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Guidelines for the Use of Animals

Guidelines for the treatment of animals in behavioural research and teaching

Behavioural studies are of great importance in increasing our understanding and appreciation of nonhuman animals. In addition to providing knowledge about the diversity and complexity of behaviour in nature, such studies also provide information crucial to improvements in the welfare of animals maintained in laboratories, agricultural settings and zoos, and as companion animals. As animals ourselves, animal research provides a window into our own behaviours. The use of animals in behavioural research and teaching does, however, raise important ethical issues. While many behavioural studies are noninvasive and involve only observations of animals in their natural habitat, some research questions cannot be addressed without manipulation of animals in the laboratory or field. Deep consideration must be given to identifying the appropriate marking techniques to allow individuals to be distinguished, and to evaluating the manipulative procedures, surgery or humane killing that may be necessary to achieve the aims of the research. Studies of captive animals may mean obtaining them from the wild and necessarily involve confinement. Other studies involving wild animals may require trapping and/or manipulation. In both cases, endpoints may result either in humane killing or subsequent release of the animals. Studies of free-living animals in their natural habitats can cause disruption to the animals' population or the wider ecosystem, particularly if feeding, capture, marking or experimental procedures are involved. While the furthering of scientific knowledge is a proper aim, and may itself advance an awareness of human responsibility towards animal life, the investigator must always weigh the potential gain in knowledge against any adverse consequences for the individual animals, populations under study and the wider ecosystem. This is equally true for the evaluation of animal use in animal behaviour teaching activities. In fact, animal behaviour courses provide an excellent opportunity to introduce students to the ethical obligations a researcher accepts when animals are studied.

To help both researchers and teachers make what are sometimes difficult ethical judgements about the procedures involved in the study of animals, the Association for the Study of Animal Behaviour and the Animal Behavior Society have formed Ethical and Animal Care Committees, respectively, and each appoints an Ethics Editor that serves on the editorial board of *Animal Behaviour*. These committees jointly produced the following guidelines for the use of all those who are engaged in behavioural research and teaching activities involving vertebrate and invertebrate animals. Given the diversity of species and the study techniques used in behavioural research, these guidelines are by necessity general in scope. There are, however, a variety of sources available that give more details on the principles on which the guidelines are based (Hubrecht & Kirkwood, 2010). These guidelines are used by the Ethics Editors of *Animal Behaviour* in assessing the acceptability of submitted manuscripts. Submitted manuscripts may be rejected by an Editor, after consultation with the appropriate Ethical or Animal Care Committee, if the content is deemed to violate either the letter or the spirit of the guidelines.

The ethical acceptability of manuscripts considered for publication in *Animal Behaviour* is assessed in terms of the cost–benefit trade-off. Costs to the animals might include compromises of animal welfare, any pain or distress (irrespective of the efforts to minimize this), reduction of likely survival rates or reproductive success. Other costs include adverse impact on the environment or reductions in the quality of science. Benefits are considered to be the value of the specific scientific insights sought to humans, other animals or the environment (i.e. whether the science is of good quality and addresses questions of importance). Any study that allows or precipitates great costs to animals for research must have both the highest potential benefits and the highest ethical justification. Great costs can be 'offset' in the cost–benefit analysis by achieving a high quality of research and/or answering very important questions.

During ethical assessment of papers submitted to *Animal Behaviour*, the costs and benefits are weighed on a case-by-case basis to assess whether costs have been minimized, the benefits maximized, and whether the benefits outweighed the costs, before making a recommendation on publication. For this review process to function effectively it is vital that authors supply detailed information on the ethical treatment of their animals (see Guide for Authors, http://www.elsevier.com/wps/find/journaldescription.cws_home/622782/authorinstructions), providing details of the capture, care, marking, treatment and subsequent release or disposal of their study animals. The process endorsed here uses the same logic that national, state or institutional ethical licensing bodies utilize (see below). It is important to note that these guidelines act to supplement the legal requirements in the country and/or state or province in which the work is carried out. They should not be considered an imposition upon the scientific freedom of individual researchers, but rather as an aid to provide an ethical framework that each investigator may use in making and defending decisions related to animal welfare.

LEGISLATION

Investigators are accountable for the care and wellbeing of animals used in their research and teaching activities, and must therefore abide by the spirit and the letter of relevant legislation. It is the investigator's responsibility to acquire knowledge about

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local legislation. Appendix 1 lists sources of information relating to the legislation of several countries; Bayne, Morris, and France (2010) provide an overview that includes additional countries. Investigators must familiarize themselves with legislation both on animal welfare and on threatened and endangered species, and conform with the spirit and letter of the laws. When submitting manuscripts to *Animal Behaviour*, all authors must verify that they have identified and adhered to the legal requirements of the country in which the study was conducted, and provide relevant permit numbers. Many nations and academic institutions require that experiments performed on captive animals or on wildlife that are manipulated in some way must first be reviewed and approved by an animal welfare, animal care and use or ethics committee of the sponsoring institution (Hagelin, Hau, & Carlsson, 2003; Jennings, 1994). A manuscript based on institutional committee-approved research may still be referred by Editors or reviewers to the Ethical and Animal Care Committees of ASAB/ABS, if they feel the manuscript raises ethical concerns. Investigators from countries without any legal requirements or guidelines are encouraged to voluntarily refer to one or more of the documents in Appendix 1 for guidance.

THE THREE RS: REPLACEMENT, REDUCTION AND REFINEMENT

Much of the current recommendations and legislation for ensuring appropriate animal care and use are based on the three guiding principles of replacement, reduction and refinement (3Rs; Buchanan-Smith et al., 2005; Kilkenny, Browne, Cuthill, Emerson, & Altman, 2010; Manciocco et al., 2008; Richmond, 2010; Russell & Burch, 1959; Vitale, Manciocco, & Alleva, 2008). Replacement refers to efforts to replace animal subjects and models with nonanimal ones, such as tissue cultures or computer models, wherever it is possible to do so while still achieving the scientific objectives. Reduction means reducing the numbers of animals affected by the experiment to the lowest number of individuals necessary while still ensuring the ability to achieve statistical power and the aims of the experiment. Careful hypothesis selection, experimental design and choice of statistics are critical to this, and researchers may find it useful to consult a resource such as those provided by the National Centre for the Replacement, Refinement and Reduction of Animals in Research (NC3Rs: https://www.nc3rs.org.uk/experimental-design). There will be occasions when it is possible to reduce the total number of animals used in an experiment, but only by increasing the degree or duration of discomfort for the fewer individuals that are used. In such cases, the investigator must find a balance between the two principles. Refinement refers to efforts to design and conduct the study as carefully as possible to maximize the scientific benefit while minimizing suffering to the animals, for example by planning and implementation of humane endpoints in the event that problems arise (Richmond, 1998; Rowan, 1998), using the least invasive technique and ensuring that housing conditions are optimal. Research on wildlife often raises particular issues and difficulties, and recent advice on practice in this area can be found in Lane and McDonald (2010) and Inglis, Mathews, and Hudson (2010). The implementation of the 3Rs in behavioural research raises issues regarding the scientific planning (PREPARE, Smith, Clutton, Lilley, Hansen, & Brattelid, 2017) and reporting (ARRIVE, Kilkenny et al., 2010) of studies. Both ASAB and ABS have taken the decision to endorse the use of the PREPARE (https://norecopa.no/prepare) and ARRIVE guidelines (http://www.nc3rs.org.uk/arrive-guidelines) for planning and reporting experiments using live animals, and recommend that authors refer to these.

CHOICE OF SPECIES AND NONANIMAL ALTERNATIVES

Investigators should choose species and strains for study that are appropriate and best suited for investigation of the questions posed. Choosing these requires knowledge of natural history, physiology and phylogenetic relationships. Knowledge of an individual animal's previous experience, such as whether or not it has spent a lifetime in captivity, is also important. Recent advances in genetic characterization of many laboratory animals may also allow the investigator to control for the effects of genotype on expected behavioural traits. The specialist characteristics and needs of some genetically altered strains must also be considered (Robinson et al., 2003); these are becoming increasingly common in behavioural studies and their welfare status and responses can be considerably different to those of nonmodified animals (Alleva & Vitale, 2000).

When research or teaching involves procedures or housing conditions that may cause pain, discomfort or stress to the animal, and when alternative species or strains can be used, the researcher should use the species or strain that is believed to be the least likely to experience pain or distress (OTA 1986; Sneddon, Elwood, & Adamo, 2014). The fact that a species being studied is locally classified as 'vermin' does not free the researcher from normal obligations to the experimental animals. The majority of invertebrate species are usually excluded from legislation regulating scientific research on animals (with the notable exception of cephalopods, Berry, Vitale, Carere, & Alleva, 2015). This does not mean that they are all unable to experience pain, discomfort or stress, but knowledge is more limited than for vertebrate groups (Gherardi, 2009; Horvath, Angeletti, Nascetti, & Carere, 2013; Sherwin, 2001; Sneddon et al., 2014). Manuscripts involving research with invertebrates that are excluded from legislation on animal use in research may still be reviewed by the Ethical and Animal Care Committees in light of the most current knowledge in this respect. Researchers using such species should seek expert advice and take any evidence on this matter relating to their species into account when designing experiments, and should endeavour to minimize potential harm wherever possible. Live animal subjects are generally essential in behavioural research, but nonanimal alternatives such as video records from previous work or computer simulations can sometimes be used (Smyth, 1978; van Zutphen & Balls, 1997). The use of webcams (especially on bird nests) is increasing rapidly and could provide excellent material for both educational and scientific purposes (e.g. www.birdcam.it; www.beleefdelente.it; http://www.looduskalender.ee/n/en, see also Porter, Lin, Smith, & Lu, 2010). Material of this kind also exists or can be produced for teaching purposes and can sometimes be used instead of live animals to teach aspects of the behavioural sciences (Smith & Allen, 2005; van der Valk et al., 1999).

NUMBER OF INDIVIDUALS

In compliance with the principles of replacement, reduction and refinement, any experiment should use the minimum number of animals necessary to test the hypotheses, without the loss of scientific rigour (Festing, Overend, Gaines Das, Cortina Borja, & Berdoy, 2002; Russell & Burch, 1959; Still, 1982). This should not only be applied to studies that involve procedures or treatments that may have a negative impact upon an animal or population, but should be adopted in the design of noninvasive experiments to ensure limited impact upon the subjects. The use of clear, theoretically driven hypotheses, pilot studies, good experimental design and statistical tests that enable several factors to be examined simultaneously are ways in which a researcher can reduce the number of animals used without compromising the research

objectives (Dell, Holleran, & Ramakrishnan, 2002; Hunt, 1980; Kramer & Font, 2017; Nakagawa & Cuthill, 2007; Still, 1982). Statistical tests, such as power analyses of pilot or previously published data, can calculate the lowest number of animals needed to obtain meaningful scientific data (Cohen, 1989; Kraemer & Theimann, 1987). However, in the absence of such data, sample sizes should be based upon related published studies and the researcher should consider preliminary statistical analyses during the experiment to determine whether additional animals are required. Employing robust experimental design and data analysis are vital when determining the number of animals needed because surveys of published studies have concluded that fewer animals could have been used to obtain the same outcomes (Douglas, Chanter, Pell, & Burney, 1986; Kilkenny et al., 2009). It is equally important, however, not to use so few animals that the research is invalid. Morris (1999) and Ruxton and Colegrave (2006), and online software packages such as G Power (http://www.gpower.hhu.de/en.html), are useful references to this end.

Despite the many important empirical benefits associated with the use of genetically modified animals, investigators should carefully consider any underlying ethical and welfare issues associated with the use of such animals. These may include the large numbers of animals used in the pre-experimental production phase and that the genetic modification may itself lead to a detrimentally altered phenotype. Investigators are encouraged to examine published discussions of the pros and cons of using genetically altered animals in research (e.g. Hubrecht, 1995; Ormandy, Dale, & Griffin, 2011; Wells et al., 2007).

PROCEDURES

Investigators are encouraged to discuss with colleagues both the scientific value of their research proposals and possible animal welfare and ethical considerations. There are several models for evaluating animal research that can be of use when making ethical decisions (Bateson, 1986; de Cock Buning & Theune, 1994; Donnelley & Nolan, 1990; Fraser, 1999; Fraser, Weary, Pajor, & Milligan, 1997; Magalhães-Sant'Ana, Sandøe, & Olsson, 2009; Orlans, 1987; Porter, 1992; Richmond, 1998; Sandøe, Crisp, & Holtug, 1997; Shapiro & Field, 1988). If procedures used in research or teaching involve animals' exposure to painful, stressful or noxious stimuli, whether through acts of commission or omission, the investigator must consider whether the knowledge that may be gained is justified, and carefully considered harm/benefit analysis should be performed. This will partly depend upon the goal of the research; for example, research designed to enhance our understanding of animal welfare issues may be judged differently to research designed for other purposes. Custom and practice, economic savings, convenience, or the fact that an animal might experience the same or similar stimuli in the wild are not adequate justifications. It should be borne in mind that the welfare costs of animal use may reflect not only the infliction of that which is unpleasant, but the denial of that which is pleasurable. There is a considerable amount of literature discussing the assessment of pain, suffering and well-being in both vertebrates and invertebrates (e.g. Bateson, 1991; Broom & Johnson, 1993; Dawkins, 2006; Flecknell, 1994; Flecknell & Waterman-Pearson, 2000; Hellbrekers, 2000; Jordan, 2005; Jones & McGreevy, 2010; Morton, 1998; Morton & Griffiths, 1985; NRC, 1992; Poole, 1995; Scott, Fitzpatrick, Nolan, Reid, & Wiseman, 2003; Sherwin, 2001; Sneddon et al., 2014; USDA, 1999).

Researchers are urged to consider the use of more refined procedures before using techniques that are likely to cause physical or psychological discomfort to the animal (Kreger, 2000; Lloyd, Foden, & Wolfensohn, 2008). When attempting to identify potential alternative procedures, the investigator will need to consider the pain- or distress-causing potential of all aspects or stages of the procedures in an experiment. The possible procedural substitutes or refinements for each may be very different. Pain or suffering should be minimized both in duration and magnitude as far as possible under the requirements of the experimental design. However, studies of factors such as housing or management procedures that may induce states of stress, anxiety and fear can be a necessary part of research aimed at improving animal welfare or scientific validity. Attention should be paid to the provision of proper pre- and postprocedural care to minimize preparatory stress, and immediate and residual effects. When a study involves any procedure or condition likely to cause more than momentary, low-intensity pain, discomfort or distress, then appropriate anaesthesia, analgesia, tranquillization and/or adjunctive relief measures should be used, sufficient to prevent or alleviate the discomfort, unless this would jeopardize the aims of the study. Use of analgesics after such procedures should also be considered to appropriately minimize pain and distress (Benson, Thurman, & Davis, 1990; Coulter, Flecknell, & Richardson, 2009; Flecknell, 1985; Flecknell & Waterman-Pearson, 2000; Stokes et al., 2009; see also Ernst, Tuchscherer, Kanitz, Puppe, & Manteuffel, 2006). Advice on the appropriate use of analgesics and anaesthetics to minimize discomfort and distress should be taken from trained veterinarians. In certain species, appropriate procedural training of animals can reduce the stress of some experimental procedures (see Conour, Murray, & Brown, 2006; Grandin, 2000; Reinhardt, 1997). The investigator should keep in mind that many forms of discomfort or suffering can involve experiences other than nociception, such as nausea, pruritus, thermal stress, social isolation or fear (McMillan, 2003). Moreover, these forms of discomfort and stress can be taxon and context specific, emphasizing the importance of being familiar with the species in question. Many of these sources of pain can be alleviated or prevented by medication, procedures or housing conditions that specifically target those experiences. Investigators should consult with trained veterinary staff or other experts for guidance on how to control or pre-empt suffering in its various forms.

The following more specific points may be of use.

Fieldwork

Field studies, whether involving observations or experimental manipulations, are a potentially powerful means to investigate animal behaviour in the natural contexts in which it evolved. However, field studies are potentially disruptive to the subjects and the wider ecosystem (e.g. Nisbet, 2000; de la Torre, Snowdon, & Bejarano, 2000; Williams, Trites, & Bain, 2002), and can interfere with the very qualities that field studies are best equipped to investigate, namely the natural character of behaviour (Martin & Bateson, 2007). Therefore, for both scientific and ethical reasons, investigators studying free-living animals are expected to take precautions to minimize the imposition of fear, distress or lasting harm on individual animals, and to minimize the impacts of the study on the populations and ecosystems of which the individual animals are a part.

Investigators should weigh the potential gain in knowledge against the adverse consequences of disruption for the subject animals, other animals and plants in the ecosystem, and the environment itself (e.g. soil compaction, damage to stream bed). A key issue to take into account is that field studies, whether observational or experimental, may have impacts reaching far beyond the

focal individuals under study. For example, 'by-catch' from trapping focal individuals often involves trapping a considerable number of nonfocal individuals in the process. It is the total impact on the ecosystem as a whole that should be balanced against the scientific gain, when evaluating the design of a proposed study.

A range of behavioural research techniques, including capture, handling and marking of wild animals, fitting with data logging or transmitting devices (e.g. radiotransmitters, geolocators), collection of physiological data (e.g. blood or tissue samples) or the experimental manipulations themselves, may have adverse consequences, such as a reduced probability of survival or reproduction (e.g. Costantini & Moller, 2013; Drolet & Savard, 2006; Jennings, 2007; Knapp & Abarca, 2009; McCarthy & Parris, 2004; Parris & McCarthy, 2001; Spotswood et al., 2012). Such impacts may be immediately apparent, or they may be delayed and thus potentially hidden from the experimenter (Putman, 1995); with wild animals it will also usually be more difficult to intervene to rectify a problem (e.g. a badly fitted device) than if the equivalent study was being conducted in the laboratory. Investigators should, therefore, consider the effects of such interference, and select the least disruptive, and the least stressful or harmful, techniques available in the context of the study (Beausoleil, Mellor, & Stafford, 2004). This may include considering minimally invasive or noninvasive techniques for monitoring physiology (Cooper, 1998; Gedir, 2001) and for identifying individuals, such as the use of phenotypic features (e.g. Scott, 1978) or genetic markers extracted from faeces (e.g. Beebee & Rowe, 2008), instead of invasive tissue sampling or marking.

Cuthill (1991) discusses the ethical issues associated with fieldwork, and recommends pilot investigations to evaluate potential negative impacts and follow-up monitoring to detect and minimize longer-term or delayed effects. For example, pilot studies may be used to determine the minimally effective doses of chemicals required for physiological manipulations under field conditions. Results from such pilot studies may be used to justify procedures that might otherwise be questioned on ethical grounds, by inclusion in the Methods section of submitted papers. Full details of the pilot studies should be made available for scrutiny by journal Editors and Reviewers.

When an experimental protocol requires that animals be removed from the population either temporarily (e.g. for fitting a tag) or on a longer-term basis (e.g. as part of a mate removal experiment), investigators should ensure that suffering or discomfort is minimized not only for the removed individuals but also those dependent on them (e.g. dependent offspring or eggs). Removed individuals and their dependants should be housed and cared for appropriately, their time in captivity minimized and their safe return ensured.

Decisions about the ethics of field studies are particularly important when they involve rare and endangered species. Investigators planning research on species at risk of extinction, or translocations or reintroductions of animals as part of behavioural studies should first consult the current IUCN Guidelines and Policy Statements on Species-Related Issues (www.iucn.org), and any other local legislation.

Marking and Tagging

Both laboratory and field studies commonly require individual identification of animals. Serious attention should be paid to identifying the least invasive method of marking such as using the natural unique coloration patterns often visible in fur or plumage. However, in many cases, naturally occurring phenotypic differences cannot be used. Great care should be taken to minimize the pain and distress if any amount of handling or invasive measures are necessary, however minimal they might be. Examples of minimally invasive procedures for marking animals include the use of metallic or plastic bands or tags, or temporarily marking animals with dyes or markers. Some semipermanent or permanent marks might be used, but can be associated with increased but acute pain and distress. Examples of these include tattooing, freeze marking or the use of radiofrequency identification (RFID) or passive integrated transponder (PIT) tags. These invasive forms of marking require damaging the animal, for example ear, toe, feather or fin clips or punches. Such methods of individual identification should only be used when absolutely necessary and no other forms of marking are possible or feasible (NRC, 2011). For instance, toe clipping is a technique that is known to produce more than temporary pain and suffering in rodents when performed after the ossification of bone tissue; for mice this occurs around postnatal day 17 (Hankenson, Garzel, Fischer, Nolan, & Hankenson, 2008; Paluch et al., 2014). In laboratory conditions, the technique is restricted in most cases to postnatal day 5–7, and is only allowable up to postnatal day 17 if the tissue collected is also collected for genetic analysis. If DNA samples are necessary, then costs of clipping might be mitigated to some degree, but such a need is not sufficient justification for this method on its own because other noninvasive or less invasive forms of DNA collection are possible. Therefore, significant and compelling rationale should be provided when justifying more invasive methods of marking. If an animal is marked using clips or punches, it is crucial to consider how this might affect the animal in both the short and long term (e.g. will feather/wing clips increase the energetic costs of flight, will ear punches alter auditory capabilities, or how will toe clipping impact an animal's ability to burrow, climb or swim?). For any method of individual identification, the experimenter should consider the potential benefits and potential costs of analgesics. For example, consumption of some analgesic creams might cause more distress during application, and can be harmful if ingested (e.g. licked), than the pain relief they might offer (Sikes, 2016). The experimenter should consult with appropriate resources (e.g. NRC, 2011) on this matter before initiating an experiment to determine the best method of individual identification.

Aggression, Predation and Intraspecific Killing

The fact that the agent causing harm may be another nonhuman animal does not free the experimenter from the normal obligations to experimental animals. Several different sources have discussed the ethical issues involved and suggest ways to minimize suffering (e.g. Bekoff, 1993; Bekoff & Jamieson, 1996; Elwood, 1991; Huntingford, 1984). Note also that the conditions in which the animals are kept may influence rates of intraspecific aggression and killing (e.g. cleaning out cages at the wrong time can prompt infanticide in rodents; Burn & Mason, 2008).

Wherever possible, field studies of natural encounters should be used in preference to staged encounters. Where staged encounters are necessary, the use of models or audio/video playback (and the like) should be considered, the number of animals should be kept to the minimum needed to accomplish the experimental goals, and the experiments should be as short as possible. Prey species can erroneously habituate to frequent attacks with models, which may change their response to natural predators. Suffering can also be reduced by continuous observation with intervention to stop aggression at predefined levels, and by providing protective barriers and escape routes for the subjects.

Aversive Stimulation and Deprivation as Motivational Procedures

Aversive stimulation, deprivation or restriction of resources can cause pain or distress to animals. To minimize suffering, the

investigator should determine whether there is an alternative reward strategy that could be used to motivate the animal in the study. Alternatives to aversive stimuli and deprivation strategies include the use of highly preferred foods and other ecologically relevant rewards that may motivate even satiated animals (Grandin, 2000; Laule, 2010; Reinhardt, 1997). If an alternative reward strategy is unavailable, or the effect of aversive stimulation is itself of interest (e.g. in studies of fear, stress or pain), investigators should ensure that the levels of restriction, deprivation or aversive stimulation used are no greater than necessary to achieve the goals of the experiment. Use of minimal aversive stimuli levels requires knowledge of the relevant technical and biological literature (e.g. quantitative studies of aversive stimulation: Church, 1971; Rushen, 1986), or the behaviour of satiated animals (Morgan, 1974). Even for invertebrate groups the design and implementation of experiments that use aversive stimuli/procedures should be considered carefully (Gherardi, 2009; Sneddon, 2015; Sneddon et al., 2014), as cephalopods, for example, can recognize individual humans (Anderson, Mather, Monette, & Zimsen, 2010; Mather & Anderson, 2007).

Before deciding in favour of aversion or deprivation, investigators should consider consulting with animal care staff, laboratory animal scientists and veterinary staff experienced in working with animals in research settings. Practical animal motivation and training is a highly specialized skill. The failure of positive reinforcement methods to motivate an animal may reflect only the strategy or tactic used, the skill of the research staff and level of rapport with the animal. Failure to properly motivate an animal does not necessarily indicate that it would be impossible for a skilled trainer to motivate the animal with positive reinforcement. Further comments on reducing distress caused by motivational procedures are to be found in Lea (1979) and Moran (1975). In practice, at all times positive training regimens should be considered before the use of aversive stimulation or deprivation. If this is not possible, then the use of aversive stimulation or deprivation must be strongly justified in submitted manuscripts.

Social Deprivation, Isolation and Crowding

Experimental designs that require keeping solitary or territorial animals in overcrowded conditions, or that involve social disruption, deprivation or isolation, may be extremely stressful to the animals involved, and may adversely impact the behaviours being studied to gregarious animals. Because the degree of stress experienced by the individual animal can vary with species, age, sex, reproductive condition, developmental history and social status, the natural social behaviour of the animals concerned and their previous social experience must be considered to minimize such stress (Poole & Dawkins, 1999; Shepherdson, Mellen, & Hutchins, 1998).

Deleterious Conditions

Studies that induce deleterious conditions can involve causing disease, increasing parasite loads and exposing animals to pesticides or other environmental stressors. These studies should address the possible treatment or alleviation of the condition induced. Animals exposed to deleterious conditions that might result in suffering or death should be monitored frequently using appropriate methods. These methods may involve the use of behavioural indicators of harm, or tests that predict the development of serious states of harm, such as impending organ failure. The advice of trained care, scientific or veterinary personnel experts on current methods for early detection of disease or harm resulting from deleterious conditions should be sought. Whenever possible, considering the aims of the research, these animals should be medically treated or humanely euthanized as soon as they show signs of distress. If the goals of the research allow it, the investigator should also consider experimental designs in which the deleterious condition is removed (e.g. removing rather than adding parasites as the experimental treatment) or in which naturally occurring instances of deleterious conditions are observed.

Special care should be taken in studies involving the creation of deleterious situations with genetically modified animals. The welfare of transgenic animals, for example, or those with induced mutations, may be compromised even if this is not the primary goal of the modification. Standard welfare assessment procedures should be established and be in place before work commences (Westh Thon, Ritskes-Hoitinga, Gates, & Prins, 2010). Genetically altered animals should be checked or screened for possible welfare problems and their suitability as the most appropriate species/strain carefully considered.

ENDANGERED SPECIES

All research on endangered or locally rare species must comply with relevant legislation, and be coordinated with official agencies responsible for the conservation effort for the species or population under study. Legislation and sources of help in identifying endangered species can be found in Appendix 1. Members of threatened species should not be placed at risk except as part of a serious attempt at conservation. Observation alone can result in serious disturbance, including higher predation rates on nests of young, or their abandonment. Careful consideration of techniques and of alternative species should be undertaken as a first step. Consideration should also be given to any further adverse consequences such as opening remote areas for subsequent access or teaching techniques of anaesthesia and capture that might be misused (e.g. by poachers).

PROCUREMENT AND TRANSPORT OF ANIMALS

When it is necessary to procure animals either by purchase or by donation from outside sources, only reputable breeders and suppliers should be used (see Appendix 1). If animals are procured by capture in the wild, this must be done using methods that minimize pain, distress and suffering, and must comply with any relevant legislation. Investigators who purchase animals from local trappers should not encourage methods of trapping that cause suffering, or methods that involve killing many individuals to obtain a few live specimens. Procurement practices also should not create a local market for culling wildlife for profit. Individuals of endangered species or populations should not be taken from the wild unless they are part of an active conservation programme. The investigator should ensure that those responsible for the handling and transport of animals to the research facilities are well qualified and experienced in the requirements of the species. This includes the provision of adequate food, water, ventilation, space and protection from waste (IATA, 2011). Any potential sources of stress associated with transport should be identified and minimized, for example, excessive climatic conditions or sensory stimulation, prolonged food or water deprivation, aggression during transport.

Generally, only healthy animals that are able to withstand the rigours of transport should be transported. Young, dependent animals are not usually suitable for transport. If the transport of dependent animals is necessary, they should usually be transported only with the mother or equivalent such as a foster mother. Special care, and additional regulations, may be relevant to the transport of pregnant animals. With particularly sensitive or social species, it may be necessary for a trained attendant or veterinarian to travel

with the animal(s). Preconditioning animals to transport containers prior to shipment will reduce stress during loading and shipping. Furthermore, the investigator should carefully consider modes of transport, transport schedules (so as to reduce or eliminate layovers, unless rest periods are desirable) and shipping containers to ensure that they are suitable for the species being shipped. Compliance with the relevant transport regulations for the species concerned (local, national and international) must be a top priority.

HOUSING AND ANIMAL CARE

The researcher's responsibilities extend to the conditions under which the animals are kept when not being studied, as well as during study. Ultimately, consideration of the natural history of the animal should be given to decisions on how to handle and house animals, acknowledging that 'standard' conditions or practices might vary between species, and that not all standards are translatable across all species.

Caging conditions and husbandry practices must meet, at the very least, minimal recommended requirements of the country in which the research is carried out (see Appendix 1). Although these publications provide general guidance, there is evidence that housing animals in larger or more enriched conditions than specified in these minimal requirements and providing them with a variety of species-specific enrichment choices improves not only animal welfare (Kessler & Turner, 1999; Mason, Cooper, & Clarebrough, 2001; Newberry, 1995; Olsson & Dahlborn, 2002; Olsson et al., 2003; Pounder et al., 2016; Sherwin & Olsson, 2004) but also the quality of science, including behavioural studies (Calisi & Bentley, 2009; Poole, 1997; Sherwin, 2004; Wurbel, 2001, 2002). Normal maintenance of captive animals should incorporate, as much as possible, aspects of the natural living conditions deemed important to maximizing welfare and survival. Consideration should be given to providing biologically relevant enrichment features such as natural material, refuges, perches, dust baths, water baths and (for aquatic species) appropriate water flows (Liss et al., 2015), although it should be borne in mind that some enrichment features can sometimes create welfare problems of their own, for instance by increasing levels of competition and aggression (e.g. Barnard, Behnke, & Sewell, 1996; Haemisch & Gartner, 1994; McGregor & Ayling, 1990).

The formation of the appropriate stable social groups of compatible individuals, for instance in rehoming schemes, is increasingly being incorporated into animal welfare legislation (for example, Directive 2010/63/EU/010 on the protection of animals used for scientific purposes). However, objective metrics, guidelines and tools to apply these recommendations are currently still lacking and should be based on personal experience and careful observation of the formed groups and the individuals involved in group allocation. Companions should be provided for social species where possible, providing that this does not lead to aggression, suffering or injury. Therefore, an assessment of the behaviour at the individual level is recommended. Frequency of cage or tank cleaning should represent a compromise between the level of species-specific cleanliness necessary to prevent diseases/stress and the amount of stress imposed by handling and exposure to unfamiliar surroundings, odours and bedding. Similar arguments apply to the rate of water turnover in aquatic species.

Standard housing and care regimens established for the commonly used laboratory animals are not necessarily suitable for all species. Wild individuals or those of wild species born in captivity may require special attention to enhance their comfort and safety. Investigators should also consult the most recent guidelines available from relevant taxon-oriented professional societies (Appendix 2).

The nature of human–animal interactions during routine care and experimentation should be considered by investigators. Depending upon species, rearing history and the nature of the interaction, animals may perceive humans as conspecifics, predators or symbionts (Estep & Hetts, 1992). Special training of animal care personnel can help in implementing procedures that foster habituation of animals to human contact and minimize stress. Stress can also be reduced by procedurally training animals to cooperate with handlers and experimenters during routine husbandry and experimental procedures (Biological Council, 1992; Reinhardt, 1997; Laule, 2010)

FINAL DISPOSITION OF ANIMALS

When research projects or teaching exercises are completed, the decision about the disposition of the animals is a vital concern. If permitted by local legislation, investigators may sometimes distribute animals to colleagues for further study or breeding. However, if animals are distributed to a colleague or another project, care must be taken to ensure that the same animals are not used repeatedly in stressful or painful experiments, and that they continue to receive a high standard of care. Animals should never be subjected to major surgery more than once unless it is an unavoidable and justifiable element of a single experiment. Except as prohibited by national, federal, state, provincial or local laws, researchers may release field-trapped animals if this is practical and feasible, especially if it is critical to conservation efforts. However, the researcher should assess whether releases into the wild might be injurious or detrimental both to the released animal and to existing populations in the area. Because of potential impacts on the genetic structure of local populations in the area, animals should be released only at the site where they were trapped (unless conservation efforts dictate otherwise), and only when their ability to survive in nature has not been impaired and when they do not constitute a health or ecological hazard to existing populations.

If animals are to be killed at the end of a study, this should be done as humanely and painlessly as possible, and death should be confirmed before their bodies are destroyed. Veterinary staff with appropriate expertise should be consulted for advice on methods of euthanasia that are appropriate for the particular species being used. When animals are killed, establishing that tissues or carcasses can be fully utilized, for example by other researchers, is in line with the principles set out in the 3Rs. Carcasses of wild animals killed with lethal anaesthetics or other toxic substances should be disposed of in a manner that would prevent scavengers from suffering secondary toxicity. Additional information on euthanasia methods can be found in the American Veterinary Medical Association's Guidelines on Euthanasia (AVMA, 2013).

OBTAINING FURTHER INFORMATION

There are a number of organizations around the world that provide publications and detailed information about the care and use of animals. Please note that ASAB/ABS do not necessarily endorse all of the recommendations proposed by these organizations, but we suggest that they may make excellent sources of information from which experimenters can inform their decisions regarding animal welfare.

Groups with an international focus that provide information on animal welfare include the World Organisation for Animal Health (OIE, http://www.oie.int/), the International Fund for Animal Welfare (IFAW, http://www.ifaw.org/) and the European Food Safety Authority (EFSA, http://www.efsa.europa.eu/). The EFSA, for example, has published a series of guidelines and scientific opinions on the care and use of animals (http://www.efsa.europa.eu/en/science/animal-health-and-welfare), particularly related to

animals farmed for food production. The International Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC, http://www.aaalac.org/) is a private, nonprofit organization with offices in Europe, Southeast Asia, and the U.S.A. that promotes the humane treatment of animals in science through voluntary accreditation and assessment programmes. For information on organizations around the world involved in animal welfare, with an emphasis on animal protection, there is a directory hosted by WorldAnimal.Net (http://worldanimal.net/).

Nationally specific policy and legislation can also be found in countries around the world. In the U.K., the Department for Environment, Food and Rural Affairs (DEFRA) provides information about the Animal Welfare Act 2006 (https://www.gov.uk/guidance/animal-welfare#legislation) and the Home Office provides information about the application of the Animals (Scientific Procedures) Act 1986 (http://www.homeoffice.gov.uk/science-research/animal-research/). The Canadian Council on Animal Care (Suite 1510–130 Albert Street, Ottawa, ON K1P 5G4, Canada, http://www.ccac.ca/), the Scientists' Center for Animal Welfare (7833 Walker Drive, Suite 410, Greenbelt, MD 20770, U.S.A., www.scaw.com), and the Universities Federation for Animal Welfare (The Old School, Brewhouse Lane, Wheathampstead, Hertfordshire AL4 8AN, U.K., http://www.ufaw.org.uk/) provide links to numerous sources within each county. In Norway, the Norecopa website (https://norecopa.no) contains a number of links to relevant resources, including a comprehensive list of guidelines, databases, information centres, journals and email lists of relevance to researchers planning procedures involving animals (https://norecopa.no/3r-guide-database). In Australia, the Animal Welfare Science Centre (http://www.animalwelfare.net.au/) has produced some publications related to the care and use of animals and provides a list of links to other organizations.

The Animal Welfare Information Center at the National Agricultural Library (10301 Baltimore Avenue, Room 410 Beltsville, MD 20705, U.S.A., http://www.nal.usda.gov/awic/) publishes a series of bibliographies on special topics, and offers individualized database searches for investigators on potential alternatives, including techniques for replacement with nonanimal models or alternative species, methods for reducing the total number of animals necessary to address the research question, and experimental refinements that can reduce pain and stress. The National Academies of Sciences Engineering and Medicine (http://www.nas.edu/) sponsored Institute of Laboratory Animal Resources (ILAR) (http://dels.nas.edu/ilar) publishes and periodically updates their Guide for Care and Use of Laboratory Animals (published by the National Academies Press, http://www.nap.edu/), which serves as a basis for most United States-based Institutional Animal Care and Use Committee (IACUC) policy on the use of animals in research. The National Institutes of Health Office of Laboratory Animal Welfare (OLAW) site (http://grants.nih.gov/grants/olaw/olaw.htm) contains considerable policy information and many links (e.g. http://grants.nih.gov/grants/olaw/links.htm), from which one can find information on alternatives to animal testing, databases, laws, regulations and policies, and government- and nongovernment-sponsored resources on ethics and the care and use of animals in research. Helpful information, such as the IACUC Guidebook published by ARENA (Applied Research Ethics National Association), is available on the OLAW links webpage. The USDA/APHIS Animal Welfare Information Center site (https://www.aphis.usda.gov/aphis/ourfocus/animalwelfare) and the National Agricultural Law Center are also both good sources for online animal welfare information. (http://nationalaglawcenter.org/aglaw-reporter/case-law-index/animalwelfare). The Association of Zoos and Aquariums also has guidelines and advice on the husbandry of various zoo and wildlife species in captivity (http://www.aza.org/). It should be noted that links can become outdated and direct an experimenter to older editions of texts or other resources, in which case newer editions should be preferentially consulted.

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Appendix 1. Some sources of legislation and regulations regarding animal use and procurement of animals

International or Multinational

Bayne, K., Morris, T. H. & France, M. P. 2010. Legislation and oversight of the conduct of research using animals: a global overview. In: *The UFAW Handbook on the Care and Management of Laboratory and Other Research Animals* (Ed. by R. Hubrecht & J. Kirkwood), pp. 107–123. Oxford, U.K.: Wiley-Blackwell.
Convention on International Trade in Endangered Species of Wild Fauna and Flora. http://www.cites.org/
International Air Transport Association (IATA). 2011. *Live Animals Regulations (LAR). The Global Standard for the Transportation of Live Animals by Air.* http://www.iata.org/publications/store/pages/live-animals-regulation.aspx.

United Kingdom

Home Office. Guidance on the operation of the U.K. legislation on animals used in research and codes of practice. https://www.gov.uk/guidance/research-and-testing-using-animals.
International Union for the Conservation of Nature, IUCN, The World Conservation Union Rue Mauverney 28, CH-1196 Gland, Switzerland. https://www.iucn.org/resources/publications.
Laboratory Animal Breeders Association of Great Britain. http://www.laba-uk.com/
Radford, M. 2001. *Animal Welfare Law in Britain*: *Regulation and Responsibility*. Oxford, U.K.: Oxford University Press.

United States of America

American Association for Laboratory Animal Science. http://www.aalas.org.
Code of Federal Regulations, *Title 9 (Animal and Animal Products), Subchapter A (Animal Welfare), Parts 1–3*. Available from: the Office of the Federal Register, National Archives and Records Administration, U. S. Government Printing Office, 732 N. Capitol Street, NW, Washington, D.C. 20401: https://www.gpo.gov/fdsys/pkg/CFR-2000-title9-vol1/content-detail.html and APHIS, U.S.D.A., Federal Building, 6505 Belcrest Road, Hyattsville, MD 20782. https://www.aphis.usda.gov/aphis/resources/lawsandregs.
Code of Federal Regulations, *Title 50 (Wildlife and Fisheries), Chapter 1* (Bureau of Sport Fisheries and Wildlife Service, Fish and Wildlife Service, Department of Interior). Washington, D.C.: U.S. Government Publishing Office. https://www.ecfr.gov/cgi-bin/text-idx?SID=78936da1947b2ec11a2345f172ad71a9&mc=true&tpl=/ecfrbrowse/Title50/50tab_02.tpl.

Federation of Animal Science Societies (FASS). 2010. *Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching*. Washington, D.C. https://www.aaalac.org/about/Ag_Guide_3rd_ed.pdf.
FELASA, the Federation of European Laboratory Animal Science Associations. http://www.felasa.eu.
NRC (National Research Council). 2010. *Guide for the Care and Use of Laboratory Animals. Committee for the Update of the Guide for the Care and Use of Animals*. Washington, D.C.: National Academies Press. http://dels.nas.edu/Report/Guide-Care/12910.
Office for Endangered Species, U.S. Department of Interior, Fish and Wildlife Service, Room 430, 4401 N Fairfax Drive, Arlington, VA 22203.
PHS (Public Health Service). 1986. *Public Health Service Policy on Humane Care and Use of Laboratory Animals*. Washington, D.C.: U.S. Department of Health and Human Services. Available from: Office for Protection from Research Risks, Building 31, Room 4809, NIH, Bethesda, MD 20892.
U.S. Department of Agriculture (USDA), Animal and Plant Inspection Service (APHIS). *Animal Welfare Electronic Freedom of Information Requests — Annual Reports*. https://www.aphis.usda.gov/aphis/ourfocus/animalwelfare/sa_awa/awa-inspection-and-annual-reports.

Europe

Council of Europe. European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes http://www.coe.int/en/web/conventions/full-list/-/conventions/treaty/123.
Council of Europe. 2006. Fourth multilateral consultation of parties to the European Convention for the protection of vertebrate animals used for experimental and other scientific purposes (ETS 123) https://search.coe.int/cm/Pages/result_details.aspx?ObjectID=09000016805c18be#RelatedDocuments.
European Commission. 2007. Commission recommendations of 18 June 2007 on guidelines for the accommodation and care of animals used for experimental and other scientific purposes. Annex II to European Council Directive 86/609. See 2007/526/EC. http://eurlex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2007:197:0001:0089:EN:PDF.
European Union. http://europa.eu/index_en.htm.
European Union. Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the Protection of Animals Used for Scientific Purposes. http://eurlex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2010:276:0033:0079:en:PDF.

Canada

Canadian Council on Animal Care. 2017. *Guide to the Care and Use of Experimental Animals. Vols 1 and 2*. Ottawa: Canadian Council on Animal Care. http://www.ccac.ca/en_/standards/guidelines.
Canadian Council on Animal Care. 2003. *Guidelines on the Care and Use of Wildlife*. Ottawa: Canadian Council on Animal Care. http://www.ccac.ca/Documents/Standards/Guidelines/Wildlife.pdf.
Committee on the Status of Endangered Wildlife in Canada, Canadian Wildlife Service, Environment Canada, Ottawa, ON K1A 0E7.
The Canadian Association for Laboratory Animal Science. http://www.calas-acsal.org/.

Australia

Commonwealth of Australia. 2005. *Australian Animal Welfare Strategy*. Department of Agriculture, Fisheries and Forestry. Canberra, Australia. http://www.agriculture.gov.au/animal/welfare/aaws.
National Health and Medical Research Council. 2004. Australian Code of Practice for the Care and Use of Animals for Scientific Purposes. Australian Government. http://www.nhmrc.gov.au/publications/synopses/ea16syn.htm.

New Zealand

Ministry of Agriculture and Forestry, New Zealand. 1999. Animal Welfare Act 1999. Public Act 1999, Number 142. http://www.legislation.govt.nz/act/public/1999/0142/latest/DLM49664.html.
Ministry for Primary Industries, New Zealand. *Codes of Welfare*. https://www.mpi.govt.nz/protection-and-response/animal-welfare/codes-of-welfare.

Appendix 2. Taxon-oriented societies with ethical or animal welfare guidelines

American Society of Mammalogists:

http://www.mammalsociety.org/uploads/Sikes%20et%20al%202011.pdf.

Ornithological Council:

http://naturalhistory.si.edu/BIRDNET/guide/index.html.
http://naturalhistory.si.edu/BIRDNET/documents/guidlines/Guidelines_August2010.pdf.
American Society of Ichthyologists and Herpetologists (ASIH), the Herpetologists' League (HL) and the Society for the Study of Amphibians and Reptiles (SSAR): Guidelines for the Use of Live Reptiles and Amphibians in Research.
http://www.asih.org/publications.
American Society of Ichthyologists and Herpetologists (ASIH), the American Fisheries Society (AFS) and the American Institute of Fisheries Research Biologists (AIFRB): Guidelines for the Use of Fishes in Research.
http://fisheries.org/policy-media/science-guidelines/guidelines-for-the-use-of-fishes-in-research/

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