



Communication

New Online Resource on the 3Rs Principles of Animal Research for Wildlife Biologists, Ecologists, and Conservation Managers

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Abstract: The Earth's biodiversity is in crisis. Without radical action to conserve habitats, the current rate of species extinction is predicted to accelerate even further. Efficient species conservation requires planning, management, and continuous biodiversity monitoring through wildlife research. Conservation biology was built on the utilitarian principle, where the well-being of species, populations, and ecosystems is given priority over the well-being of individual animals. However, this tenet has been increasingly under discussion and it has been argued that wildlife researchers need to safeguard the welfare of the individual animals traditionally subjected to invasive or lethal research procedures. The 3Rs principles of animal use (Replacement, Reduction, and Refinement) have become the cornerstone of ethical scientific conduct that could minimize the potential negative impact of research practices. One of the obvious strategies to implement the 3Rs in wildlife studies is to use non-invasive or non-lethal research methods. However, in contrast to toxicological or pharmacological research on laboratory animal models, up to now no 3Rs databases or online resources designed specifically for wildlife biologists, ecologists, and conservation managers have been available. To aid the implementation of the 3Rs principles into research on wildlife, I developed an online resource whose structure is outlined in this paper. The website contains a curated database of peer-reviewed articles that have implemented non-invasive or non-lethal research methods that could be used as a guideline for future studies.

Keywords: 3Rs; database; non-invasive sampling; non-lethal sampling; website; wildlife welfare



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1. Introduction

Although the importance of preserving nature has been recognised for a long time, the accelerating impact of anthropogenic activities on ecosystems during the last decades has prompted an upsurge in efforts to mitigate the so-called sixth mass extinction [1–3]. For example, mammals are currently one of the most endangered taxa, with 25% of species for which we have sufficient data to assess their conservation status classified as endangered or declining [4,5]. In order to protect and manage animal species and populations, we need to understand their role and interactions within the ecosystem [6–9] and their responses to anthropogenic activities [10–12]. Therefore, a vital component of our attempts to tackle the global biodiversity crisis is conservation biology research [13–15], including studies on wildlife [16–18]. How many animals are being used in conservation biology research is difficult to estimate. However, for example, within the EU Member States several thousands of vertebrate and cephalopod species are used in species preservation research every year (Table 1).

Table 1. The number of animals used in research procedures¹ with the aim of “preservation of species” in the EU Member States [19].

Year	2015	2016	2017
Animals Used	38,070	71,852	78,893

¹ A procedure is defined by the EU Directive 2010/63 as “any use, invasive or non-invasive, of an animal for experimental or other scientific purposes, with a known or unknown outcome, or educational purposes, which may cause the animal a level of pain, suffering, distress or lasting harm equivalent to, or higher than, that caused by the introduction of a needle in accordance with good veterinary practice”.

For a long time, conservation has been concerned primarily with the preservation of species, populations, or ecosystems, not individual animals [20]. However, over recent years, concerns have been raised over the impact on the individual animal under study, posed by traditionally invasive or lethal research methods [21–23]. Here, I would like to provide a few examples. One illustration of an invasive technique is blood or tissue sampling for genetic or physiological assessment. Although generally considered safe, it has been reported that blood sampling has resulted in increased mortality in cliff swallows (*Petrochelidon pyrrhonota*) [24]. Another potentially problematic research technique is toe-clipping, used for marking and identifying individual amphibians, lizards, and rodents. This method continues to be implemented even though it has been already shown that toe-clipping can cause the inflammation and infection of limbs [25,26], affect locomotor performance [27,28], and lower survival rates [29]. Even non-invasive methods (i.e., not penetrating the skin), such as the attachment of radio-transmitters to track animals, could result in poor animal welfare. It has been described that the attachment of radio-transmitters might cause skin lesions and obstruct movement [30]. Other studies have shown that radio and GPS collars can affect mobility [31] and feeding behaviour [32]. Lastly, before we can actually perform the blood or tissue sampling, marking, or attach an instrument, we need to catch the animal. Unlike laboratory and domesticated animals, wildlife species are usually not accustomed to interactions with humans. Therefore, even capture alone can be very stressful for the free-living animal [33]. This stress can in some cases lead to capture myopathy, a metabolic disease associated with the stress of capture [34–36]. To improve the safety of both wild animals and scientists, anaesthesia can be used during capture or handling. Anaesthesia, however, can increase the risk of mortality [37] and result in behavioural changes [38]. Fatalities have been also reported as a result of a physical injury caused by the net or trap [39–41] or due to extreme temperatures within the trap [42].

Research practices that might negatively affect animal welfare can lead to public outrage [43–45] and disagreement within the scientific community [44,46–48]. For example, the editors of the journal *Biological Conservation* reported that they decided to reject several manuscripts for publication on ethical grounds because the authors used lethal methods that the editors considered “unnecessary and inappropriate” [22]. In some instances, lethal sampling might also add pressure to already small populations of threatened species [49]. Furthermore, methods that cause pain or distress will likely compromise animal welfare, behaviour, or fitness [50–52]. Poor animal welfare is often reflected in atypical behaviour, leading to biased results [39]. Unfortunately, there seems to be a lack of guidance and education in ethics, animal welfare, and responsible wildlife research [53].

An important landmark in thinking about the welfare of animals used in research was achieved more than 60 years ago when Russell and Burch developed the so-called 3Rs principles (Replacement, Reduction, Refinement) [54]. While these principles were originally proposed for working with laboratory animal models, they can—and should—also be applied in wildlife research to help overcome the divide between species conservation and animal welfare [23]. Nevertheless, the application of the 3Rs principles to wildlife research has significantly lagged behind that of research on laboratory animal models. For instance, a recent study by Field et al. [55] reviewed animal care policies in 206 biodiversity and wildlife-related journals and found that only 6% required authors to adopt the 3Rs principles in their research. Moreover, even though many non-invasive research methods

that could replace the traditional, invasive techniques have been developed, their potential seems not to have been fully implemented [56]. One of the main reasons for the low implementation rate might be lack of awareness. While there are several databases available describing the 3Rs principles and alternative methods for laboratory and toxicology studies (e.g., the NAT database available at <https://nat-datenbank.de/>, accessed on 20 April 2021), there is no equivalent resource that could help guide researchers working with wildlife. Therefore, to promote the appropriate implementation of the 3Rs principles into wildlife research, I developed an online resource aimed specifically at wildlife biologists, ecologists, and conservation managers.

2. Materials and Methods

As guidelines on animal experimentation that focus primarily on laboratory animals [57–60] are insufficient for wildlife research due to vast differences between species and research methodology, the goal of this project was to create an overview of how the 3Rs principles can be applied in wildlife research. The website consists of two main parts: (1) background information and (2) examples of the 3Rs implementation, plus links to other useful resources, FAQ, information about the author, and a contact form (Figure 1).

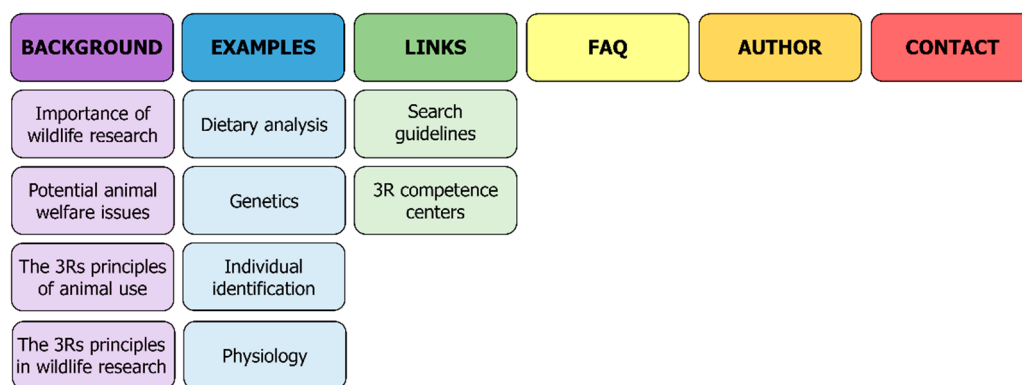


Figure 1. The current content structure of the 3Rs Principles in Wildlife Research website (<https://3RsWildlife.info>, accessed on 15 May 2021).

The first main part encompasses the background information on (i) the importance of wildlife research; (ii) potential animal welfare issues when using capture, invasive methods, or lethal sampling; (iii) the 3Rs principles of animal use; and (iv) how the 3Rs can be applied to research on wildlife. The second main part contains specific examples of peer-reviewed studies that either developed or implemented a non-invasive or non-lethal technique. The studies were identified through a systematic search of the literature conducted between March 2020 and May 2021. I searched the Web of Science and Google Scholar databases using the query (“dietary analysis” OR “diet” OR “genetics” OR “DNA” OR “identification” OR “marking” OR “recognition” OR “physiology” OR “stress” OR “hormone”) AND (“non-invasive” OR “non-lethal”). The generated list of publications was then scanned for suitable studies.

3. Results

The informational website 3Rs Principles in Wildlife Research is freely accessible at <https://3RsWildlife.info> (Figure 2; accessed on 15 May 2021). To the best of my knowledge, it is the first 3Rs online resource of its kind developed specifically for researchers working with free-living animals. The website currently features approximately six hundred peer-reviewed wildlife studies, covering more than seventy non-invasive and non-lethal methods across almost one thousand different species. There are now four main areas covered: dietary analysis, genetics, individual identification, and physiology (Figure 1).

The content is being continuously updated and expanded. Future development of this resource will include the addition of other types of studies (e.g., ecotoxicology).

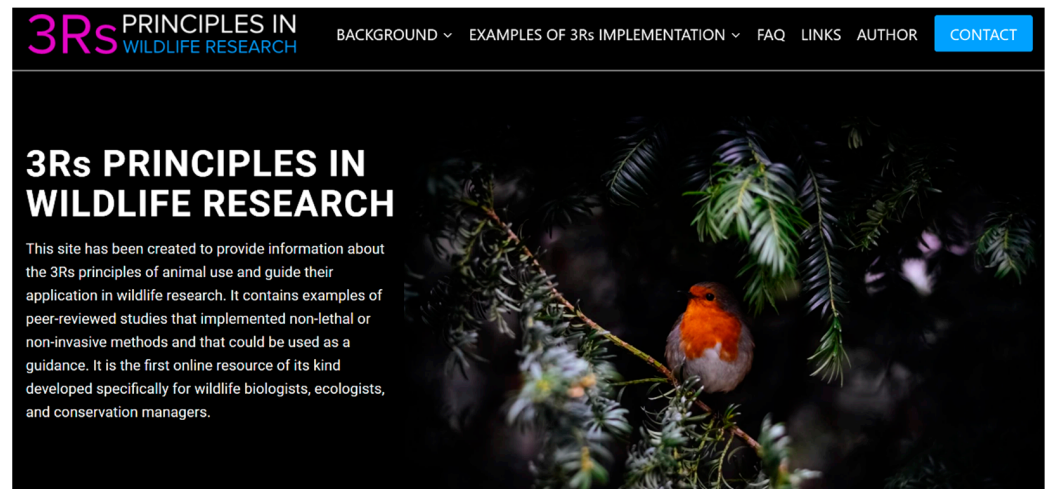


Figure 2. Homepage of the informational website and database 3Rs Principles in Wildlife Research (<https://3RsWildlife.info>, accessed on 15 May 2021).

4. Discussion

The preservation of animal and plant species is one of the greatest challenges of the 21st century [61] and wildlife conservation research has never been more important than it is now [13]. At the same time, animal welfare has become an increasingly discussed topic among both researchers and the general public, as evidenced by initiatives such as One Welfare [62]. While animal welfare science has traditionally focused on pet, farm, and laboratory animals, concern about the well-being of animals has recently expanded to include wildlife as well [22,23,63]. It is crucial that our research practices do not excessively compromise the welfare of the individual animals under study [64,65]. The availability of evidence-based guidelines and studies that promote the use of research methods with no or only minimal harm to the animal is therefore a critical part of efficient and ethical wildlife research.

Wildlife biologists, ecologists, and conservation managers might benefit from freely available information on how to best design their studies. The core priority for the informational website is to provide a single source for the evidence base of non-invasive or non-lethal approaches that have been either developed or implemented in different types of studies and for a variety of animal species. It is important to note that the online resource cannot provide explicit instructions that would suit every type of a research project and it is advisable to obtain additional guidance from a local animal welfare officer or ethics committee. Nevertheless, the website 3Rs Principles in Wildlife Research reduces the burden associated with searching, retrieving, and evaluating evidence for its relevance and appropriateness.

This work also aims to promote animal welfare in wildlife research by (1) increasing the sensitivity of wildlife biologists to the potential issues in animal research; (2) collecting, summarizing, and making accessible the knowledge and experience with non-invasive research methods; (3) increasing the efficiency of searching for alternative methods and study design; (4) reducing the number of animals that need to be “sacrificed” or invasively handled; and (5) encouraging further research into non-invasive methods and animal welfare.

Lastly, the informational website can also play a significant role in promoting the 3Rs principles outside of the laboratory settings and reinforce the position that responsible animal research is crucial across disciplines.

5. Conclusions

The availability of relevant 3Rs resources that are freely accessible is a crucial part of promoting responsible animal use in research. I hope that this resource will help inform those wishing to refine their research methods and stimulate further development of non-invasive approaches to wildlife research and management.

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