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ANIMAL WELFARE FROM MOUSE TO MOOSE—IMPLEMENTING THE PRINCIPLES OF THE 3RS IN WILDLIFE RESEARCH

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ABSTRACT: The concept of the 3Rs (replacement, reduction, and refinement) was originally developed for improving laboratory animal welfare and is well known in biomedical and toxicologic research. The 3Rs have so far gained little attention in wildlife research, and there could be several reasons for this. First, researchers may prioritize the welfare of populations and ecosystems over the welfare of individual animals. The effects of research on individual animals can, however, impact welfare and research quality at group and population levels. Second, researchers may find it difficult to apply the 3Rs to studies of free-living wildlife because of the differences between laboratory and wild animals, species, research environment, and purpose and design of the studies. There are, however, several areas where it is possible to transfer the 3R principles to wildlife research, including replacement with noninvasive research techniques, reduction with optimized experimental design, and refinement with better methods of capture, anesthesia, and handling. Third, researchers may not have been trained in applying the 3Rs in wildlife research. This training is needed since ethics committees, employers, journal publishers, and funding agencies increasingly require researchers to consider the welfare implications of their research. In this paper, we compare the principles of the 3Rs in various research areas to better understand the possibilities and challenges of the 3Rs in wildlife research. We emphasize the importance of applying the 3Rs systematically throughout the research process. Based on experiences from laboratory research, we suggest three key factors to enhance implementation of the 3Rs in wildlife research: 1) organizational structure and management, 2) 3R awareness, and 3) research innovation, validation, and implementation. Finally, we encourage an interdisciplinary approach to incorporate the 3R principles in wildlife research. For improved animal welfare and increased research quality, researchers have moral obligations to include the 3Rs into all research areas, including wildlife research.

Key words: Animal welfare, reduction, refinement, replacement, wildlife research, 3Rs.

INTRODUCTION

The principles of the 3Rs (replacement, reduction, and refinement) guide scientists on the ethical use of animals in research, testing, and education. Research areas where animals are used have developed and changed since the principles were first proposed (Russell and Burch 1959); however, the principles have been adapted to modern research, and they still are applicable. "Replacement" is defined as methods that permit a given purpose to be achieved without conducting experiments or other procedures on animals. "Reduction" refers to methods for obtaining comparable levels of information from the use of fewer animals in scientific procedures or for obtaining more information from the same number of animals. "Refinement" involves methods that alleviate or minimize potential pain, suffering, and distress, and that enhance animal well-being (ATLA, Executive Committee of the Congress 2009).

In the last decade, the 3Rs have been incorporated into various legislative actions for animal protection in research using animals, for example, in Japan (Kurosawa 2008), Brazil (Filipecki et al. 2011), and the European Union (EU) directive 2010/63/EU (European Union [EU] 2010). The EU directive covers 3R implementation in practice and the need for specific training of any staff handling research animals and encompasses all types of research, including laboratory, farm, and field research (EU 2010). National 3R advisory boards, or 3R centers, are active in several countries, such as in Canada since 1968 (Canadian Council on Animal Care in Science [CCAC] 2015), in Australia and New Zealand since 1987 (Australian and New Zealand Council for the Care of Animals in Research and Teaching Ltd. [ANZCCART] 2015), in Norway since 1999 (Norwegian Consensus Platform for Replacement, Reduction, and Refinement of Animal Experiments [NORECOPA] 2015), and in the UK since 2004 (National Centre for the Replacement Refinement & Reduction of Animals in Research [NC3Rs] 2015). Each 3R center is tasked with funding 3R research, performing surveys on attitudes towards animal ethics, and promoting open dialogue about research on animals. Although the 3Rs have originated from the laboratory research field, several 3R centers also have a special focus on the 3Rs in wildlife research (CCAC 2015; NC3Rs 2015; NORECOPA 2015). For example, efforts have been made to compile guidelines for improved animal welfare in wildlife research from professional societies and research councils (Guidelines for Wildlife Research 2008; CCAC 2015).

Ethical animal use in all fields of research requires consideration of animal welfare needs. Presently, there are no globally recognized avenues for reporting animal use in research and education, which makes it difficult to analyze trends in global animal use concerning research areas and species (Törnqvist et al. 2014). However, since 1996, animal use in EU member states is collated every third year. Approximately 11.5 million laboratory animals were used in 2011, and the percentage of animals used in biomedical research and toxicity testing remains rather stable over time (about 60% and 9%, respectively). Similar information on animal use in wildlife research is not available in the EU (European Commission 2013).

The purpose of animal-based research models differs between laboratory (biomedical and toxicologic) and wildlife research (Curzer et al. 2013; Sikes and Paul 2013). In laboratory research, animals are typically used as models for humans, to increase the knowledge of physiologic or pathologic mechanisms, for example, safety testing of new drugs and fundamental physiologic research. In wildlife research, defined here as studies on free-ranging vertebrates, animals themselves are the subject of investigation to better understand their biology, ecology, abundance, behavior, and health (Cooch et al. 2012; Kjellander et al. 2012; Sanders and Trost 2013). Research involving wild animals can also be applied to areas where results are significant for species and populations other than the studied animals. Such research includes investigation of disease transmission among humans, domestic animals, and wild animals (Buttke et al. 2015; Jenkins et al. 2015), wildlife as an indicator of environmental health (Ryan et al. 2009; Durkalec et al. 2015), and recently also wildlife as a model for human diseases (Fröbert et al. 2010; Fink et al. 2011). Laboratory studies frequently include experimental manipulations, whereas wildlife research is often descriptive (Sikes and Paul 2013). In addition, research conditions differ between laboratory- and field-based studies (Lane and McDonald 2010). Standardized conditions with inbred species, regulated housing and environmental settings, acclimatization, and training of animals to reduce stress are desired in laboratory research to facilitate interpretation of data from different animal models. Wildlife research conditions, on the other hand, are more heterogeneous with genetic variations within species, interspecies variations in behavior, physiology, and population size, and nonstandardized environmental conditions (Mulcahy 2003a). Free-ranging wild animals are not accustomed to humans and the research process. Capture, handling, marking, and sampling are likely perceived as major stressors by the animal (Wilson and McMahon 2006).

Centers practicing the 3Rs emphasize that good animal welfare practice, including the 3Rs, is characterized by the same features for both wildlife and laboratory research, even though the scientific aims and experimental conditions differ between the two areas (CCAC 2015; NC3Rs 2015; NORECOPA 2015). Curzer et al. (2013) suggested tailor-made 3Rs for wildlife research, translated from 3R tradition in biomedicine. Different approaches and procedures may be needed for wild animals compared to laboratory-bred animals because of the differences in experimental conditions and research purposes (Griffin and Gauthier 2004). However, some strategies for the 3Rs could be applied to both settings. Examples include computer modelling, improved statistical design, development of new sampling and analytical techniques, and educational programs for improved handling of the animals before, during, and after experimental procedures. The 3Rs should be applied systematically throughout the research process, preferably by using checklists or guidelines. This approach is practiced when teaching Australian graduate students about ethics and practical components of wildlife research, with a strong focus on the 3Rs during the early stages of the experimental and sampling design (Monamy and Gott 2001). Critical steps during the research process where a 3R review may enhance animal welfare and data quality are:

1) Define research question and hypothesis.
2) Choose appropriate methods to address research questions with well-defined end results.
3) Optimize study design, including data sharing.
4) Consult a statistician.
5) Plan and practice experimental procedures, including handling of animals before, during and after the experiment.
6) Decide humane endpoints.
7) Choose methods for euthanasia, if needed.
8) Properly report and publish according to the ARRIVE guidelines for reporting in vivo experiments (Kilkenny et al. 2010), including publishing negative data to avoid duplication.

ROLE AND CHALLENGES OF THE 3RS IN VARIOUS RESEARCH FIELDS

Biomedical and Toxicologic Research

Technical and scientific advances in laboratory research, such as new in vitro methods with stem cells and in silico method developments within computational science, are driving the development of methods for replacement of research on animals or reduction of animal use. There is also an increased interest in assessing the overall concept of animal welfare instead of a one-dimensional focus on estimations of pain or distress with regards to refinement of experimental procedures. Other trends include increased focus on collaborations among authorities, industry, and academia on increased development, validation, and use of alternative methods resulting in replacement, reduction, or refinement (Altex Proceedings 2014).

Certain fields of biomedical research are not necessarily moving toward reduced animal use, despite increased availability of in vitro or in silico methods. The number of scientific procedures using animals such as genetically modified mice and fish has recently increased, and there is continued reliance on in vivo models (NC3Rs 2014). However, one example of an important refinement initiative is work on a severity assessment framework document by a European Commission expert working group (EU 2012). This document contains examples illustrating and promoting the use of severity classification, day-to-day assessment, and actual severity assessment in biomedical animal models.

Toxicologic research is often regulated by legislation and guidelines, including standard operating procedures (SOPs) for toxicity testing and safety assessments of new drugs and chemicals (EU 2008, 2009; Organization for Economic Co-operation and Development [OECD] 2014). Such regulation likely both enhances development of new methods and enables acceptance and implementation of alternative methods, resulting in increased use of the 3Rs (Törnqvist et al. 2014). In vitro and in silico methods to study toxicology mechanisms and markers for toxicity are already used today, and animal use is predicted to decrease in the future (NC3Rs 2014).

Wildlife research

"Wildlife" obviously encompasses a very diverse group with regards to taxa and ecologic, physical, physiologic, and behavioral characteristics. This diversity poses challenges when trying to generalize the needs and responses of animals used in wildlife research (Lane and McDonald 2010). It is clear, however, that there are increasing awareness and consideration of the welfare of wild animals used in research (Brivio et al. 2015). Negative animal welfare effects in field research can be minimized by application of the 3Rs. Use of the 3Rs benefits the welfare of individual animals and may also decrease the risk of negative effects on a larger scale, because impact on individual welfare and survival certainly has consequences for social groups or populations (Bekoff 2002; Sikes and Paul 2013). The 3Rs also benefit research quality. Capture, handling, and marking can affect short- and long-term health, behavior, and welfare, with potential impacts on research data (Mulcahy 2003b; Cattet et al. 2008). Refined research methods may therefore decrease confounding effects in study results (Powell and Proulx 2003). Within wildlife research, there are examples of projects specifically aimed at development and validation of methods that address the 3Rs (Cattet et al. 2006; Long et al. 2007), and projects where 3R methods have been applied (Sharma et al. 2014; Harms et al. 2015). Several professional societies provide guidelines on wildlife research (Guidelines for Wildlife Research 2008), and the implementation of the 3Rs in wildlife research has been presented in a consensus paper (NORECOPA 2008). However, the process of incorporating the 3Rs into wildlife research is slow (Draper and Bekoff 2013) and is only occurring on a small scale compared to laboratory research. Importantly, referral to the concept of the 3Rs in peer-reviewed wildlife research literature is uncommon.

One explanation for the limited presence of the 3Rs in wildlife research could be that the principles are not, or are not perceived as, applicable to wild animals in field settings (Griffin and Gauthier 2004; Lane and McDonald 2010). In addition, animal welfare implications of field research are often unknown, and welfare indicators are insufficiently known for many wild species (Cattet 2013), further complicating the inclusion and validation of the 3Rs in wildlife research (NORECOPA 2008). Another reason is that some researchers may work under the perception that using individual animals for "the better good" of wildlife populations and ecologic systems is acceptable without considering how the end justifies the means (Paquet and Darimont 2010). Finally, awareness and knowledge of the 3Rs among wildlife researchers may simply be missing through lack of education and absence of the 3Rs in significant professional literature (Curzer et al. 2011; Cattet 2013). Lane and Jackson (2013) stressed the need to train all personnel interacting with wildlife in best practices for capture, handling, marking, and release. Proulx et al. (2012) suggested that field personnel should be trained in the concept and implementation of the 3Rs prior to beginning work. In fact, animal welfare is a growing concern for stakeholders in wildlife research, and ethical committees, employers, journal publishers, and funding agencies increasingly require that researchers consider and address welfare implications of their research (McMahon et al. 2012;

Cattet 2013). While replacing wildlife with suitable substitutes is not always feasible at present, refining research methods and reducing the number of animals are possible in most wildlife research projects.

IMPLEMENTATION OF THE 3RS IN WILDLIFE RESEARCH

Key factors—organization, awareness, and research development

The moral and ethical implications of using wild animals in research, such as the welfare of individual animals vs. species, population and ecosystem welfare, are debated (McMahon et al. 2012, 2013; Draper and Bekoff 2013). Nevertheless, given societal concern and the impacts of animal welfare on research quality, implementation of the 3Rs in wildlife research should be as strict as for other research areas (Lane and McDonald 2010). Proulx et al. (2012) suggested that collaboration in the development of formal training courses, protocols, and guidelines for wildlife species will help promote the implementation of the 3Rs.

We have identified three key factors for successful incorporation and implementation of the 3Rs in wildlife research: 1) enhanced organizational structure and management commitment to welfare, 2) increased 3R awareness, and 3) research innovation, validation, and implementation.

These factors do not stand alone, and they obviously overlap. An organizational structure and management with high standards regarding animal welfare and the 3Rs will increase the 3R awareness and empower staff at all levels of organization to be innovative in their efforts to enhance welfare. Comprehensive 3R visions and goals, education, and internal workshops on the 3Rs, and recognition of good examples of implementation would further increase awareness. Increased 3R awareness and organizational expectations stimulate staff to act on ideas regarding the 3Rs, and to test ideas during the research process. To enable implementation of new methods that result in reduction, refinement, or replacement, an organization must provide tools to facilitate the process from innovation to implementation. Within an organization, this requires a process for testing ideas, validating and implementing them into SOPs, and communicating them to all staff.

When it comes to research innovation of the 3Rs, it is crucial to uphold high-quality research both within general research projects, and in those specifically investigating the 3Rs. In fact, when the inclusion of 3R methods improves research quality, incorporation of the 3Rs is further stimulated. In addition, the 3Rs should be included in research and project planning and in publishing results to enable sharing of information and interdisciplinary collaborations among academia, industry, authorities, equipment manufacturers, and animal welfare organizations.

Törnqvist et al. (2014) showed that organizational culture and 3R awareness were key factors for successful implementation of the 3Rs in the daily practices of researchers and animal technicians. The importance of organizational culture rather than legal demands to achieve 3R improvements was suggested by Brønstad and Berg (2011). The gap between intentions and practices was revealed by *Views on the 3Rs*, a United Kingdom survey in which laboratory researchers and animal care staff were asked when during the research process the 3Rs were considered (NC3Rs 2008). Almost all respondents (95%) considered the 3Rs when initiating their projects, but this dropped to 50% during the experimental procedures and to 25% when presenting data at conferences or meetings. To our knowledge, similar data from wildlife researchers are lacking, but surveys could be developed and implemented, and results shared among the wildlife professional community.

Schuppli and Fraser (2005) reported that researchers worry that in some instances, reducing the number of animals used in a study may result in increased pain and distress in the animals used. This conflict between reduction and refinement was illustrated in a study where 20 painful procedures on one mouse (*Mus musculus*) were equally preferred by students to the same procedure performed once on 20 mice (Franco et al. 2014). Guidance is certainly needed on how to prioritize between the 3Rs when planning animal experiments and in ethical committee discussions (de Boo et al. 2005).

In wildlife research, a research design allowing several samples to be collected from one animal results in reduction (Proulx et al. 2012) but may lead to longer handling times and postoperative discomfort and pain, thus contradicting refinement. Even though reduction is not accompanied by refinement per se, Törnqvist et al. (2014) showed examples of synergies between these two Rs. A new microsampling technique for collection of blood resulted in a reduced number of rats (*Rattus norvegicus*) and mice used. Compared to the technique for larger-volume samples, it also substantially reduced discomfort, with shortened times of restraint and collection, reduced risks for hematoma, and decreased total blood loss, and in some study designs, fewer samples per individual were required (Jonsson et al. 2012a, b). In addition, micro-sampling resulted in higher data quality with simultaneous evaluation of toxicity and toxicokinetic information, all together strengthening the implementation of the 3Rs.

A Canadian survey on 3R awareness showed a concern among researchers that incorporation of the 3Rs affects scientific quality and increases costs (Fenwick et al. 2011). Törnqvist et al. (2014) showed that implementation of 3R projects in toxicity testing resulted in a reduction by half the number of rats and substantially increased cost-effectiveness yet maintained scientific rigor. Reduction of animal use usually results in reduced costs, with a reduced number of purchased animals, and decreased use of cages and laboratory space, man-hours, and amount of test substance used. However, some scientists worry that incorporating refinement in their projects would increase costs (Fenwick et al. 2011). To our knowledge, there are no peer-reviewed publications supporting these assumptions. On the contrary, increased welfare before, during, and after animal experimentation could decrease costs. New routines for group-housing of male mice, traditionally single-housed because of aggression, resulted in increased animal welfare and an improved working environment for the staff due to calmer animals, fewer cages to clean, and reduced costs for housing material (Annas et al. 2013). Similarly, improvement of capture, handling, and anesthesia techniques minimizing morbidity and mortality are examples of refinement that would likely reduce costs as well as improve wildlife welfare.

3R strategies in wildlife research—an interdisciplinary approach

Researchers are ethically obliged to ask themselves: 1) Do I need to use traditional animal-based methods to test my hypothesis (replacement); 2) if I do, how can I design my study to obtain high-quality data using as few animals as possible (reduction); and 3) how can I minimize pain, suffering, and distress in the animals used (refinement). Here, we present 3R strategies and examples from laboratory and wildlife research for comparison and inspiration. Some strategies apply to more than one 3R principle. For instance, noninvasive methods can be considered as replacement, but also part of reduction and refinement.

Strategies for replacement

Replacement of animals in research is the most debated of the 3Rs in both the research community and society, with a wide range of opinions from "no research on animals is ever acceptable" to "it will never be possible to replace animal models with alternatives." In laboratory research, physiologic or pathologic processes are commonly studied in animal models, and the use of animal models in biomedical research is predicted to increase (NC3Rs 2014). In toxicology testing and safety assessments, however, a paradigm shift is occurring, from in vivo studies with pathology read-outs to "adverse outcome pathways" (AOPs) (Vinken 2013). Adverse outcome pathways provide a framework within which to assess toxicity processes from the initial molecular initiating event (MIE) by a chemical to an adverse outcome for the organism through a set of key events. In vitro methods evaluating cellular responses to chemicals and in silico methods predicting the toxicity of chemicals can be used to identify and evaluate events within the pathways (OECD 2013). Because MIEs and key events can be conserved across species, it is possible to develop models that are applicable across a diverse range of organisms, including wildlife. Madden et al. (2014) discussed the application of AOPs for wildlife and emphasized the opportunities to make predictions for wild species that are unlikely to otherwise become available.

In wildlife research, the animals are usually the object of the study and may therefore be difficult to replace (Griffin and Gauthier 2004). Nevertheless, today there are several noninvasive sampling methods available that, where appropriate, can replace the need to capture and handle wild animals. Sign surveys (tracks, scats, bones, etc.), genetic and endocrine sampling, and camera trap observations are continuously improving (Kelly et al. 2012). Olson et al. (2012) detected Eastern hellbenders (*Cryptobranchus a. alleganiensis*) by extracting DNA from water and soil. Cortisol and reproductive hormone levels were analyzed in grizzly bear (*Ursus arctos*) hair collected via hair snags (Bryan et al. 2013), and thyroid hormones were measured in feces of several mammalian and avian species (Wasser et al. 2010). Saliva samples for viral disease detection in free-ranging primates collected from ropes (Smiley Evans et al. 2015), and collection of urine on snow for wolf (*Canis lupus*) DNA analyses (Sastre et al. 2009) are other methods replacing animal use.

In silico techniques are commonly used in laboratory research (NC3Rs 2014). In wildlife research, computer modelling methods are used for population studies, including those aimed at evaluating methods of lethal or fertility control, investigating animal movements, and predicting disease transmission, enabling both replacement and reduction (Alexander et al. 2012; Heinonen et al. 2014). The use of immature vertebrates or invertebrates in laboratory research, such as fruit flies (*Drosophila melanogaster*), may replace the use of mature vertebrates (Ong et al. 2015) in appropriate settings. In wildlife research, the use of a less sentient or nonprotected species as a substitute is usually defined as replacement (Lane and McDonald, 2010). This approach, debated among researchers, has potential in ecotoxicologic studies where less sentient invertebrate models can be used for assessment of environmental pollutants (Ashauer et al. 2007). Furthermore, Curzer et al. (2013) suggested that replacement includes using species other than keystone species, which have a large impact on other species and their ecosystems.

Strategies for reduction

Many of the principles and techniques used to reduce the numbers of animals in laboratory research are applicable to wildlife research. It is essential to have appropriate and well-planned experimental design, with consideration of the study aims, species, and individual animal characteristics, and previous results from the field if possible. Consultation with a statistician can ensure that sample sizes are sufficient but not excessive, so the collected data will be valid. Wildlife research may necessarily involve fewer animals than biomedical research because of the myriad difficulties associated with capturing wild animals. Research that investigates general patterns in species or across populations may, however, require larger sample sizes because of greater variability within and between populations and habitat (Sikes and Paul 2013). Optimization of study design has proved to be an effective strategy for reduction in toxicology research (Törnqvist et al. 2014). Nine traditional protocols for in vivo safety assessments of genetic and reproductive toxicity were updated and validated to ensure the scientific quality and resulted in an 8–80% reduction in number of animals used.

Coordination between research groups with similar goals can reduce the number of animals used. In order to further minimize the need for separate, similar studies, establishment of biobanks is useful to maximize information gained from each animal used in field experiments (Lane and McDonald 2010). Törnqvist et al. (2014) used coordination between research areas and research units as a strategy to achieve reduction of number of animals needed from 13% to 100% in 11 projects. This was the result of recognized increase in scientific outcomes, establishment and use of biobanks, and enhanced communication and collaboration between different research groups. Publishing and data sharing in general are essential to improve study design and avoid repeating experiments (Kilkenny et al. 2010). Pilot studies that use a smaller number of animals are appropriate to ensure that a project of a larger scale is feasible (Mulcahy 2003a).

Reduction of animal numbers can be obtained by combining traditional sampling methods with noninvasive methods, such as simultaneous use of telemetry, camera traps, fecal and hair collection, and sign surveys (Kelly et al. 2012). A noninvasive alternative to the capture-recapture method combines empirical and simulation evaluations of animal detection probabilities and abundances in relation to environmental variables. These methods may be used to extrapolate abundances across a species' distribution and uncover trends (Nielsen et al. 2010). Simulations based on marsh bird data enabled determination of the number of routes, proportion of survey stations along a route, and survey times needed per year to successfully monitor abundance trends (Steidl et al. 2013).

There is continuous technical development of in silico products, field equipment, and tissue and body fluid analysis equipment. Global positioning system (GPS) transmitters provide more detailed information from an animal than very high frequency (VHF) transmitters (Markham 2008). Improved capture methods can result in fewer captures of nontarget animals (Brand Phillips and Winchell 2011). Collection of blood samples is a common experimental procedure in research on wild birds, but there are concerns regarding the impact on bird survival (Brown and Brown 2009). Improved techniques and routines for sampling and analyzing blood in laboratory animals can substantially reduce the number of animals used in toxicity testing (Törnqvist et al. 2014). The development of microsampling and use of microliter-analysis of rat blood (Jonsson et al. 2012a, b) resulted in a reduction of up to 50% of the number of animals sampled, depending on scientific aim and study design (Törnqvist et al. 2014). Importantly, this microsampling method also resulted in refinement. If introduced in research on wild rodents and birds, it would likely result in significant reduction and refinement in wildlife research.

Strategies for refinement

Refinement decreases negative impacts on animals and enhances welfare. Refining the handling and care of laboratory animals can reduce stress, but increasing the well-being of animals before, during, and after experimental procedures is also important (Baumans and Van Loo 2013). Because interaction with humans is a stressful event for a wild animal, the focus is on minimizing the negative short- and long-term effects on health and welfare (Lane and McDonald 2010). Refinement strategies in wildlife research include conducting retrospective reviews, assessing potential sources of harm to target and nontarget species, and determining how these can be eliminated or minimized. Negative impacts on animal welfare can often be reduced by careful experimental design, and by choosing less invasive sampling techniques (Powell and Proulx 2003). Remote biopsy darting of polar bears (*Ursus maritimus*) is less invasive than physical capture (Pagano et al. 2014), and swab sampling compared to blood collection in birds is less invasive (Beja-Pereira et al. 2009). Disturbing animals may result in abandoned nests, den sites, or home ranges. Uher-Koch et al. (2015) found that nest survival decreased when incubating Yellow-billed Loons (*Gavia adamsii*) and Pacific Loons (*Gavia pacifica*) were disturbed.

There are several strategies for refinement during the capture and handling process. Intense pursuit and trap captures may cause detrimental physiologic effects and physical injuries (Iossa et al. 2007; Cattet et al. 2008), thus researchers should ensure that the least stressful and harmful capture methods are used. Physical capture can be refined by the use of tranquilization for handling, to decrease the animals' stress level (Montané et al. 2003; Wolfe and Miller, this supplement). Evaluation of capture and anesthesia, including measurement of physiologic variables such as pulse rate, respiratory rate, and body temperature, is imperative for refinement of wildlife handling (Fahlman et al. 2011; Brivio et al. 2015). Providing intranasal oxygen supplementation to immobilized or anesthetized animals is an example of refinement in wildlife studies that can prevent morbidity and mortality during and after capture (Fahlman et al. 2014a, b), as is providing appropriate analgesia. Methods used for marking must be chosen carefully to avoid pain, discomfort, and long-term welfare problems such as infections, entanglement, or growth-related issues, such as animals outgrowing radiocollars. Field personnel should make sure that study animals are protected from weather and predators during recovery (Lane and McDonald 2010). Determination of humane endpoints and appropriate methods for euthanasia of injured animals is equally important in research on wildlife (Lane and McDonald 2010) and laboratory animals (Baumans and Van Loo 2013). In wildlife research, this must be determined for both target and nontarget species. It is vital that all field personnel know how to handle injured animals (Lane and McDonald 2010) and that a plan for rehabilitation or euthanasia of injured animals is in place.

Wild animals are completely dependent on the handling skills of field personnel during capture, experimental procedures, and release. Similar to laboratory settings, training of field personnel in best practices is necessary for refining the research process (Lane and Jackson 2013). However, in contrast to the handling of free-ranging wildlife in the field, handling of laboratory animals is a part of acclimatization to the experimental procedure. Taming rats over 2 wk, including the critical socialization period of life for rats between 4 and 5 wk of age, resulted in a long-lasting higher level of tameness and trust in humans (Maurer et al. 2008). This type of training is not available and indeed not desired in most wildlife studies.

Basic needs are similar in laboratory and wild animals, such as social contact, nest building, hiding, exploring, foraging, and resting. Behavioral and physiologic needs of laboratory animals are translated into adequate housing, husbandry, and environmental enrichment (Baumans and Van Loo 2013). Refinement of housing and husbandry is not applicable to research on free-ranging wildlife. Nevertheless, it is important to consider basic needs and behavior in wild animals when the research includes capture, transportation, manipulation of environment or social grouping, social deprivation, methods of attracting animals, and disturbing interactions between and within species (Ferreira et al. 2013).

RECOMMENDATIONS

To enable further achievements in combining ethics, animal welfare, and high-quality science in wildlife research, we suggest:

1) an early and systematic incorporation of the 3Rs throughout the research process, considering the characteristics of the species and research conditions;
2) consideration of the following three key factors for successful implementation of the 3Rs:
   a) organizational structure and management,
   b) 3R awareness, and
   c) research innovation, validation, and implementation; and
3) an interdisciplinary approach to the 3Rs, resulting in increased transfer of knowledge between laboratory and wildlife research.

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