house mice in south-eastern Australia. *Wildlife Research,* **29,** 101-103.

Jenkins, C., McGarigal, K. & Gamble, L. (2003) Comparative effectiveness of two trapping techniques for surveying the abundance and diversity of reptiles and amphibians along drift fence arrays. *Herpetological Review,* **34,** 39.

Jung, T.S. (2016) Comparative efficacy of Longworth, Sherman, and Ugglan live-traps for capturing small mammals in the Nearctic boreal forest. *Mammal Research,* **61,** 57-64.

Kilkenny, C., Browne, W.J., Cuthill, I.C., Emerson, M. & Altman, D.G. (2010) Improving bioscience research reporting: The ARRIVE guidelines for reporting animal research. *Plos Biology,* **8**.

Kirkwood, J.K., Sainsbury, A.W. & Bennett, P.M. (1994) The welfare of free-living wild animals - methods of assessment. *Animal Welfare,* **3,** 257-273.

Kissling, W.D., Pattemore, D.E. & Hagen, M. (2014) Challenges and prospects in the telemetry of insects. *Biological Reviews,* **89,** 511-530.

Krebs, J.R. (1982) Territorial defense in the great tit (*Parus major*) - do residents always win. *Behavioral Ecology And Sociobiology,* **11,** 185-194.

Kukalová, M., Gazárková, A. & Adamík, P. (2013) Should I stay or should I go? The influence of handling by researchers on den use in an arboreal nocturnal rodent. . *Ethology,* **119,** 848-859.

Lane, J.M. & MacDonald, R.A. (2010) Welfare and 'best practice' in field studies of wildlife. . *The UFAW handbook on the care and management of laboratory and other research animals* (eds Kirkwood J & H. R), pp. 92–107. Universities Federation for Animal Welfare, Oxford.

Langkilde, T. & Shine, R. (2006) How much stress do researchers inflict on their study animals? A case study using a scincid lizard, *Eulamprus heatwolei*. *Journal Of Experimental Biology,* **209,** 1035-1043.

Le Gouar, P.J., Dubois, G.F., Vignon, V., Brustel, H. & Vernon, P. (2015) Demographic parameters of sexes in an elusive insect: implications for monitoring methods. *Population Ecology,* **57,** 227-236.

Leary, S.L., Underwood, W., Anthony, R., Gwaltney-Brant, S., Poison, A. & Meyer, R. (2013) AVMA guidelines for the euthanasia of animals: 2013 edition. American Veterinary Medical Association Schaumburg, IL.

Lechenne, M.S., Arnemo, J.M., Brojer, C., Andren, H. & Agren, E.O. (2012) Mortalities due to constipation and dystocia caused by intraperitoneal radio-transmitters in Eurasian lynx (*Lynx lynx*). *European Journal of Wildlife Research,* **58,** 503-506.

Lentini, A.M., Crawshaw, G.J., Licht, L.E. & McLelland, D.J. (2011) Pathologic and hematologic responses to surgically implanted transmitters in eastern massasauga rattlesnakes (*Sistrurus catenatus catenatus*). *Journal of Wildlife Diseases,* **47,** 107-125.

Lewbart, G.A. & Mosley, C. (2012) Clinical anesthesia and analgesia in invertebrates. *Journal of Exotic Pet Medicine*, **21**, 59-70.

Lillywhite, H.B., Shine, R., Jacobson, E., DeNardo, D.F., Gordon, M.S., Navas, C.A., Wang, T., Seymour, R.S., Storey, K.B., Heatwole, H., Heard, D., Brattstrom, B. & Burghardt, G.M. (2017) Anesthesia and euthanasia of amphibians and reptiles used in scientific research: Should hypothermia and freezing be prohibited? *BioScience,* **67,** 53-61.

Lindsjö, J., Fahlman, Å. & Törnqvist, E. (2016) Animal welfare from mouse to moose - implementing the principles of the 3Rs in wildlife research. *Journal of Wildlife Diseases,* **52,** S65-S77.

Loru, L., Sassu, A., Fois, X. & Pantaleoni, R.A. (2010) Ethyl acetate: a possible alternative for anaesthetizing insects. *International Journal of Entomology,* **46**, 422–424.

Mantor, M., Krause, S. & Hart, L.A. (2014) Trapping and handling squirrels: Trap modification and handling restraint to minimize injuries and stress. *Wildlife Society Bulletin,* **38,** 152-159.

Marks, C., Allen, L., Gigliotti, F., Busana, F., Gonzalez, T., Lindeman, M. & Fisher, P. (2004) Evaluation of the tranquilliser trap device (TTD) for improving the humaneness of dingo trapping. *Animal Welfare,* **13,** 393-400.

Matsubara, S., Deeming, D.C. & Wilkinson, A. (2017) Cold-blooded cognition: new directions in reptile cognition. *Current Opinion in Behavioral Sciences*, **16**, 126-130.

McCarthy, M.A. & Parris, K.M. (2004) Clarifying the effect of toe clipping on frogs with Bayesian statistics. *Journal of Applied Ecology,* **41,** 780-786.

McGregor, M. & Jones, D. (2016) Tattoo pens as a low-cost approach to in-field permanent identification of medium-sized mammals. *Wildlife Society Bulletin,* **40,** 169-173.

Mellish, J.A.E., Calkins, D.G., Christen, D.R., Horning, M., Rea, L.D. & Atkinson, S.K. (2006) Temporary captivity as a research tool: comprehensive study of wild pinnipeds under controlled conditions. *Aquatic Mammals,* **32,** 58.

Melton, D.A. (1980) Baboon (*Papio ursinus*) capture using a blow-dart system. *South African Journal of Wildlife Research,* **10,** 67-70.

Moberg, G.P. (1999) When does stress become distress? *Lab animal,* **28,** 422 – 426.

Mock, D.W. (1984) Siblicidal aggression and resource monopolization in birds. *Science,* **225,** 731-733.

Monaghan, P., Nager, R.G. & Houston, D.C. (1998) The price of eggs: increased investment in egg production reduces the offspring rearing capacity of parents. *Proceedings Of The Royal Society B-Biological Sciences,* **265,** 1731-1735.

Mullineaux, E. (2014) Veterinary treatment and rehabilitation of indigenous wildlife. *Journal of Small Animal Practice*, **55**, 293-300.

Mulcahy, D.M. (2013) Legal, ethical, and procedural bases for the use of aseptic techniques to implant electronic devices. *Journal of Fish and Wildlife Management,* **4,** 211-219.

Murray, D.L. & Fuller, M.R. (2000) A critical review of the effects of marking on the biology of vertebrates. *Research techniques in animal ecology: controversies and consequences* (eds L. Boitani & T. Fuller), pp. 15-64. Columbia University Press, New York.

Murray, M.J. (2006) Euthanasia. *Invertebrate Medicine* (ed. G.A. Lewbart), pp. 303-304. Blackwell Publishing, Oxford, UK.

Nager, R.G., Monaghan, P., Houston, D.C. & Genovart, M. (2000) Parental condition, brood sex ratio and and differential young survival: an experimental study in gulls (*Larus fuscus*). *Behavioral Ecology And Sociobiology,* **48,** 452-457.

Narayan, E.J., Molinia, F.C., Kindermann, C., Cockrem, J.F. & Hero, J.M. (2011) Urinary corticosterone responses to capture and toe-clipping in the cane toad (Rhinella marina) indicate that toe-clipping is a stressor for amphibians. *General and Comparative Endocrinology,* **174,** 238-245.

National Research Council (2006) *Guidelines for the humane transportation of research animals*. National Academies Press. https://www.nap.edu/read/11557/chapter/1 May 2018

National Research Council (2010) *Guide for the care and use of laboratory animals*. National Academies Press.

O'Mara, M.T., Wikelski, M. & Dechmann, D.K.N. (2014) 50 years of bat tracking: device attachment and future directions. *Methods in Ecology and Evolution,* **5,** 311-319.

O'Brien, M.F., Lee, R., Cromie, R. & Brown, M.J. (2016) Assessment of the rates of injury and mortality in waterfowl captured with five methods of capture and techniques for minimizing risks. *Journal of Wildlife Diseases,* **52,** S86-S95.

OIE (2017) (Office International des Epizooties) Terrestrial Animal Health Code. http://www.oie.int/en/standard-setting/terrestrial-code/ May, 2018.

Pavone, L.V. & Boonstra, R. (1985) The effects of toe clipping on the survival of the meadow vole (*Microtus pennsylvanicus*). *Canadian Journal of Zoology-Revue Canadienne De Zoologie,* **63,** 499-501.

Perry, G., Wallace, M.C., Perry, D., Curzer, H. & Muhlberger, P. (2011) Toe clipping of amphibians and reptiles: Science, ethics, and the law. *Journal of Herpetology,* **45,** 547-555.

Petit, S., Waudby, H.P., Walker, A.T., Zanker, R. & Rau, G. (2012) A non-mutilating method for marking small wild mammals and reptiles. *Australian Journal of Zoology,* **60,** 64-71.

Phillips, R.A., Xavier, J.C. & Croxall, J.P. (2003) Effects of satellite transmitters on albatrosses and petrels. *The Auk,* **120,** 1082-1090.

Piyankarage, S.C., Featherstone, D.E. & Shippy, S.A. (2012) Nanoliter hemolymph sampling and analysis of individual adult *Drosophila melanogaster*. *Analytical Chemistry,* **84,** 4460-4466.

Ponjoan, A., Bota, G., De La Morena, E.L.G., Morales, M.B., Wolff, A., Marco, I. & Manosa, S. (2008) Adverse effects of capture and handling little bustard. *Journal of Wildlife Management,* **72,** 315-319.

Portugal, S.J. & White, C.R. (2018) Miniaturization of biologgers is not alleviating the 5% rule. *Methods in Ecology and Evolution*.

Powell, R.A. & Proulx, G. (2003) Trapping and marking terrestrial mammals for research: Integrating ethics, performance criteria, techniques, and common sense. *ILAR Journal,* **44,** 259-276.

Quinn, J.L., Patrick, S.C., Bouwhuis, S., Wilkin, T.A. & Sheldon, B.C. (2009) Heterogeneous selection on a heritable temperament trait in a variable environment. *Journal Of Animal Ecology,* **78,** 1203-1215.

Rachlow, J.L., Peter, R.M., Shipley, L.A. & Johnson, T.R. (2014) Sub-lethal effects of capture and collaring on wildlife: Experimental and field evidence. *Wildlife Society Bulletin,* **38,** 458-465.

Read, J.L., Pedler, R.D. & Kearney, M.R. (2018) Too much hot air? Informing ethical trapping in hot, dry environments. *Wildlife Research*, **45,** 16-30.

Rode, K.D., Pagano, A.M., Bromaghin, J.F., Atwood, T.C., Durner, G.M., Simac, K.S. & Amstrup, S.C. (2014) Effects of capturing and collaring on polar bears: findings from long-term research on the southern Beaufort Sea population. *Wildlife Research,* **41,** 311-322.

Russell, W.M.S., Burch, R.L. & Hume, C.W. (1959) The principles of humane experimental technique.

Schemnitz, S.D., Batcheller, G.R., Lovallo, M.J., White, H.B. & Fall, M.W. (2009) Capturing and handling wild animals. *The wildlife techniques manual* (ed. N.J. Silvy), pp. 232-269. Johns Hopkins University Press,, Baltimore, MD.

Schmidt, M.F. (2010) An IACUC perspective on songbirds and their use in neurobiological research. *ILAR Journal,* **51,** 424-430.

Sharpe, F., Bolton, M., Sheldon, R. & Ratcliffe, N. (2009) Effects of color banding, radio tagging, and repeated handling on the condition and survival of Lapwing chicks and consequences for estimates of breeding productivity. *Journal of Field Ornithology,* **80,** 101-110.

Siitari, H., Alatalo, R.V., Halme, P., Buchanan, K.L. & Kilpimaa, J. (2007) Color signals in the black grouse (*Tetrao tetrix*): signal properties and their condition dependency. *American Naturalist* **169**, S81-S92.

Sikes, R.S. & Paul, E. (2013) Fundamental differences between wildlife and biomedical research. *ILAR Journal*, **54**, 5-13.

Silvy, N.J., Lopez, R.R. & Peterson, M.J. (2005) Wildlife marking techniques. *Techniques for wildlife investigation and management.* (ed. C.E. Braun), pp. 339–376 The Wildlife Society, Bethesda , Maryland

Soulsbury, C.D., Siitari, H. & Lebigre, C. (2018) Stabilising selection on immune response in male black grouse *Lyrurus tetrix*. *Oecologia,* **186,** 405-414.

Spotswood, E.N., Goodman, K.R., Carlisle, J., Cormier, R.L., Humple, D.L., Rousseau, J., Guers, S.L. & Barton, G.G. (2012) How safe is mist netting? evaluating the risk of injury and mortality to birds. *Methods in Ecology and Evolution,* **3,** 29-38.

Steidl, R.J., Hayes, J.P. & Schauber, E. (1997) Statistical power analysis in wildlife research. *Journal of Wildlife Management,* **61,** 270-279.

Stokesbury, M.J.W., Neilson, J.D., Susko, E. & Cooke, S.J. (2011) Estimating mortality of Atlantic bluefin tuna (*Thunnus thynnus*) in an experimental recreational catch-and-release fishery. *Biological Conservation,* **144,** 2684-2691.

Swallow, J., Anderson, D., Buckwell, A.C., Harris, T., Hawkins, P., Kirkwood, J., Lomas, M., Meacham, S., Peters, A. & Prescott, M. (2005) Guidance on the transport of laboratory animals. *Laboratory animals,* **39,** 1-39.

Thomas, B., Holland, J.D. & Minot, E.O. (2012) Wildlife tracking technology options and cost considerations. *Wildlife Research,* **38,** 653-663.

Thompson, S.D. (1982) Structure and species composition of desert heteromyid rodent species assemblages - effects of a simple habitat manipulation. *Ecology,* **63,** 1313-1321.

Tinbergen, J., Tinbergen, J. & Ubels, R. (2014) Is fitness affected by ring colour? *Ardea,* **101,** 153-163.

Todd Jones, T., Van Houtan, K.S., Bostrom, B.L., Ostafichuk, P., Mikkelsen, J., Tezcan, E., Carey, M., Imlach, B. & Seminoff, J.A. (2013) Calculating the ecological impacts of animal-borne instruments on aquatic organisms. *Methods in Ecology and Evolution,* **4,** 1178-1186.

Veldhuizen, L.J.L., Berentsen, P.B.M., De Boer, I.J.M., Van De Vis, J.W. & Bokkers, E.A.M., (2018) Fish welfare in capture fisheries: A review of injuries and mortality. *Fisheries Research*, **204**, 41-48.

Venarsky, M.P. & Wilhelm, F.M. (2006) Use of clove oil to anaesthetize freshwater amphipods. *Hydrobiologia*, **568**, 425-432.

Vucetich, J.A. & Nelson, M.P. (2007) What are 60 warblers worth? Killing in the name of conservation. *Oikos*, **116**, 1267-1278.

Walker, K.A., Trites, A.W., Haulena, M. & Weary, D.M. (2012) A review of the effects of different marking and tagging techniques on marine mammals. *Wildlife Research,* **39,** 15-30.

Weiser, E.L. *et al.* (2016) Effects of geolocators on hatching success, return rates, breeding movements, and change in body mass in 16 species of Arctic-breeding shorebirds. *Movement Ecology,* **4**.

Willson, J.D., Winne, C.T. & Fedewa, L.A. (2005) Unveiling escape and capture rates of aquatic snakes and salamanders (*Siren* spp. and *Amphiuma means*) in commercial funnel traps. *Journal of Freshwater Ecology,* **20,** 397-403.

Wilson, R.P. & McMahon, C.R. (2006) Measuring devices on wild animals: what constitutes acceptable practice? *Frontiers in Ecology and the Environment,* **4,** 147-154.

Wilson, R.P., Holton, M., Wilson, V.L., Gunner, R., Tysse, B., Wilson, G.I., Quintana, F., Duarte, C. & Scantlebury, D.M. (2019) Towards informed metrics for examining the role of human-induced animal responses in tag studies on wild animals. *Integrative Zoology***14,** 17–29.

Wimsatt, J., O'Shea, T.J., Ellison, L.E., Pearce, R.D. & Price, V.R. (2005) Anesthesia and blood sampling of wild big brown bats (*Eptesicus fuscus*) with an assessment of impacts on survival. *Journal of Wildlife Diseases,* **41,** 87-95.

FIGURE LEGENDS

Figure 1: Decision tree for marking wild animals

Figure 2: End-point decision tree: the consideration of release, rehoming and euthanasia for wild animals

Table 1: Examples of capture-related mortality and injury across different methods in vertebrates and invertebrates

Taxa	**Method**	**% injury**	**% mortality**	**Reference**
Birds	Mist netting	0.59%	0.23%	Spotswood *et al.* 2012
Birds	Canon-netting	0.42%	0.1%	O'Brien *et al.* 2016
Mammals	Longworth traps		<1%-10.4%	Jacob *et al*. 2002; Anthony *et al.* 2005; Jung 2016
Mammals	Sherman traps		10-93%	Schonfield et al. 2013
Mammals	Box trap	0-87%	0%	Iossa *et al.* 2007
Mammals	Leg hold snare	18-100%	0-3%	Iossa *et al.* 2007
Mammals	Leg-hold snare			Iossa *et al*. 2007
Mammals	Darting		0-20%	Haulton, Porter & Rudolph 2001
Mammals	Box trap		0-7.6%	Haulton, Porter & Rudolph 2001
Mammals	Clover trap		0.9-20.7%	Haulton, Porter & Rudolph 2001
Mammals	Canon net		4.6-10%	Haulton, Porter & Rudolph 2001
Fish	Electrofishing	0-50.3%		Culver & Chick 2015
Fish	Trammel net		44%	Chopin, Arimoto & Inoue 1996
Fish	Rod and line		3.4-4.3%	Chopin et al. 1996; Albin & Karpov 1998
Herptiles	Funnel trap		1.1-23.4%	Enge 2001; Jenkins, McGarigal & Gamble 2003
Herptiles	Pitfall trap		1.0-19.4%	Enge 2001; Jenkins, McGarigal & Gamble 2003
Crustacean	Trawl		1.2-21%	Blackburn & Schmidt 1988

Table 2. Examples of impacts of marking and tagging to the health and welfare of wild animals.

Taxa	**Mark or device**	**Impact category**	**Details**	**Reference**
Echinoidea	Fluorochrome markers	Survival; Health	Some markers resulted in a growth slowing in the month post-marking. Six-months post-marking there were no differences between controls and marked individuals in growth rate, survival, gonad production or jaw weight.	Ellers & Johnson 2009
Arthropoda	Nail varnish; queen bee marker	Survival; Behaviour	No impacts of marking on survival, but marked individuals showed reduced activity and increased hiding compared to controls.	Drahokoupilova &Tuf 2012
Gastropoda	Glued plastic marks; gouache paint; car body paint; nail varnish; corrective fluid	Reproduction; Survival	There were no effects of any of the marking treatments on life history traits or survival of the animals.	Henry & Jarne 2007
Fish	Surgically or gastrically implanted radio transmitter	Behaviour; Health	Devices weighing 2.3-5% of body mass. Gastrically implanted fish had slower growth, mouth abrasions caused by antennae and impaired feeding behaviour. Inflammation was present for 22% of fish that had surgery.	Adams *et al.* 1998
Mammals	GPS collar	Behaviour	Distances travelled and home range sizes were smaller when cats wore a collar weighing ~ 3% of body mass, compared to those weighing <1% or ~2%.	Coughlin & van Heezik 2014
Mammals	Radio collar	Social	Changes in dominance structure were not affected by collars weighing < 10% body mass, but voles lost dominance when their collar was > 10% body mass.	Berteaux *et al.* 1994

Birds	Transmitter in a back harness	Behaviour; Health; Physiology	Transmitters weighing either 2.5% or 5% of the bird's body mass slowed down flight times to a similar extent on 90 and 320km journeys. Pigeons produced 85-100% more CO_2 on the longer journey with a transmitter than with no equipment attached.	Gessaman & Nagy 1988
Mammals	Toe clipping	Survival	Males lived 2.1 weeks less than non-clipped controls. No effects on female survival.	Pavone & Boonstra 1985
Mammals	Toe clipping	Health; Survival	No infection caused by toe clipping, no growth impacts and no effects on survival in captivity or the wild.	Fisher & Bloomberg 2009
Mammals	Toe clipping	Behaviour; Health; Survival	No impact of toe clipping on body weight or survival. Newly clipped animals travelled further, but may be due to handling effects.	Borremans *et al.* 2015
Herptiles	Toe clipping	Survival	Toe clipping decreased the return rate of animals as a function of the number of toes removed	McCarthy & Parris 2004
Birds	Ringing	Survival	Decreased life expectancy (28% shorter) for individuals without conspicuous rings than for those with inconspicuous rings.	Tinbergen *et al.* 2014
Birds	Flipper bands	Survival	Banded penguins had lower breeding probability and lower chick production. Survival rate of banded chicks after 2–3 years was significantly reduced.	Gauthier–Clerc *et al.* (2004)
Birds	Geolocator in backpack-style harnesses	Aerodynamics	Increased drag for backpack-style harnesses, compared with no harness. Drag was higher when the device was between the wings than when on the rump.	Bowlin *et al*. 2010
Birds	Geolocator attached to leg	Reproduction	Reduced return rates; reduced nesting success; increased partial clutch failure for three out of 23 taxa studied.	Weiser *et al.* 2016

			Mounting perpendicular to the leg increased negative effects on nesting, compared with parallel to the leg. No impact for 20 of the taxa studied.	
Birds	Implantation of intracoelomic devices	Reproduction	Three years post-implantation, 16% lower yearly survival than non-implanted group. Only three eggs were found from two implanted birds and all three were deformed.	Hooijmeijer *et al.* 2014
Fish	Implanted interperitoneal acoustic transmitter	Behaviour and physical health	Short term effects (first five days post-tagging) on behaviour, though not seen long-term. Incisions for implantation were well-healed and clean upon recapture.	Gardner *et al.* 2015
Herptiles	Multiple electronic tags attached to shell	Behaviour; hydrodynamics	Tags had negligible impacts on adult drag (< 5% additional drag), but increased drag significantly (> 100%) for juvenile turtles. Potential negative impact on an individual's ability to conduct standard behavioural repertoire	Todd Jones *et al.* 2013
Herptiles	Implantation of intracoelomic devices	Health	Inflammation in 66% of tested snakes and bacterial infection in 33%.	Lentini *et al*. 2011
Mammals	GPS collar	Behaviour	Negative impact on feeding behaviour, with heavier collars reducing the animals' rate of travel by > 50% when in the foraging patch and drinking area.	Brooks *et al.* 2008
Mammals	Implanted intraperitorneal radio-transmitter	Health	Mortality caused by severe constipation in two animals (the device compressed the colon) and dystocia in another.	Lechenne *et al.* 2012

Table 3: Key considerations for choosing to capture and kill animals for scientific research.

3Rs	**Theme**	**Priority**	**Considerations**
Replacement	Research Question	1	Does the research question require animals to be captured and killed? Can alternatives be used – with non-animals or live animals?
Refinement	Techniques	2	Can different research techniques be used? Cost should not be used as justification for killing animals, compared to other, non-lethal techniques.
Refinement	Source	3	Can existing samples or sources of dead animals be used? Can sample collection avoid collecting new animals?
Reduction	Sample size	4	Can minimal sample sizes be used? If large numbers are needed, then these need to be clearly justifiable with a power analysis.
Refinement	Method	5	The most humane, selective method must be used to kill animals.

Table 4: Examples of different manipulation type experiments and direct and long-term effects on individuals

Manipulation type	**Direct Effect**	**Long term effect**	**Reference**
Vaccination study	Increasing immune response	Reduced survival	Soulsbury *et al.* 2018
Increased egg production	Reduced breeding female condition Reduced chick production Smaller chick size		Monaghan *et al.* 1998
Breeding female removal	Infanticide		Emlen *et al.* 1989
Hormone increase	Increased breeding attempt Sexual ornament size increase	Reduced survival Reduced sexual ornament size	Siitari *et al.* 2007
Playback of predator calls	Reduced incubation behaviour		Ibanez-Alamo & Soler 2012
Playback of predator calls	Reduced clutch size		Egger *et al.* 2006
Reduced female plumage brightness	Reduced offspring quality		Berzin & Dawson 2018
Induced tail loss in lizards	Reduced survival		Fox & McCoy 2000
Food supplementation	Altered egg composition		Siitari *et al.* 2014

BOX 1: 3Rs CHALLENGES FOR WILD ANIMAL RESEARCH

Reduction: A key aim of the 3Rs is to minimise the number of animals used. It is challenging to translate Reduction into practice in wild animal research for several reasons: (i) genetic variation is generally greater in wild animals, meaning they respond more heterogeneously to a given set of conditions. This increased variation often necessitates larger sample sizes than captive populations; (ii) the environmental variation of animals is considerably greater than in controlled laboratory conditions, meaning larger sample sizes are required; (iii) in wild-based studies, animals will be lost due to natural mortality or other random events. Conducting pre-study power analysis is therefore especially important (Steidl, Hayes & Schauber 1997).

Replacement: In laboratory-based research, 98% of all animals used are rodents (UK Home Office 2014). The 3Rs principles promote the use of the lowest sentient forms where possible. In biomedical research, the typical targets are to move towards more *in vitro* and *in silico* research. This is possible because the research focus is a physiological, genetic or other biochemical response within the animal. In wild animal research, Replacement is often not possible as the study focus is often at the level of individual animals, and their interactions within the wider ecosystem. There are scenarios where a species considered less sentient or less protected could be used to test hypotheses (Lane & MacDonald, 2010; Sneddon, Halsey & Bury 2017); in practise such scenarios are likely to be rare, or difficult to generalise with confidence without confirmation at the higher/more protected level.

Refinement: A greater diversity of non-invasive methods has been devised in wild studies, compared to lab-based studies. One driver of this is the need to return animals to the wild as quickly as possible or because techniques may harm the species or population. Approaches such as DNA analysis from the collection of hair or faeces have been well established. There is still a need to collaborate with other disciplines to improve and refine techniques (Cattet 2013). These include greater use of remote methods of monitoring such as camera trapping (Burton *et al*. 2015) or passive acoustic monitoring (Gibb *et al*. 2019), and advances in analytical methods (e.g. machine learning: Tabak *et al*. 2019). Though, there must be awareness that these may still have a negative effect (e.g. drones: Bennitt *et al*. 2019).

BOX 2: WELFARE CONSIDERATIONS FOR CAPTURING AND HANDLING WILD ANIMALS

1. **Capture methods**: Capture techniques should be as selective as possible to minimise the risk of capturing non-target species. They should be species-appropriate to minimise injury and mortality during capture and reduce welfare impacts. For example, considering whether the study species' would benefit from being held in darkness prior to handling.
2. **Appropriate checking**: Capture devices should be checked frequently, at appropriate intervals for the target species.
3. **Location:** Even if the capture technique itself has little welfare impact, undertaking capture in an inappropriate location places the user and animals at risk. This includes placing traps on slopes or near water. Being aware of potential predators is also important. Trapping individuals near breeding sites may lead to offspring abandonment.
4. **Seasonal timing:** Some species are sensitive to disturbance during key parts of their life cycle. This includes keeping animals away from dependent young for long periods.
5. **Time of day:** Animal's circadian activities should be considered. Nocturnal animals should not be released during daytime, and individuals should have enough time to forage after release.
6. **Weather**: Researchers should avoid capturing animals when weather conditions may lead to hyper- or hypothermia. If necessary, regular monitoring of capture sites and provision of bedding should be considered. Researchers should avoid using capture sites with high sun exposed for parts of the day.
7. **How many times**: Capture events should be minimised, but where captures are necessary, researchers should take care to avoid repeated capture of the same individual. This may mean moving capture locations, or cessation of capturing for set time periods. If capture is for removal of tags/devices, consider whether self-removing tags/devices can be used.
8. **Contingency planning**: Before trapping begins, researchers must have management plans in place for animals that are injured or killed during capture. Plans should include evaluating injuries, determining when euthanasia is appropriate, and ensuring that persons who will conduct this are trained and licensed.
9. **How many animals?** A clear maximum number of animals caught at any one time must be considered and numbers should be based upon power analyses. This ensures researchers can safely process animals in as short a time as possible to minimise capture and handling time.

10. **Minimise the number of procedures:** The cumulative impacts of procedures (even minor procedures) on study animals is a poorly understood area for most laboratory species, and unknown for wild species. Reducing the number of procedures an individual is subjected to has the benefit of reducing direct handling time.

BOX 3: KEY QUESTIONS WHEN MARKING/TAGGING WILD ANIMALS

1. If using natural marks, will data collection interfere with the species biology?
2. How long does the mark or tag need to last to complete the study; and how durable is the proposed marking method?
3. Will the proposed marking/tagging method interfere with other studies?
4. Will the marks/tag promote public concern about the study; and will the marks/tag have to be removed after study completion?
5. Have the appropriate approvals (animal welfare and state and/or federal permits) to mark/tag animals been obtained?
6. Will the mark have any direct or indirect effect on survival or behaviour? Can alternative methods be used or mitigated e.g. reducing size of mark?

BOX 4: NC3Rs BEST PRACTICE FOR WILD VERTEBRATE TRANSPORT GUIDELINES

Some wild animals will undergo transportation from the field to a captive housing location. Although longer distances need additional planning and care, it is important to note that any transport can be a significant stressor that may impact animal welfare and study research outcomes. The primary objective should be to move the animals in a manner that does not jeopardise their well-being and ensures their safe arrival at their destination in good health, with minimal distress. Many aspects of the transport process need to be considered, including: the route and journey plan; container design; vehicle design; the competence and attitude of drivers and others involved in the transportation; travel duration; the nature of food and water supplies; arrangements for acclimatisation after transport.
Critical appraisal and refinement of the logistical aspects of transport is essential if animal welfare is to be safeguarded during journeys. Guidance is available from a working group of the UK Laboratory Animal Science Association (LASA) (Swallow *et al.* 2005) and the US Institute for Laboratory Animal Research (ILAR) guidelines for the humane transportation of research animals (National Research Council 2006).

It is important that all relevant legislation on animal transport is followed - designating a person in each establishment with responsibilities on understanding and implementing transport legislation will help to ensure compliance.

Within Europe, Council Regulation (EC) No. 1/2005 on the protection of animals during transport and related operations determines minimum standards for the welfare of animals during transport. The Regulation applies to the transport of all live vertebrate animals for the purposes of economic activity, i.e. a business or trade. It is implemented in England by The Welfare of Animals (Transport) (England) Order 2006 and by parallel legislation in Scotland, Wales and Northern Ireland. Defra has published an overview of the requirements of the Regulation. European Convention for the Protection of Animals during International Transport (Revised) (2006) also applies to the movement of live animals within the EU. The transport of live animals by air is governed by the Live Animals Regulations of the International Air Transport Association (IATA). CITES permits must be obtained for all movements (import and export) of CITES listed species (e.g. non-human primates) between countries signed up to the Convention.

BOX 5: MINIMUM CHECKLIST OF WELFARE CONSIDERATIONS FOR HOUSING WILD ANIMALS

If your study design requires wild animals to be housed in captivity, the following checklist should be completed alongside ethical approval documentation.

Housing arrangements

☐ How do the housing arrangements meet the daily needs of your study species?

☐ housing type

☐ space allowance per individual

☐ temperature

☐ humidity

☐ lighting

☐ noise levels

☐ food and water access

☐ social conditions

☐ Have the housing conditions been checked by a suitable expert (e.g. veterinarian)?

☐ How do the proposed cleaning regimes for the housing meet the needs of your study species and help to prevent the spread of infection?

☐ cleaning schedule

☐ cleaning products to be used

☐ protocol for moving animals during cleaning

☐ Has the proposed cleaning regimes checked and approved by a relevant expert (e.g. a veterinarian)?

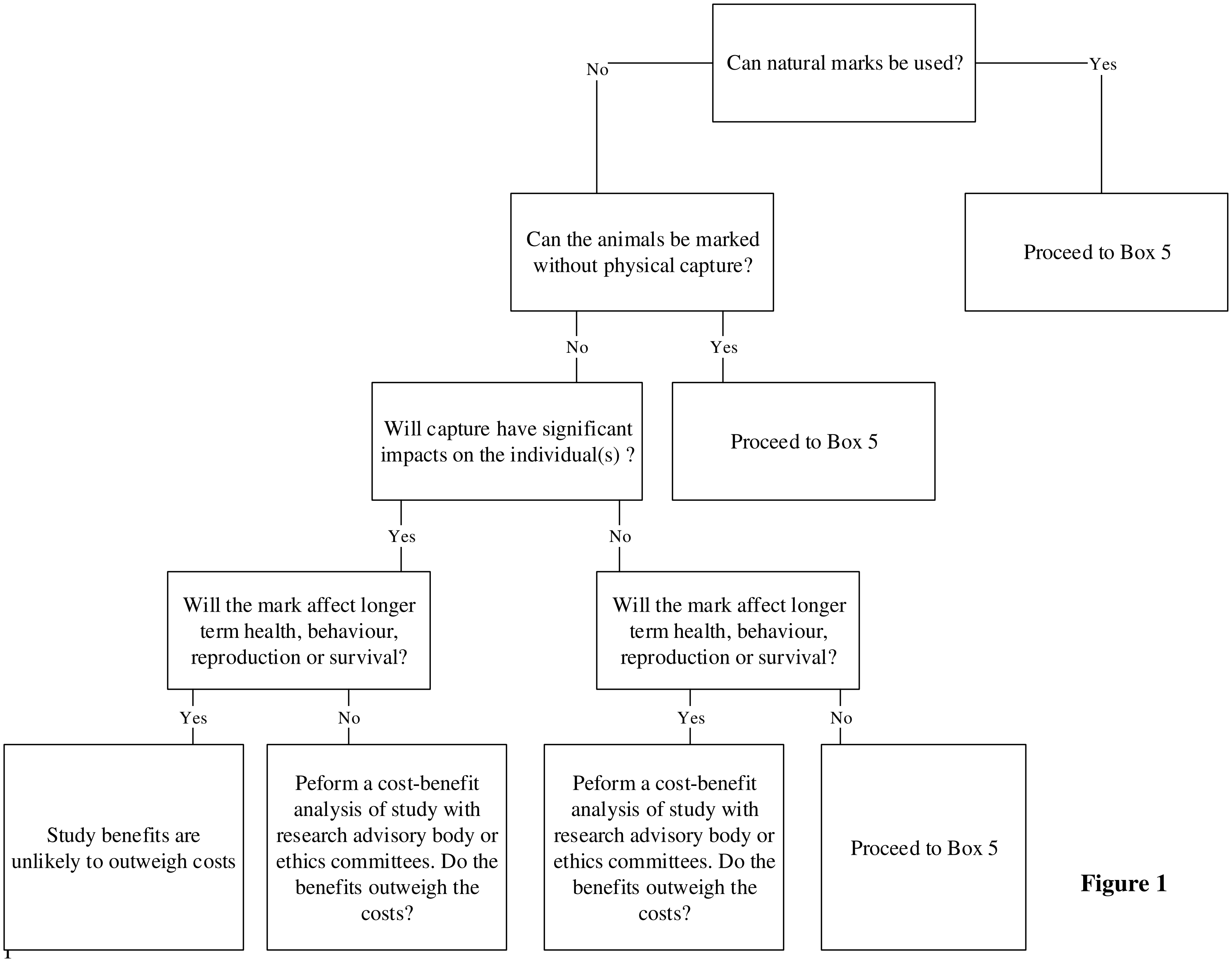
☐ Will individual animals be checked for infections prior to entering housing?

☐ What is the protocol for housing infected animals?

☐ What biosecurity procedures are in place upon entry and exit of the housing area?

BOX 6: WELFARE CONSIDERATIONS FOR RELEASE OF WILD ANIMALS

1. Check legislation regarding release of wild animals. Is it legal?
2. Are animals healthy enough to be released, including having recovered fully from any procedures or anaesthesia?
3. Release the animal as soon as it is feasible to do so, with attention paid to:
    a. conspecifics and dependent young
    b. time of day
    c. likely harm to animal
4. Release site should be as close to capture site as is safe for the animal.
5. Confirm that:
    a) it is legal to release the animals
    b) that the animal's state of health allows it to be released or re-homed;
    c) that the animal poses no danger to public health, animal health or to the environment;
    d) that there is an adequate scheme in place for ensuring the socialisation of the animal upon being released or re-homed where appropriate;
    e) that appropriate measures have been taken to safeguard the animal's welfare when released or re-homed.



Can natural marks be used?
No
Yes
Can the animals be marked without physical capture?
Proceed to Box 5
No
Yes
Will capture have significant impacts on the individual(s) ?
Proceed to Box 5
Yes
No
Will the mark affect longer term health, behaviour, reproduction or survival?
Will the mark affect longer term health, behaviour, reproduction or survival?
Yes
No
Yes
No
Study benefits are unlikely to outweigh costs
Peform a cost-benefit analysis of study with research advisory body or ethics committees. Do the benefits outweigh the costs?
Peform a cost-benefit analysis of study with research advisory body or ethics committees. Do the benefits outweigh the costs?
Proceed to Box 5

Figure 1

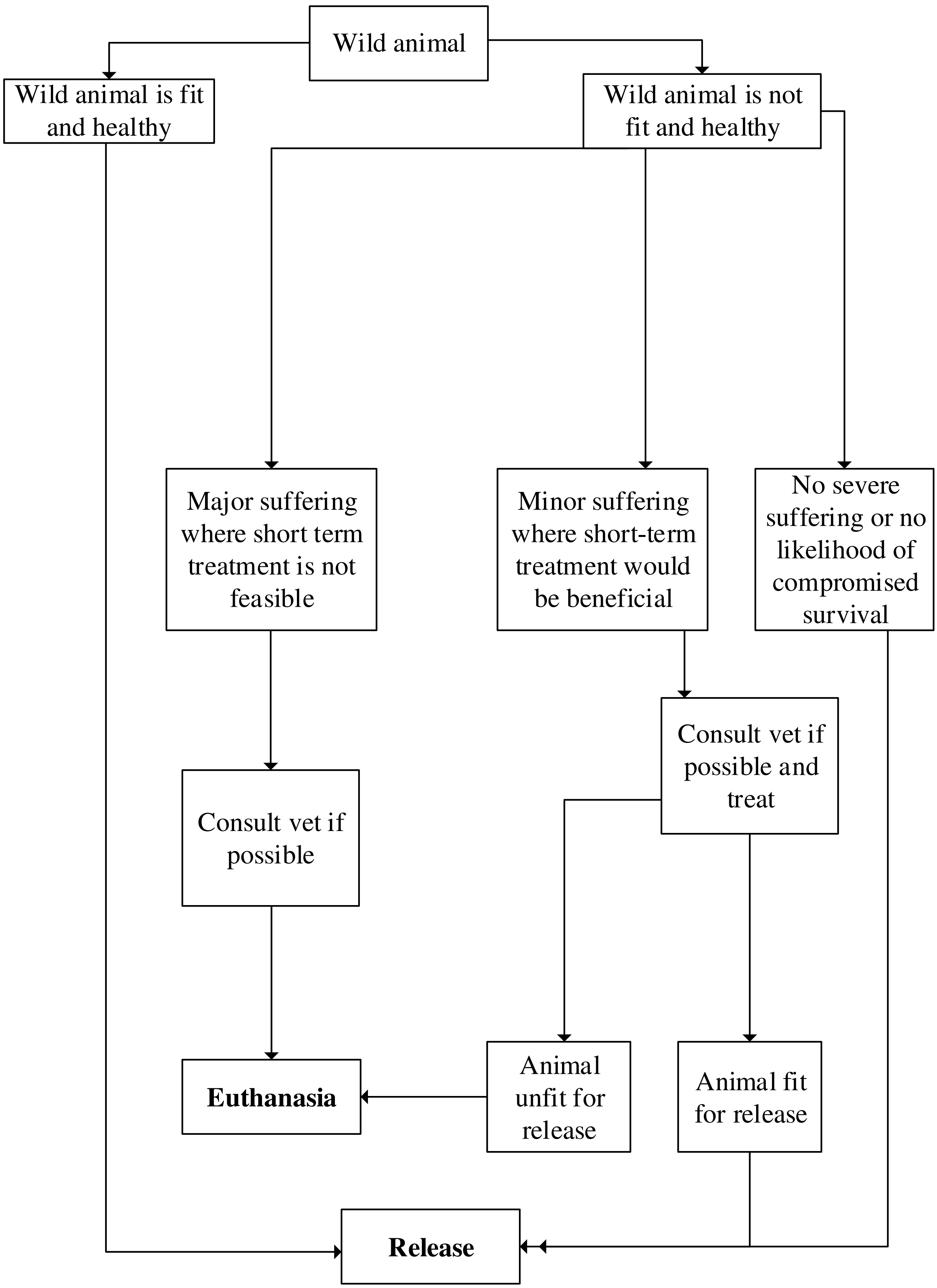

Wild animal
Wild animal is fit and healthy
Wild animal is not fit and healthy
Major suffering where short term treatment is not feasible
Minor suffering where short-term treatment would be beneficial
No severe suffering or no likelihood of compromised survival
Consult vet if possible and treat
Consult vet if possible
Animal unfit for release
Animal fit for release
Euthanasia
Release

Figure 2