Towards more compassionate wildlife research through the 3Rs principles: moving from invasive to non-invasive methods

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Research in ecology and wildlife biology remains crucial for increasing our knowledge and improving species management and conservation in the midst of the current biodiversity crisis. However, obtaining information on population status often involves invasive sampling of a certain number of individual animals. Marking and sampling practices include taking blood and tissue samples, toe-clipping of amphibians and rodents, or using implants and radio-transmitters—techniques that can negatively affect the animal. Wildlife research may then result in a fundamental conflict between individual animal welfare and the welfare of the population or ecosystem, which could be significantly reduced if non-invasive research practices were more broadly implemented. Implementation of non-invasive methods could be guided by the so-called 3Rs principles for animal research (replace, reduce, refine), which were proposed by Russell and Burch 60 years ago and have become a part of many animal protection legislations worldwide. However, the process of incorporating the 3Rs principles into wildlife research has been unfortunately rather slow and their importance overlooked. In order to help alleviate this situation, here I provide an overview of the most common practices in wildlife research, discuss their potential impact on animal welfare, and present available non-invasive alternatives.

Keywords: 3Rs principles, animal welfare, ecology, reduction, refinement, replacement

Ecosystems worldwide are currently experiencing a dramatic species extinction process, which has been largely attributed to human activities (Harrop 2011, Ceballos et al. 2015). Recognizing the critical situation, several international conventions have been implemented with the aim to halt the biodiversity crisis and support conservation measures (e.g., Convention on Biological Diversity, Bonn Convention on the Conservation of Migratory Species of Wild Animals, Bern Convention on the Conservation of European Wildlife and Natural Habitats, Convention on International Trade in Endangered Species of Wild Fauna and Flora). These conservation efforts depend on accurate data on species distribution, population size and impact of global changes, and it is therefore necessary to continuously monitor populations of various plant and animal species.

In the noble pursuit of knowledge that is important for preserving wildlife populations, scientists can unfortunately inflict distress on animals, because wildlife biodiversity monitoring has traditionally employed some invasive or even destructive techniques (Vucetich and Nelson 2007, Minteer et al. 2014b, Field et al. 2019). Examples of research activities that might influence animal welfare are chasing, capturing, blood and tissue sampling, marking, attachment of data loggers and lethal sampling (Donnelly et al. 1994, Sutherland et al. 2004, Wilson and McMahon 2006, Walker et al. 2010). The impact on animal welfare is a problem not only for the affected animal but also for the reliability of study results (Powell and Proulx 2003, Catter 2013, Jewell 2013). It has been shown that pain negatively affects data quality through behavioural, physiological and neurobiological changes (Jirkof 2017, Sneddon 2017). Because sound information about wildlife is of utmost importance for sound management decisions, it is crucial that the research procedures affect animal welfare as little as possible.

An important milestone in thinking about animal welfare in research was achieved 60 years ago, when Russell and Burch proposed the 3Rs principles (replace, reduce, refine; Russell and Burch 1959). These principles encourage scientists to replace the use of animals with alternative methods whenever possible, to reduce the number of animals in experiments to the absolute minimum, and to refine or limit the pain and distress that animals are exposed to.

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The 3Rs have since become an integral part of legislation and guidelines on animal experiments in many countries (Sneddon et al. 2017) and the research community itself has encouraged the development of guidelines to improve the use of animals for scientific purposes (Kilkenny et al. 2010, Buchanan et al. 2015, Mellor 2016). While the 3Rs principles were originally proposed for laboratory animals, they can be – and should be – applied also in wildlife research. One example is the use of non-invasive research methods, i.e. methods that do not affect the physical integrity of the animal (Lefort et al. 2019). Some non-invasive methods do not even require capturing and handling. Even though efforts have been made to support the process of incorporating the 3Rs into wildlife research (NORECOPA 2008, Lindsjö et al. 2016, Field et al. 2019, Sloman et al. 2019), there have been recently published several articles that indicate that wildlife biologists may be struggling with implementing these principles (Costello et al. 2016, Waugh and Monamy 2016, Russo et al. 2017, Lindsjö et al. 2019, Zemanova 2019).

In order to encourage the implementation of the 3Rs principles in wildlife research, I carried out a review of the literature on the potential impact of research methods on animal welfare, and provide here specific examples where the 3Rs principles have been successfully applied.

Methods

I derived my synthesis based on published journal articles and books. Specifically, I searched for relevant literature on the Web of Science and Google Scholar until March 2019 and also used references cited in the papers I found. For publications on impact of research methods on animal welfare, my search strings consisted of: Topic=(“welfare” OR “impact” OR “effect” OR “detrimental” OR “lethal” OR “survival”) AND “wildlife” OR “ecology” AND (“method” OR “technique”). To identify publications describing available non-invasive techniques and their implementation, I used the search terms: Topic=(“non-invasive” OR “non-lethal” OR “non-destructive” OR “alternative” OR “replacement” OR “reduction” OR “refinement” OR “improved”) AND “wildlife” OR “ecology” AND (“method” OR “technique”). I excluded publications in which content was not relevant to this synthesis.

Potential impact of commonly used methods in wildlife research on animal welfare

Capturing, trapping and experiments in captivity

In contrast with laboratory animals, wildlife animals are not used to interaction with humans, so any capture or handling can be very stressful (Wilson and McMahon 2006). Increased cortisol levels have been reported for example in captured Weddell seals Leptonychotes weddellii (Harcourt et al. 2010). Cattet et al. (2008) showed that grizzly bears Ursus arctos that have been repeatedly captured significantly differed in their body condition compared with bears that have been captured only once. Nest survival in captured seabirds, yellow-billed loon Gavia adamsii and pacific loon G. pacifica, was 30% lower than in non-captured adults (Uber-Koch et al. 2015). Stress of capture can even lead to capture myopathy, a metabolic muscle disease that often results in death (Nuvoli et al. 2014, Green-Barber et al. 2018).

Capturing can also change the animal’s behaviour, which then significantly affects data collected in behavioural and recapture studies. For instance, polar bears Ursus maritimus in the study by Rode et al. (2014) displayed reduction in activity and movement rates 3.5 days post-capture. Lindhart et al. (2012) showed that willow warblers Phylloscopus trochilus could recall the capture event by mist netting even a year later and learn to avoid mist nests.

Apart from stress and impact on behaviour, capturing can also result in physical damages. These damages can range from skin abrasions to broken limbs (Phillips et al. 1996, Fleming et al. 1998, Grisham et al. 2015). Trapped animals are also vulnerable to predation (Hilario et al. 2017).

Many experiments on behavior and cognition in animals take place in captive settings. While these conditions allow for logistical control, they often lead to harms for the captive animals (Marino and Frohoff 2011). The damaging effects of captivity have been well documented. Animals can exhibit stereotyped behavior (Wechsler 1991, Callard et al. 2000, Shyne 2006, Jett et al. 2017, Poirier and Bateson 2017, Williams et al. 2018a), and suffer from increased stress (Bordeleau et al. 2018, Ferreira et al. 2018), which can eventually result in higher incidence of diseases and mortality (Terio et al. 2004, Mitchell et al. 2018).

Marking

Research on wildlife often requires marking of animals to obtain data on behaviour, survival, reproduction or home range size. Virtually all marking methods require capture, which is stressful to wild animals, and many methods also involve tissue damage. Common marking techniques include for instance hot- or freeze-branding, mutilations, tags and bands, and the use of radio-transmitters.

Branding

Hot-branding and freeze-branding have been used for marking cattle Bos taurus and horses Equus caballus for centuries (Macpherson and Penner 1967), and have been modified for marking pinnipeds. Not surprisingly, hot-branding is a painful procedure, reflected in the behavioural changes of branded Stellar sea lions Eumetopias jubatus (Walker et al. 2010). Public concerns about animal welfare have resulted in lawsuits and withdrawal of research permits for sea lion research involving hot-branding (Dalton 2005). Moreover, the development of skin tumours following freeze- or hot-branding has been observed in cattle (Yeruham et al. 1996), raising caution for wildlife branding.

Mutilations

Toe clipping is a classic method for marking small vertebrates such as lizards, amphibians and rodents. Unique marking is achieved by clipping toes in different combinations on different limbs. Although considered harmless by some authors...
mitters implanted into the abdominal cavities of brown anole Anolis carolinensis pad-bearing lizards, which was documented in the Carolina anole Anolis carolinensis (Bloch and Irsich 2005).

Tags and bands
Another common method of marking animals is with tags or bands. Tags can be made from a variety of materials – most commonly metal or plastic – and are usually augmented by alphanumeric codes for individual or group recognition. Larger animals often require immobilization before marking and attaching tags, and the procedure of tag attachment can be painful (Cramer 2017, MacRae et al. 2018). In small animals, for instance, fish, tagging can affect the survival rate (Burdick 2011, Hoye et al. 2015).

Tags can be applied to many different parts of the body depending on the anatomy of the animal, most often to wings (Tre fry et al. 2013), fins (Son ne et al. 2012) or flippers (Haze kamp et al. 2010). The study by Robinson and Jones (2014) revealed that tagged seabirds, crested auklets Aethia cristatella, showed reduced return rates and provisioning behaviour. Tags can increase the cost of swimming due to drag in grey seals Halichoerus grypus (Haze kamp et al. 2010), and tagged Magellanic penguins Spheniscus magellanicus have been observed to experience foraging difficulties during low food abundance periods (Wilson et al. 2015). Moreover, tags damaged flippers of Adélie penguins Pygoscelis adeliae (Jackson and Wilson 2002), and modified diving behaviour and decreased survival in the first year after banding in little penguins Eudyptula minor (Fallow et al. 2009).

Radio-transmitters
Radiotelemetry has been key to track the movement of animals. This method uses the transmission of radio signals to locate a radio-transmitter that has been attached to an animal. Radio-transmitters can be glued to the skin, designed as a GPS collar or a harness, or surgically implanted. To track mule deer Odocoileus hemionus, elk Cervus elaphus nelsoni and moose Alces alces females, even vaginal implant transmitters are being used (Bishop et al. 2007, Barbknecht et al. 2009, Thompson et al. 2018).

Several issues have been identified with radio-transmitters. For instance, Dixon et al. (2016) found evidence of decreased survival rate associated with harness-mounted satellite transmitters on falcons Falco cherrug. In passerine birds, both entanglement with vegetation or body parts and non-entanglement injuries have been observed (Hill and Elphick 2011).

Another issue is the method of attachment and weight of the instrument. If the radio-transmitter is attached by glue, this can lead to lesions and abrasions on the skin (Field et al. 2012). The study by Rasiulis et al. (2014) showed that heavy collars decreased survival rate in caribou Rangifer tarandus, and by Brooks et al. (2008) that grazing behavior of Burchell’s zebra Equus burchelli antiquorum is affected by collar weight.

Particularly problematic is the use of implanted transmitters as this involves additional trauma to the animal. The recent study by Arnold et al. (2018) showed that transmitters implanted into the abdominal cavities of brown bears Ursus arctos performed poorly and were not biocompatible, in several cases causing the animal’s death. Several cases of mortality caused by implanted radio-transmitters have been reported also in European lynx Lynx lynx (Lechenné et al. 2012), Harlequin ducks Histrionicus histrionicus (Mulcahy and Esler 1999) and American badgers Taxidea taxus (Quinn et al. 2010).

Blood and tissue sampling
Genetic tools have become indispensable for biodiversity assessment and monitoring (Stetz et al. 2011). Genetics is important to assess abundance, occupancy, hybridization, genetic diversity, population structure and effective population size (Stetz et al. 2011, Carroll et al. 2018). Common methods used for DNA collection are blood and non-lethal tissue sampling, such as toe-clipping or fin-clipping. Blood is also commonly used for assessing levels of potential detrimental elements, such as heavy metals, and in physiology studies to assess hormonal levels (Bryan et al. 2007, Berglund 2018). Blood sampling could be however difficult in small animals, such as zebrafish Danio rerio (Zang et al. 2013), and has been even linked to lower survival rates during the first year after sampling in American cliff swallows Petrochelidon pyrrhonota (Brown and Brown 2009). Fin-clipping has been shown to be painful for fish, common carp Cyprinus carpio and Atlantic salmon Salmo salar, and may affect their survival (Hansen 1988, Roques et al. 2010).

Lethal sampling
The use of lethal means for tissue sampling and collection of voucher specimens has a long tradition in wildlife research. Besides the obvious harm to the individual animal, removing a key member of the group in species with complex social formations can result in impaired well-being of the remaining individuals (Shannon et al. 2013). Moreover, lethal methods are unfortunately often used even in cases when this is not necessary, such as in gathering data on abundance, DNA sampling or dietary analysis (Vucetich and Nelson 2007, Hammerschlag and Sulikowski 2011, Costello et al. 2016, Russo et al. 2017, Zemanova 2019).

Application of the 3Rs into wildlife research
There is a significant difference between research on laboratory animals and on wildlife in that the former is used as models for humans, for example, in testing toxicity or effectiveness of new drugs. Wildlife research, on the other hand, focuses on the study animal itself, in order to understand its biology, behavior and health. Moreover, wildlife encompasses a very broad range of species with different ecological and physiological traits, which makes generalizations of guidelines challenging. Nevertheless, the 3Rs principles can be applied to wildlife research in several ways.

Replacement may not be always possible, because the animals are the objects of the study. However, individual identification with natural marking, use of camera traps, or non-invasive sampling can provide data without the necessity of handling an animal (Fig. 1, Table 1).
Reduction (Fig. 1, Table 2) can be achieved, for example, through efficient experimental design and planning, calculating the minimum sample size, avoiding repetition through meta-analyses of previously published studies, sharing data and resources (NC3Rs 2018). Individual animals can also be used for multiple purposes – for instance by combining capture–mark–recapture and genotyping studies (Lampert et al. 2003). Another strategy for reduction is the implementation of in silico methods, which could be used for species distribution, population modelling in response to climate change or disease spread predictions (Smith and Cheeseman 2002, Zemanova et al. 2018).

Non-invasive methods can be considered as replacement, but also a part of reduction and refinement strategies (Lind-sjo et al. 2016), depending on how the methods are used (Fig. 1, Table 1–3). Refinement (Table 3) includes, for example, the use anesthesia, tranquilization and light-weight radio-transmitters (Harcourt et al. 2010, McGuire et al. 2014). For minimum injuries and capture of non-target species, it has been recommended to use call playback and taxidermy decoys (Veltheim et al. 2015), and to use traps that have been shown to cause no or minimal injuries, for instance, replacing foothold traps with box traps (Kolbe et al. 2003, Bergvall et al. 2017).

**Alternatives to capturing and trapping**

Many data that used to require trapping can nowadays be achieved by different means. For instance, the presence/absence data can be collected using camera traps or drones, and DNA can be sourced from hair traps or faeces.

**Camera traps**

Camera traps can be applied to estimate species richness, habitat occupancy, population density or behavior, with little effort by the researcher (Di Cerbo and Biancardi 2013). The absence of a researcher is particularly beneficial in the study of wild primates, where habituation to human presence could be detrimental due to threat of hunting (Bezerra et al. 2014). Camera traps can be even a more efficient method of detection than other methods, such as hair traps, cage traps or scat count surveys (Monterroso et al. 2014, Welbourne et al. 2015, Day et al. 2016). This efficiency improves when using a lure or bait (Boulerice and Van Fleet 2016, McLean et al. 2017). Modern camera traps can record also videos that can be used in behavioural studies (Lobo et al. 2013, Flagg et al. 2016).

Camera traps have been used for detection of many species, including small terrestrial and arboreal mammals such as red and eastern grey squirrels, *Sciurus vulgaris* and *S. carolinensis* (Di Cerbo and Biancardi 2013), foxes *Vulpes velox* (Stratman and Apker 2014), feral cats and European wildcats, *Felis catus* and *F. silvestris* (Amile et al. 2014, Stokeld et al. 2015), dogs *Canis familiaris* (Rasambainarivo et al. 2017), Hermann’s tortoises *Testudo hermanni* (Ballouard et al. 2016), northern flying squirrels *Glaucomys sabrinus* (Boulerice and Van Fleet 2016), North American river otters *Lontra canadensis* (Day et al. 2016) and grey wolves *Canis lupus* (Sver et al. 2016).
| Replacement method | Instead of | Species | Animal class | Reference |
|--------------------|------------|---------|--------------|-----------|
| Individual identification by footprints | Marking with invasive methods (e.g. tags, collars) | white rhinocero *Ceratotherium simum* | mammals | Alibhai et al. 2008 |
| Individual identification by natural markings | Marking with invasive methods (e.g. toe-clipping) | giant panda *Ailuropoda melanoleuca* | mammals | Li et al. 2018 |
| | | South American tapir *Tapirus terrestris adaeleichis* | mammals | Moreira et al. 2018 |
| | | pygmy blue-tongue skink *Tiliqua adelaidensis* | reptiles | Li et al. 2009 |
| | | grey seal *Halichoerus grypus* | mammals | Vincent et al. 2001 |
| | | fire salamander *Salamandra salamandra* | amphibians | Sukalo et al. 2013 |
| | | alpine longhorn beetle *Rosalia alpina* | insects | Caci et al. 2013 |
| | | sperm whale *Physeter macrocephalus* | mammals | Alessi et al. 2014 |
| | | wunderpus octopus *Wunderpus photogenicus* | cephalopods | Huffard et al. 2008 |
| Using faeces for DNA collection | Blood or tissue sampling | bumblebees *Bombus* spp. | insects | Scriven et al. 2013 |
| | | mountain gorilla *Gorilla beringei beringei* | mammals | Roy et al. 2014 |
| | | western capercaillie *Tetrao urogallus* | birds | Rosner et al. 2014 |
| | | Asian elephant *Elephas maximus* | mammals | Gray et al. 2014 |
| | | Cabrera’s vole *Microtus cabrerae* | mammals | Proença-Ferreira et al. 2019 |
| Using faeces for dietary analysis | (Lethal) intestinal content sampling or stomach flushing | Hawaiian tree snails *Achatinella* spp. | molluscs | Price et al. 2017 |
| Using faeces for ecotoxicology | Blood or tissue sampling | wolf spiders *Pardosa* spp. | arachnids | Sint et al. 2015 |
| | | smooth snake *Coronella austrialia* | reptiles | Brown et al. 2014 |
| | | lesser horseshoe bat *Rhinolophus hipposideros* | mammals | Afonso et al. 2016 |
| | | European pied flycatcher *Ficedula hypoleuca* | birds | Berglund 2018 |
| Using faeces for stress assessment | Blood sampling | red deer *Cervus elaphus* | mammals | Millsapugh et al. 2001 |
| | | African bush elephant *Loxodonta africana* | mammals | Ahlering et al. 2013 |
| | | mourning dove *Zenaida macroura* | birds | Washburn et al. 2003 |
| | | North Atlantic right whale *Eubalaena glacialis* | mammals | Hunt et al. 2006 |
| | | Columbian ground squirrel *Urocitellus columbianus* | mammals | Bosson et al. 2009 |
| Camera traps | Capture | European wildcat *Felis silvestris* | mammals | Stokeld et al. 2015 |
| | | Hermann’s tortoise *Testudo hermannii* | reptiles | Ballouard et al. 2016 |
| | | North American river otter *Lontra canadensis* | mammals | Day et al. 2016 |
| | | black rat *Rattus rattus* | mammals | Rendall et al. 2014 |
| Environmental DNA (eDNA) from water | Blood or tissue sampling | freshwater mussels (Unionidae) | bivalves | Cho et al. 2016 |
| Using hair and feathers for DNA collection | Blood or tissue sampling | platypus *Ornithorhynchus anatinus* | mammals | Lugg et al. 2018 |
| | | wild boar *Sus scrofa* | mammals | Williams et al. 2018b |
| | | grey wolf *Canis lupus* | mammals | Ausband et al. 2011 |
| | | American black bear *Ursus americanus* | mammals | Gould et al. 2018 |
| | | cougar *Puma concolor* | mammals | Sawaya et al. 2011 |
| | | kiwis *Apteryx* spp. | birds | Ramon-Laca et al. 2018 |
| | | American black bear *Ursus americanus*, brown bear *U. arctos* | mammals | Bryan et al. 2014 |
| Using hair and feathers for endocrinology | Blood or tissue sampling | Clark’s nutcrackers *Nucifraga columbiana* | birds | Fairhurst et al. 2011 |
| Using saliva for endocrinology | Blood or tissue sampling | Indian rhinoceros *Rhinoceros unicornis* | mammals | Gomez et al. 2004 |
| | | rhesus macaque *Macaca mulatta* | mammals | Higham et al. 2010 |
To estimate density, individuals are marked or identified by natural markings (Jordan et al. 2011, Thornton and Pekins 2015).

**Drones**

One recent technological advance applied in wildlife monitoring has been the unmanned aerial vehicles, also known as drones. Drones are particularly useful in approaching sensitive wildlife in inaccessible areas. Some studies revealed that drone-derived data are more accurate than data from ground-counting methods (Ezat et al. 2018, Hodgson et al. 2018). Moreover, drone technology can be cheaper than radio-collars (Mulero-Pazmany et al. 2015). Drones have been successfully implemented in monitoring populations of polar bears *Ursus maritimus* (Barnas et al. 2018b), saltwater crocodiles *Crocodylus porosus* (Bevan et al. 2018, Ezat et al. 2018), or snow geese *Anser caerulescens* (Barnas et al. 2018a). Drones can be also used for collecting exhaled breath condensate of humpback whales *Megaptera novaeangliae* for microbiome analysis (Apprill et al. 2017).

**Alternatives to invasive marking**

**Dyes**

For short-term studies, paint can be used to mark individual animals, as has been demonstrated in studies on lizards, *Anolis cristatellus*, *A. gundlachi*, *A. krugi* and *Sceloporus undulates* (Johnson 2005) or rainbow trout *Oncorhynchus mykiss* (Frenkel et al. 2002). Birds can be marked with dyes placed on eggs or nests (Cramer 2017).

**Natural markings**

For identification of individual animals, natural markings can be used, such as unique patterns and scars, fungal patches or pelage markings (Vincent et al. 2001, Manings et al. 2006, Li et al. 2009). Identification based on natural markings has been successfully implemented in studies on e.g. fish (Arzoumanian et al. 2005, Meekan et al. 2006, Auger-Methe et al. 2011, Martin-Smith 2011, Correia et al. 2014, Monteiro et al. 2014, Gonzalez-Ramos et al. 2017), Indo-Pacific bottlenose dolphins *Tursiops aduncus* (Gomez-Salazar et al. 2011, Bichell et al. 2018), sperm whales *Physeter macrocephalus* (Alessi et al. 2014), Asian black bears *Ursus thibetanus* (Higashide et al. 2012), polar bears *Ursus maritimus* (Anderson et al. 2007), Australian sea lions *Neophoca cinerea* (Osterrieder et al. 2015), cougars *Puma concolor* (Alexander and Gese 2018), tigers *Panthera tigris* (Karanth et al. 2006), cheetahs*Acinonyx jubatus* (Kelly 2001), giant pandas *Ailuropoda melanoleuca* (Zheng et al. 2016), salamanders, *Eurycea tonkawae*, *Ambystoma opacum* and *Salamandrina perspicillata* (Gamble et al. 2008, Ben-dik et al. 2013, Romiti et al. 2017), crustaceans *Rhynochocinetes typus* and *Chionoecetes opilio* (Gallardo-Escarate et al. 2007, Gosselin et al. 2007), manatees *Trichechus manatus latirostris* (Langimm et al. 2004), Majorcan midwife toads *Alytes muletensis* (Pinya and Perez-Mellado 2009), common European vipers *Vipera berus* (Bauwens et al. 2018), green sea turtles *Chelonia mydas* (Gatto et al. 2018), wunderpus octopuses *Wunderpus photogenicus* (Huffard et al. 2008), little brown bats *Myotis lucifugus* (Amelon et al. 2017), jewelled geckos *Naultinus gemmeus* (Knox et al. 2013), newts *Ichthyosaura alpestris* and *Lissotriton vulgaris* (Metzouris et al. 2016), and even beetles *Lucanus cervus* (Caci et al. 2013, Romiti et al. 2017, Diaz-Calafat et al. 2018).

**Identification by footprints**

Some mammal species can leave signs that are sufficiently distinctive for identification purposes. Footprints have been used as a tracking method for millennia (Pimm et al. 2015), and current specialized software allows for individual, sex and age group classification with more than 90% accuracy (Jewell et al. 2016). Shape and size of footprints were used to identify individual white rhinos *Ceratotherium simum* (Alihaji et al. 2008, Law et al. 2013), fishers *Martes pennanti* (Herzog et al. 2007), giant pandas *Ailuropoda melanoleuca* (Li et al. 2018), tigers *Panthera tigris* (Gu et al. 2014) or South American tapirs *Tapirus terrestris* (Moreira et al. 2018).

*Table 2. Examples of studies implementing the 3Rs principle of Reduction. See Fig. 1 and the main text for more detail.*

| Reduction method | Instead of | Species | Animal class | Reference |
|-----------------|------------|---------|--------------|-----------|
| Improved statistics methods and minimum sample size calculation | Using an excessive number of animals | moose *Alces alces* | mammals | Girard et al. 2002 |
| Computer modelling (in silico) | Using an excessive number of animals | lesser black-backed gull *Larus fuscus* | birds | Thaxter et al. 2017 |
| Using a single individual or sample for multiple purposes | Using multiple individuals for one purpose only | black slug *Arion ater*, red slug *A. rufus*, Spanish slug *A. pustulosus* | molluscs | Zemanova et al. 2018 |
| Conducting meta-analysis of previous studies | Duplication of studies | American badger *Taxidea taxus*, coyote *Canis latrans* | mammals | Smith and Cheeseman 2002, Prugh et al. 2008 |
| | | African bush elephant *Loxodonta africana*, passerine bird species | mammals | Guldemand and Van Aarde 2008 |

**Table 2:** Examples of studies implementing the 3Rs principle of Reduction. See Fig. 1 and the main text for more detail.
Common swift

Invertebrate-derived DNA (iDNA)

Blood or tissue sampling

Suction cups for attaching devices

Feral cat

In wood mice

...applications in several ecotoxicology studies. For instance, heavy metal levels used to be typically assessed from blood (Hood et al. 1998, Mathies et al. 2001), but this often requires the capture of the animal, which could influence the results. An additional drawback of blood samples is that they may not represent long-term hormone levels (Millspaugh and Washburn 2004). Glucocorticoid surges are used to be typically assessed from blood (Hood et al. 1998, Mathies et al. 2001), but this often requires the capture of the animal, which could influence the results. An additional drawback of blood samples is that they may not represent long-term hormone levels (Millspaugh and Washburn 2004).

Vocal individuality

Instead of marking, individual animals of certain species can be distinguished by their vocalization features (Terry et al. 2005). This method has been successfully applied not only in birds, such as the great grey owl Strix nebulosa (Rognan et al. 2009), but also in marmots Marmota olympus and Richardson’s ground squirrels Spermophilus richardsonii (Pollard et al. 2010).

Alternatives to invasive blood and tissue sampling

Ecotoxicology

Improving our knowledge of the potential impacts of chemical pollutants on wildlife is an important aspect of biological conservation. Unfortunately, traditional methods of obtaining samples in ecotoxicology are invasive (Jasinska et al. 2015, Mathies et al. 2001), but also in marmots Marmota olympus and Richardson’s ground squirrels Spermophilus richardsonii (Pollard et al. 2010).

Nevertheless, non-invasive methods have already been applied in several ecotoxicology studies. For instance, heavy metals can be determined from hair samples, which was done in wood mice Apodemus sylvaticus (Tete et al. 2014), brown rats Rattus norvegicus (McLean et al. 2009), bats Artibeus spp., Myotis bechsteinii, Myotis daubentonii, Myotis myotis and Pipistrellus pipistrellus (Flache et al. 2015, Becker et al. 2018) or European hedgehogs Erinaceus europaeus (Vermeulen et al. 2009). Heavy metals can be also detected in faeces (Afonso et al. 2016, Berglund 2018). Mingo et al. (2017) were able to detect pesticide exposure in common wall lizards Podarcis muralis measured through buccal swabs. The in silico modelling of toxicity pathways has been recently applied to constructing adverse outcomes in wildlife (Madden et al. 2014).

Physiology

Chronic stress can have potentially deleterious effects (please see above for more details). Stress can be quantified by measuring the level of glucocorticoids, a class of steroid hormones (Millspaugh and Washburn 2004). Glucocorticoid levels used to be typically assessed from blood (Hood et al. 1998, Mathies et al. 2001), but this often requires the capture of the animal, which could influence the results. An additional drawback of blood samples is that they may not represent long-term hormone levels (Millspaugh and Washburn 2004).

Table 3. Examples of studies implementing the 3Rs principle of Refinement. See Fig. 1 and the main text for more detail.

| Refinement method                       | Instead of                          | Species                                      | Animal class | Reference         |
|----------------------------------------|--------------------------------------|----------------------------------------------|--------------|-------------------|
| Using buccal swabs for DNA collection  | Toe clipping, blood sampling         | European tree frog Hyla arborea              | amphibians   | Angelon and Holderegger 2009 |
|                                        |                                      | red-cockaded woodpecker Leuconotopicus borealis |              | Vilstrup et al. 2018 |
|                                        |                                      | rodent species (Muridae, Heteromyidae, Sciuoridae) |              | Parmenter et al. 1998 |
|                                        |                                      | redside dace Clinostomus elongates, channel darter Percina copelandi |              | Reid et al. 2012 |
|                                        |                                      | rock dove Columba livia                      | birds        | Shepherd and Somers 2012 |
| Using buccal swabs for ecotoxicity     | Blood or tissue sampling             | common wall lizard Podarcis muralis myotis bats Myotis spp. | reptiles | Mingo et al. 2017 |
|                                        |                                      | Sierra Nevada yellow-legged frog Rana sierra | mammals | Player et al. 2017 |
| Using skin swabs for DNA collection    | Toe clipping, blood sampling         | Neotropical fruit bats Artibeus spp.         | mammals | Becker et al. 2018 |
| Using hair samples for ecotoxicology   | Tissue sampling                      | European hedgehog Erinaceus europaeus gopher tortoise Cophurus polychromus | mammals | Vermeulen et al. 2009 |
|                                        |                                      | marbled newt Triturus marmoratus              | amphibians | Le Chevalier et al. 2017 |
|                                        |                                      | timber rattlesnake Crotalus horridus Weddell seal Leptonychotes weddelli | reptiles | Hale et al. 2017 |
|                                        |                                      | zebrafish Danio rerio                        | fish         | de Abreu et al. 2014 |
|                                        |                                      | European lynx lynx                          | mammals | Kolbe et al. 2003 |
| Improved trapping                      | Trapping methods that could lead to injuries | roe deer Capreolus capreolus               | mammals | Bergvall et al. 2017 |
| Improved trapping                      | Trapping methods that could lead to injuries | European lynx lynx                          | mammals | Kolbe et al. 2003 |
| Improved trapping                      | Trapping methods that could lead to injuries | roe deer Capreolus capreolus               | mammals | Bergvall et al. 2017 |
| Smaller tracking instruments           | Using heavy and robust instruments   | California spotted owl Strix occidentalis occidentalis | birds | Atuo et al. 2019 |
| Smaller tracking instruments           | Using heavy and robust instruments   | feral cat Felix catus                       | mammals | Recio et al. 2011 |
| Suction cups for attaching devices on  | Skin penetrating instruments         | Heaviside’s dolphin Cephalorhynchus beavisitti | mammals | Sakai et al. 2011 |
| cetaceans                               | Blood or tissue sampling with         | meerkat Suricata suricatta                  | mammals | Habicher et al. 2013 |
|                                        | instruments                          | common swift Apus apus                      | birds        | Bauch et al. 2013 |
A non-invasive alternative is the use of faecal samples. Studies in which faecal glucocorticoids were assessed were conducted in elks *Cervus elaphus* (Millspaugh et al. 2001), mourning doves *Zenaida macroura* (Washburn et al. 2003), greater sage grouse *Centrocercus urophasianus* (Jankowski et al. 2009), African bush elephants *Loxodonta africana* (Munshi-South et al. 2008, Ahlering et al. 2013), Columbian ground squirrels *Urocitellus columbianus* (Bosson et al. 2009), common degus *Octodon degus* (Soto-Gamboa et al. 2009), giant pandas *Ailuropoda melanoleuca* (Yu et al. 2011), aardwolves *Proteles cristata* (Ganswindt et al. 2012), eastern chipmunks *Tamias striatus* (Montiglio et al. 2012), coyotes *Canis latrans* (Schell et al. 2013), crab-eating foxes *Cerdocyon thous* (Paz et al. 2015), marmots *Marmota flaviventris* (Wey et al. 2015), woylies *Bettongia penicillata* (Hing et al. 2017), pikas *Ochotona princeps* (Wilkening et al. 2016), North Atlantic right whales *Eubalaena glacialis* (Hunt et al. 2006) or primates (Behringer and Deschner 2017). In frogs, dermal swabs (Santymire et al. 2018) or urine samples (Narayan et al. 2010, Narayan 2013) collected by gentle massage of the lower abdomen can be used for the analysis.

Due to their small molecular weight and lipid solubility, glucocorticoids pass quickly from blood serum to saliva, where it can be directly measured (Romano et al. 2010). Stress assessment from saliva has been implemented in Indian rhinoceros *Rhinoceros unicornis* (Gomez et al. 2004), and rhesus macaques *Macaca mulatta* (Higham et al. 2010).

One of the latest methods of non-invasive sampling is body odour collection. A wide range of volatile and semi-volatile organic compounds create chemical profiles, which can be used in studies on chemical signatures of health as well as kinship, diet and reproduction (Nair et al. 2018, Weiss et al. 2018a, b).

**DNA sampling**

Non-invasive genetic sampling has a great potential in wildlife biology, with a variety of applications (Waits and Paetkau 2005). Advancements in forensics, medical research and ancient DNA techniques generate new methods that can be relatively easily applied to improve data production and analysis of non-invasive genetic samples also in wildlife research (Beja-Pereira et al. 2009).

Faecal DNA-based sampling to identify individuals and estimate the population size was implemented in e.g. Asian elephants *Elephas maximus* (Gray et al. 2014), mountain gorillas *Gorilla beringei beringei* (Roy et al. 2014), Indian rhinoceros *Rhinoceros unicornis* (Das et al. 2015), Cabrera's voles *Microtus cabrerae* (Proença-Ferreira et al. 2019), African golden wolves *Canis anthus* (Karsseme et al. 2018), kit foxes *Vulpes macrotis mutica* (Willert et al. 2015) or birds *Apteryx spp.*, *Otis tarda*, *Tetrao urogallus* (Idaghdour et al. 2003, Perez et al. 2011, Rosner et al. 2014, Ramon-Laca et al. 2018, Vallant et al. 2018). Faecal DNA has been also used for identifying insects like *Bombus* spp. and *Cetorrhynchus assimilis* (Fumanal et al. 2005, Scriver et al. 2013) and spiders *Pardosa* spp. (Sint et al. 2015). To direct the survey efforts detection dogs may be used for locating faecal samples (Arandjelovic et al. 2015, Wilbert et al. 2015).

Another method of non-invasive genetic sampling is hair trapping. Hair can be collected either by catching an animal and plugging the hair, with baited methods, or passively through natural rubs or travel route snares. Baited methods of hair collection can be divided into four main types: 1) hair corral with barbed wire encircling a bait, 2) rub stations, which are structures saturated with scent to induce rubbing, 3) trees wrapped with barbed wire or 4) boxes or tubes containing attractants and fitted with hair snaring devices (Kendall and McKeIvey 2012). Baited hair collection has been often applied in large carnivores (*Canis latrans*, *C. lupus*, *Puma concolor*) (Auband et al. 2011, Sawaya et al. 2011). Another method was introduced by Keeley and Keeley (2012), who developed a modified blowgun dart with sticky ends to collect hair from variegated squirrels *Sciurus variegatoides* without penetrating their skin.

An increasingly common non-invasive genetic sampling technique used primarily in frogs is buccal swabbing (Broquet et al. 2007, Angelone and Holderegger 2009, Gallardo et al. 2012). This method can be however applied also to other types of animals, for instance, birds (Leucostomus borealis) (Vilstrup et al. 2018), lizards (*Coronella austriaca*, *Lacerta agilis*, *Podarcis muralis*) (Beebee 2008, Schulte et al. 2011) and fish (*Clinostomus elongates*, *Percina copelandi*) (Reid et al. 2012). However, buccal swabbing may not be safe for certain reptiles, such as tortoises (due to their head retraction escape response) and snakes. In these species, cloacal swabbing can be used instead (Mucci et al. 2014, Ford et al. 2017).

Alternatively, skin and mucus swabbing can be implemented. Skin swabbing drastically limits handling in comparison to buccal swabbing, and it is particularly useful for vulnerable and small animals, which was shown in alpine newts *Ichthyosaura alpestris* (Prunier et al. 2012), fire salamanders *Salamandra salamandra* (Pichlmuller et al. 2013) and Sierra Nevada yellow-legged frogs *Rana sierrae* (Poorten et al. 2017). Wing swabbing has been successfully used for DNA collection in bats (*Myotis evotis*, *M. septentrionalis*, *M. yumanensis*, *M. lucifugus*) (Playez et al. 2017). Mucus swabbing has been used to collect DNA in cephalopods (*Enterociotopus dofleini*, *Sepia officinalis*) (Holmenbeck et al. 2017, Sykes et al. 2017), land snails (*Arianta arbustorum*) (Armbruster et al. 2005) and slugs (*Arion spp.*, *Geomalacus maculosus*) (Morinha et al. 2014), intertidal snails (*Nucella spp.*) (Kawai et al. 2004), polyclaplachoran molluscs (*Ischnochiton spp.*) (Palmer et al. 2008), freshwater pearl mussels *Margaritifera margaritifera* (Karlsson et al. 2013) and fish (*Manta birostris*, *Oreochromis niloticus*) (Kashiwagi et al. 2015, Taslim et al. 2016).

In birds, eggshells (Strausberger and Ashley 2001, Eglhoff et al. 2009, Kjelland and Kraemmer 2012, Maia et al. 2017) or feathers (Rudnick et al. 2007, Kjelland and Kraemmer 2012, Olah et al. 2016) can be used as a source of DNA. Feathers can be collected opportunistically or through a feather-trap (Maurer et al. 2010).

Saliva is also a great source of DNA that can be collected non-invasively by using e.g. baits and porous material (*Vargas et al. 2009*, *Lobo et al. 2015*). Additionally, DNA samples can be obtained from mineral lick (*Schoenecker et al. 2015*), rests of prey (*Harms et al. 2015*, *Wheat et al. 2016*) or damaged crop (*Saito et al. 2008*).

Other potential sources of DNA include scent marks (*Malherbe et al. 2009*), snow footprints (*Dalen et al. 2007*), urine (*Nagai et al. 2014*, *Nakamura et al. 2017*), insect extr...
vias (Kranzfelder et al. 2016, Nguyen et al. 2017), spider webs (Xu et al. 2015, Blake et al. 2016), antlers (Hoffmann and Griebeler 2013, Kim et al. 2015) or shed skin (Swanson et al. 2006, Horreo et al. 2015).

As organisms move through the environment, they also leave some DNA traces behind. This environmental DNA (eDNA) can be used for detection of targeted organisms, and it is particularly useful for detection of invasive (Collins et al. 2013, Hunter et al. 2015) or rare species (Jerde et al. 2011). Most eDNA applications have targeted aquatic environments, for instance, in studies on harbor porpoises Phocoena phocoena (Foote et al. 2012), mussels (Unionidae) (Cho et al. 2016), hellbenders Cryptobranchus alleganiensis (Olson et al. 2012), fish (Cyprinus carpio, Oncorhynchus mykiss) (Eichmiller et al. 2016, Fernandez et al. 2018) or platypus Ornithorhynchus anatinus (Lugg et al. 2018). However, eDNA techniques have been used also in deer Capreolus capreolus (Nichols et al. 2012) or wild boar Sus scrofa studies (Williams et al. 2018b), using saliva from twigs or water from drinking reservoirs as the DNA source.

While not completely non-invasive method, blood-sucking insects have been used as a ‘gentle’ stress-free method of DNA collection in several mammalian species (Voigt et al. 2005, Calvignac-Spencer et al. 2013, Habicher et al. 2013, Lee et al. 2015, Rodgers et al. 2017), so-called invertebrate-derived DNA (iDNA). The potential of using terrestrial leeches (Haemadipsa spp.) for the same purpose has been also assessed (Schnell et al. 2015).

Alternatives to lethal sampling

Alternatives to collecting voucher specimen

One of the best methods as an alternative to voucher collection is a series of high-quality photographs, which can be even used to describe a new species (Athreya 2006, Minteer et al. 2014a), especially in combination with other lines of evidence (e.g. DNA from skin or buccal samples and recording a species’ mating call).

Species abundance

Biodiversity assessment could be done through count surveys and visual sampling (Lecq et al. 2015, Ksiazkiewicz-Parulska and Goldyn 2017). Methodology on estimating species abundance from occurrence maps has been also recently published (Yin and He 2014).

Dietary composition

The recent application of next generation sequencing and enrichment methods to trophic ecology can enable rapid resolution to questions about diets of practically any animal from their faeces (O’Rorke et al. 2012, Pompanon et al. 2012). Faecal genotyping as a method to examine dietary composition was used in e.g. coyotes Canis latrans (Prugh et al. 2008), European pine martens Martes martes (O’Meara et al. 2014), seals (Arctocephalus forsteri, Phoca vitulina) (Emami-Khoiy et al. 2016, Hui et al. 2017), fish (Barbus barbus, Chondrostoma toxostoma toxostoma, Chondrostoma nasus nasus) (Corse et al. 2010), snakes (Coronella austricaca) (Brown et al. 2014), snails (Achatinella spp.) (O’Rorke et al. 2015, Price et al. 2017), fruit flies Drosophila melanogaster (Fink et al. 2013) or spiders (Pardosa spp.) (Sint et al. 2015).

Concluding remarks

Studies on wildlife are regularly conducted with the assumption that they have an insignificant impact on the studied animals (Jewell 2013) or that the impact is outweighed by any potential benefits to the population or species (Vucetich and Nelson 2007, Parrish et al. 2010). Such assumptions however raise concerns for animal welfare, a topic that has been increasingly discussed among public, ethical committees, journal publishers and funding agencies (McMahon et al. 2012, Zemanova 2017).

In this review, I outlined the potential implications of commonly used invasive research methods for wildlife welfare. Some of the research practices can, however, have delayed consequences and monitoring of animals for any adverse impact should be required (Putman 1995). It is also important to note that in many cases, animal welfare implications of research methods are simply not known. In this case it is imperative to exercise the precautionary principle (Crozier and Schulte-Hostede 2015).

In the past, a high level of invasiveness was necessary to obtain reliable data for understanding and designing management measures for wildlife. However, research methods have to be adjusted as our technical advancement and our understanding of species ability to feel pain grows (Costello et al. 2016, Waugh and Monamy 2016), and wildlife researchers need to limit the harm to the animals in order to ensure ethical acceptability of their work (Crettaz von Roten 2009, Lund et al. 2012). Even though non-invasive methods may not be yet suitable for all types of wildlife research, we should strive to implement them whenever possible. As I showed in this review, many researchers have already succeeded to do so.

Building upon the 3Rs principles (Fig. 1, Table 1–3), Curzer et al. (2013) proposed another R: refusal. Refused should be studies with badly conceived research plans, studies with no prospect of contributing significant knowledge, or studies in which the harm to the animal clearly exceeds any benefit of new knowledge. Some research practices might then have to be rejected simply on ethical grounds (Bekoff 2002).

In conclusion, the 3Rs principles are just as relevant to wildlife research as they are to laboratory animal studies. The current wildlife research needs to shift from using invasive and lethal methods to prioritizing non-invasive alternatives. Study and management of wildlife are necessary, but in doing so, we bear responsibility for ensuring that welfare of the studied animals is compromised as little as possible through our work.

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References

Afonso, E. et al. 2016. Is the lesser horseshoe bat (*Rhinolophus hipposideros*) exposed to causes that may have contributed to its decline? A non-invasive approach. – Global Ecol. Conserv. 8: 123–137.

Ahlering, M. A. et al. 2013. Conservation outside protected areas and the effect of human-dominated landscapes on stress hormones in savannah elephants. – Conserv. Biol. 27: 569–575.

Alessi, J. et al. 2014. Photo-identification of sperm whales in the north-western Mediterranean Sea: an assessment of natural markings. – Aquat. Conserv. 24: 11–22.

Alexander, P. D. and Gese, E. M. 2018. Identifying individual cougars (*Puma concolor*) in remote camera images – implications for population estimates. – Wildl. Res. 45: 274–281.

Alibhai, S. et al. 2008. Identifying white rhino (*Ceratotherium simum*) by a footprint identification technique, at the individual and species levels. – Endanger. Species Res. 4: 205–218.

Amelon, S. K. et al. 2017. Bat wing biometrics: using collagen-elastin bundles in bat wings as a unique individual identifier. – J. Mammal. 98: 744–751.

Anderson, C. J. R. et al. 2007. Can whisker spot patterns be used to identify individual polar bears? – J. Zool. 273: 333–339.

Angelone, S. and Holderegger, R. 2009. Population genetics suggests effectiveness of habitat connectivity measures for the European tree frog in Switzerland. – J. Appl. Ecol. 46: 879–887.

Anile, S. et al. 2014. Wildcat population density on the Etna volcano, Italy: a comparison of density estimation methods. – J. Zool. 293: 252–261.

Apprill, A. et al. 2017. Extensive core microbiome in drone-captured whale blow supports a framework for health monitoring. – mSystems 2: e00119-17.

Arandjelovic, M. et al. 2015. Detection dog efficacy for collecting faecal samples from the critically endangered Cross River gorilla (*Gorilla gorilla diehl*) for genetic censusing. – R. Soc. Open Sci. 2: 140423.

Armbruster, G. F. J. et al. 2005. Foot mucus and peristomal fraction as non-destructive source of DNA in the land snail *Arianta arbustorum*, and the development of new microsatellite loci. – Conserv. Genet. 6: 313–316.

Arnemo, J. M. et al. 2018. Long-term safety of intraperitoneal radio transmitter implants in brown bears (*Ursus arctos*). – Front. Vet. Sci. 5: 252.

Arzoumanian, Z. et al. 2005. An astronomical pattern-matching algorithm for computer-aided identification of whale sharks *Rhincodon typus*. – J. Appl. Ecol. 42: 999–1011.

Atthaya, R. 2006. A new species of *Liocichla* (Aves: Timaliidae) from Eaglenest Wildlife Sanctuary. – Indian Birds 2: 82–94.

Atuo, F. A. et al. 2019. Resource selection by GPS-tagged California spotted owls in mixed-ownership forests. – For. Ecol. Manage. 433: 295–304.

Auger-Methe, M. et al. 2011. Computer-assisted photo-identification of nearwashes. – Arctic 64: 342–352.

Aushand, D. E. et al. 2011. Hair of the dog: obtaining samples from coyotes and wolves non-invasively. – Wildl. Soc. Bull. 35: 105–111.

Ballouard, J. M. et al. 2016. Artificial water ponds and camera trapping of tortoises, and other vertebrates, in a dry Mediterranean landscape. – Wildl. Res. 43: 533–543.

Barbknecht, A. E. et al. 2009. Effectiveness of vaginal-implant transmitters for locating elk parturition sites. – J. Wildl. Manage. 73: 144–148.

Barnas, A. et al. 2018a. Evaluating behavioral responses of nesting lesser snow geese to unmanned aircraft surveys. – Ecol. Evol. 8: 1328–1338.

Barnas, A. F. et al. 2018b. A pilot(less) study on the use of an unmanned aircraft system for studying polar bears (*Ursus maritimus*). – Polar Biol. 41: 1055–1062.

Bauch, C. et al. 2013. ‘Bug-eggs’ for common swifts and other small birds: minimally-invasive and stress-free blood sampling during incubation. – J. Ornithol. 154: 581–585.

Bauwens, D. et al. 2018. Genotyping validates photo-identification by the head scale pattern in a large population of the European adder (*Vipera berus*). – Ecol. Evol. 8: 2985–2992.

Becker, D. J. et al. 2018. Mercury bioaccumulation in bats reflects dietary connectivity to aquatic food webs. – Environ. Pollut. 233: 1076–1085.

Beebee, T. J. C. 2008. Buccal swabbing as a source of DNA from squamate reptiles. – Conserv. Genet. 9: 1087–1088.

Behringer, V. and Deschner, T. 2017. Non-invasive monitoring of physiological markers in primates. – Horm. Behav. 91: 3–18.

Bezerra, A. M. et al. 2009. Advancing ecological understandings through technological transformations in noninvasive genetics. – Mol. Ecol. Res. 9: 1279–1301.

Bekoff, M. 2002. The importance of ethics in conservation biology: let’s be ethicists, not ostriches. – Endanger. Species Update 19: 23–26.

Bendik, N. F. et al. 2013. Computer-assisted photo identification outperforms visible implant elastomers in an endangered salamander, *Eurycea tonkawae*. – PLoS One 8: e59424.

Berglund, A. M. M. 2018. Evaluating blood and excrement as bioindicators for metal accumulation in birds. – Environ. Pollut. 233: 1198–1206.

Bergvall, U. A. et al. 2017. The use of box-traps for wild roe deer: behaviour, injuries and recaptures. – Eur. J. Wildl. Res. 63: 67.

Bevan, E. et al. 2018. Measuring behavioral responses of sea turtles, saltwater crocodiles and crested terns to drone disturbance to define ethical operating thresholds. – PLoS One 13: e0194460.

Bezerra, B. M. et al. 2014. Camera trap observations of nonhabituated critically endangered wild blonde capuchins, *Sapajus flavus* (formerly *Cebus flavus*). – Int. J. Primatol. 35: 895–907.

Bichell, L. M. V. et al. 2018. The reliability of pigment pattern-based identification of wild bottlenose dolphins. – Mar. Mamm. Sci. 34: 113–124.

Bishop, C. J. et al. 2007. Using vaginal implant transmitters to aid in capture of mule deer neonates. – J. Wildl. Manage. 71: 945–954.

Blake, M. et al. 2016. DNA extraction from spider webs. – Conserv. Gen. Res. 8: 219–221.

Bloch, N. and Irschick, D. J. 2005. Toe-clipping dramatically reduces clinging performance in a pad-bearing lizard (*Anolis carolinensis*). – J. Herpetol. 39: 288–293.

Boisvert, G. et al. 2019. Bioaccumulation and biomagnification of perfluoroalkyl acids and precursors in East Greenland polar bears and their ringed seal prey. – Environ. Pollut. 252: 1335–1343.

Bordeleau, X. et al. 2018. Consequences of captive breeding: fitness implications for wild-origin, hatchery-spawned Atlantic salmon kelts upon their return to the wild. – Biol. Conserv. 225: 144–153.

Bosson, C. O. et al. 2009. Assessment of the stress response in Columbian ground squirrels: laboratory and field validation of an enzyme immunoassay for fecal cortisol metabolites. – Physiol. Biochem. Zool. 82: 291–301.

Boulerice, J. T. and Van Fleet, L. A. 2016. A novel technique for detecting northern flying squirrels. – Wildl. Soc. Bull. 40: 786–791.

Brooks, C. et al. 2008. Effects of global positioning system collar weight on zebra behavior and location error. – J. Wildl. Manage. 72: 527–534.

Broquet, T. et al. 2007. Buccal swabs allow efficient and reliable microsatellite genotyping in amphibians. – Conserv. Genet. 8: 509–511.

Brown, M. B. and Brown, C. R. 2009. Blood sampling reduces annual survival in cliff swallows (*Petrochelidon pyrrhonota*). – Auk 126: 853–861.
Brown, D. S. et al. 2014. Molecular analysis of the diets of snakes: changes in prey exploitation during development of the rare smooth snake Coronella austriaca. – Mol. Ecol. 23: 3734–3743.

Bryan, C. E. et al. 2007. Establishing baseline levels of trace elements in blood and skin of bottlenose dolphins in Sarasota Bay, Florida: implications for non-invasive monitoring. – Sci. Total Environ. 388: 325–342.

Bryan, H. M. et al. 2014. Stress and reproductive hormones reflect inter-specific social and nutritional conditions mediated by resource availability in a bear-salmon system. – Conserv. Physiol. 2: cou010.

Buchanan, K. et al. 2015. Guidelines for the treatment of animals in neuroscience and teaching. – Ani. Behav. 99: 1–DX.

Bunck, S. M. 2011. Tag loss and short-term mortality associated with passive integrated transponder tagging of juvenile lost river suckers. – N. Am. J. Fish Manage. 31: 1088–1092.

Caci, G. et al. 2013. Spotting the right spot: computer-aided individual identification of the threatened cerambycid beetle Rosalia alpina. – J. Insect Conserv. 17: 787–795.

Callard, M. D. et al. 2000. Repetitive backflip behaviour in captive roof rats (Rattus rattus) and the effects of cage enrichment. – Anim. Welf. 9: 139–152.

Calvignac-Spencer, S. et al. 2013. Carrion fly-derived DNA as a tool for comprehensive and cost–effective assessment of mammalian biodiversity. – Mol. Ecol. 22: 915–924.

Carroll, E. L. et al. 2018. Genetic and genomic monitoring with minimally invasive sampling methods. – Evol. Appl. 11: 1094–1119.

Cattet, M. R. L. 2013. Falling through the cracks: shortcomings in the collaboration between biologists and veterinarians and their consequences for wildlife. – ILAR J. 54: 33–40.

Cattet, M. et al. 2008. An evaluation of long-term capture effects in urides: implications for wildlife welfare and research. – J. Mammal. 89: 973–990.

Ceballos, G. et al. 2015. Accelerated modern human-induced species losses: entering the sixth mass extinction. – Sci. Adv. 1: e1400253.

Collins, R. A. et al. 2013. Something in the water: biosecurity monitoring of ornamental fish imports using environmental DNA. – Biol. Invas. 15: 1209–1215.

Correia, M. et al. 2014. The use of a non-invasive tool for capture–recapture studies on a seahorse Hippocampus guttulatus population. – J. Fish Biol. 84: 872–884.

Corse, E. et al. 2010. A PCR-based method for diet analysis in freshwater organisms using 18S rDNA barcoding on faeces. – Mol. Ecol. Res. 10: 96–108.

Costello, M. J. et al. 2016. Field work ethics in biological research. – Biol. Conserv. 203: 268–271.

Cramer, M. J. 2017. Considerations for use of vertebrates in field studies. – In: Suckow, M. A. and Stewart, K. L. (eds), Principles of animal research for graduate and undergraduate students. Academic Press, pp. 199–223.

Crettaz von Roten, F. 2009. European attitudes towards animal research: overview and consequences for science. – Sci. Technol. Soc. 14: 349–364.

Crozier, G. K. D. and Schulte-Hostedde, A. I. 2015. Towards improving the ethics of ecological research. – Sci. Eng. Ethics 21: 577–594.

Curzer, H. J. et al. 2013. The ethics of wildlife research: a nine R theory. – ILAR J. 54: 52–57.

da Costa Araujo, A. P. et al. 2020. How much are microplastics harmful to the health of amphibians? A study with pristine polyethylene microplastics and Physalaemus cuvieri. – J. Hazard Mater. 382: 121066.

Dalen, L. et al. 2007. Recovery of DNA from footprints in the snow. – Can. Field-Nat. 121: 321–324.

Dalton, R. 2005. Animal-rights group sues over ‘disturbing’ work on sea lions. – Nature 436: 315–315.

Das, P. K. et al. 2015. Population genetic assessment of extant populations of greater one-horned rhinoceros (Rhinoceros unicornis) in India. – Eur. J. Wildl. Res. 61: 841–851.

Day, C. C. et al. 2016. Comparing direct and indirect methods to estimate detection rates and site use of a cryptic semi-aquatic carnivore. – Ecol. Indic. 66: 230–234.

de Abreu, M. S. et al. 2014. Diazepam and fluoxetine decrease the stress response in zebrafish. – PLoS One 9: e103232.

Di Cerbo, A. R. and Biancardi, C. M. 2013. Monitoring small and arboreal mammals by camera traps: effectiveness and applications. – Acta Theriol. 58: 279–283.

Diaz-Calafat, J. et al. 2018. Individual unique colour patterns of the prionom of Rhynchophorus ferrugineus (Coleoptera: Curculionidae) allow for photographic identification methods (PIM). – J. Asia-Pac. Entomol. 21: 519–526.

Dixon, A. et al. 2016. Evidence for deleterious effects of harness-mounted satellite transmitters on Saker falcons Falco cherrug. – Bird Study 63: 96–106.

Donnelly, M. A. et al. 1994. Techniques for marking amphibians. – Smithsonian Inst. Press.

Egloff, C. et al. 2009. A nondestructive method for obtaining maternal DNA from avian eggshells and its application to embryonic viability determination in herring gulls (Larus argentatus). – Mol. Ecol. Res. 9: 19–27.

Eichmiller, J. J. et al. 2016. Optimizing techniques to capture and extract environmental DNA for detection and quantification of fish. – Mol. Ecol. Res. 16: 56–68.

Emami-Khozy, A. et al. 2016. Identifying prey items from New Zealand fur seal (Arctocephalus forsteri) faeces using massive parallel sequencing. – Conserv. Gen. Res. 8: 343–352.

Ezat, M. A. et al. 2018. Use of an unmanned aerial vehicle (drone) to survey Nile crocodile populations: a case study at Lake Nyamithi, Ndumu game reserve, South Africa. – Biol. Conserv. 223: 76–81.

Fairhurst, G. D. et al. 2011. Does environmental enrichment reduce stress? An integrated measure of corticosterone from feathers provides a novel perspective. – PLoS One 6: e17663.

Fallow, P. M. et al. 2009. Flipper bands modify the short-term diving behavior of little penguins. – J. Wildl. Manage. 73: 1348–1354.

Fernandez, S. et al. 2018. Environmental DNA for freshwater fish monitoring: insights for conservation within a protected area. – PeerJ 6: e4486.

Ferreira, V. H. B. et al. 2018. Hormonal correlates of behavioural profiles and coping strategies in captive capuchin monkeys (Sapajus libidinosus). – Appl. Anim. Behav. Sci. 207: 108–115.

Field, I. C. et al. 2012. Refining instrument attachment on phocid seals. – Mar. Mamm. Sci. 28: E325–E332.

Field, K. A. et al. 2019. Publication reform to safeguard wildlife research. – J. Insect Conserv. 17: 787–795.

Flagel, D. G. et al. 2016. Natural and experimental tests of trophic cascades: gray wolves and white-tailed deer in a Great Lakes forest. – Oecologia 180: 1183–1194.

Flach, L. et al. 2015. Hair samples as monitoring units for assessing metal exposure of bats: a new tool for risk assessment. – Mamm. Biol. 80: 178–181.

Fleming, P. J. S. et al. 1998. The performance of wild-canid traps in Australia: efficiency, selectivity and trap-related injuries. – Wildl. Res. 25: 327–338.

Foote, A. D. et al. 2012. Investigating the potential use of environmental DNA (eDNA) for genetic monitoring of marine mammals. – PLoS One 7: e41781.

Ford, B. et al. 2017. Evaluating the efficacy of non-invasive genetic sampling of the Northern Pacific rattlesnake with implications for other venomous squamates. – Conserv. Gen. Res. 9: 13–15.
Gomez-Salazar, C. et al. 2011. Photo-identification: a reliable and non-invasive tool for studying pink river dolphins (Inia geoffrensis). – Aquat. Mamm. 35: 362–367.

Gould, M. J. et al. 2018. Density of American black bears in New Mexico. – J. Wildl. Manage. 82: 775–788.

Graf, T. U. et al. 2011. Putting toe clipping into perspective: a viable method for marking anurans. – J. Herpetol. 45: 28–35.

Gray, T. N. E. et al. 2014. Population size estimation of an Asian elephant population in eastern Cambodia through non-invasive mark–recapture sampling. – Conserv. Genet. 15: 803–810.

Green-Barber, J. M. et al. 2018. A suspected case of myopathy in a free-ranging eastern grey kangaroo (Macropus giganteus). – Aust. Mammal. 40: 122–126.

Grisham, B. A. et al. 2015. Evaluation of capture techniques on lesser prairie-chicken trap injury and survival. – J. Fish Wildl. Manage. 6: 318–326.

Gu, J. et al. 2014. Sex determination of amur tigers (Panthera tigris altaica) from footprints in snow. – Wildl. Soc. Bull. 38: 495–502.

Guldemond, R. and Van Aarde, R. 2008. A meta-analysis of the impact of African elephants on savanna vegetation. – J. Wildl. Manage. 72: 892–899.

Habicher, A. et al. 2013. Tsetse flies as tools for minimally invasive blood sampling. – Wildl. Soc. Bull. 37: 423–427.

Hale, V. L. et al. 2017. Radio transmitter implantation and movement in the wild timber rattlesnake (Crotalus horridus). – J. Wildl. Dis. 53: 591–595.

Hammerschlag, N. and Sulikowski, J. 2011. Killing for conservation: the need for alternatives to lethal sampling of apex predatory sharks. – Endanger. Species Res. 14: 135–140.

Hansen, L. P. 1988. Effects of carfin tagging and fin clipping on survival of Atlantic salmon (Salmo salar) released as smolts. – Aquaculture 70: 391–394.

Harcourt, R. G. et al. 2010. Effects of capture stress on free-ranging, reproductively active male Weddell seals. – J. Comp. Physiol. 196: 147–154.

Harms, V. et al. 2015. Experimental evaluation of genetic predator identification from saliva traces on wildlife kills. – J. Mammal. 96: 138–143.

Harrop, S. 2011. Climate change, conservation and the place for wild animal welfare in international law. – J. Environ. Law 23: 441–462.

Humphries, A. H. et al. 2010. Flow simulation along a seal: the impact of an external device. – Eur. J. Wildl. Res. 56: 131–140.

Hergoz, C. J. et al. 2007. Using patterns in track-plate footprints to identify individual fishers. – J. Wildl. Manage. 71: 955–963.

Higashide, D. et al. 2012. Are chest marks unique to Asian black bear individuals? – J. Zool. 288: 199–206.

Higham, J. P. et al. 2010. Measuring salivary analytes from free-ranging monkeys. – Physiol. Behav. 101: 601–607.

Hilario, R. R. et al. 2017. Preadation of birds in mist nets by Caliitrichids (primates): how to prevent similar events. – Stud. Neotrop. Fauna Environ. 52: 168–172.

Hill, J. M. and Elphick, C. S. 2011. Are grassland passerines especially susceptible to negative transmitter impacts? – Wildl. Soc. Bull. 35: 362–367.

Hing, S. et al. 2017. Identifying factors that influence stress physiology of the woylie, a critically endangered marsupial. – J. Zool. 302: 49–56.

Hodgson, J. C. et al. 2018. Drones count wildlife more accurately and precisely than humans. – Methods Ecol. Evol. 9: 1160–1167.

Hoffmann, G. S. and Griebeler, E. M. 2013. An improved high yield method to obtain microsatellite genotypes from red deer antlers up to 200 years old. – Mol. Ecol. Res. 13: 440–446.

Hollenbeck, N. et al. 2017. Use of swabs for sampling epithelial cells for molecular genetics analyses in Enterococcus. – Am. Malacol. Bull. 35: 145–157.

Hood, L. C. et al. 1998. The adrenocortical response to stress in incubating Magellanic penguins (Spheniscus magellanicus). – Auk 115: 76–84.

Horreo, J. L. et al. 2015. Skin sheds as a useful DNA source for identifying individual northern goshawks (Accipiter gentilis). – J. Avian Biol. 47: 443–447.

Hoy, S. D. et al. 2015. Covariates of release mortality and tag loss in large-scale tuna tagging experiments. – Fish. Res. 163: 106–118.

Huffard, C. L. et al. 2008. Individually unique body color patterns in octopus (Wunderpus photogenicus) allow for photoidentification. – PLoS One 3: e3732.

Hui, T. C. Y. et al. 2017. Dietary analysis of harbour seals (Phoca vitulina) from faecal samples and overlap with fisheries in Erimo, Japan. – Mar. Ecol. Evol. Perspect. 38: e12431.

Hunt, K. E. et al. 2006. Analysis of fecal glucocorticoids in the dual northern goshawks (Accipiter gentilis). – J. Avian Biol. 37: 73–77.

Hoy, S. et al. 2016. Genomic markers validate using the natural phenotypic characteristics of shed feathers to identify individual northern goshawks Accipiter gentilis. – J. Avian Biol. 47: 821–829.
Maia, T. A. et al. 2017. DNA sampling from eggshells and microsatellite genotyping in rare tropical birds: case study on Brazilian merganser. – Genet. Mol. Biol. 40: 808–812.

Malherbe, G. P. et al. 2009. Genetic clues from olfactory cues: brown hyaena scent marks provide a non-invasive source of DNA for genetic profiling. – Conserv. Genet. 10: 759–762.

Maniscalco, J. M. et al. 2006. Interseasional and interannual measures of maternal care among individual Steller sea lions (Eumetopias jubatus). – J. Mammal. 87: 304–311.

Marino, L. and Frohoff, T. 2011. Towards a new paradigm of non-captive research on cetacean cognition. – PLoS One 6: e24121.

Maurer, G. et al. 2010. A ‘feather-trap’ for collecting DNA samples from birds. – Mol. Ecol. Res. 10: 129–134.

McCarthy, M. A. and Parris, K. M. 2004. Clarifying the effect of toe clipping on frogs with Bayesian statistics. – J. Appl. Ecol. 41: 780–786.

McGuire, J. L. et al. 2014. Safety and utility of an anesthetic protocol for the collection of biological samples from gopher tortoises. – Wildl. Soc. Bull. 32: 43–50.

McLean, C. M. et al. 2009. Mammalian hair as an accumulative bioindicator of metal bioavailability in Australian terrestrial environments. – Sci. Total. Environ. 407: 3588–3596.

McLean, W. R. et al. 2017. Visual cues increase camera-trap detection of the southern cassowary (Casuarius casuarius johnsonii). – Wildl. Res. 44: 230–237.

McMahon, C. R. et al. 2012. Publish or perish: why it’s important to publicise how, and if, research activities affect animals. – Wildl. Res. 39: 375–377.

Meekan, M. G. et al. 2006. Population size and structure of whale sharks Rhincodon typus at Ningaloo Reef, Western Australia. – Mar. Ecol. Prog. Ser. 319: 275–285.

Mellor, D. J. 2016. Updating animal welfare thinking: moving beyond the ‘five freedoms’ towards ‘a life worth living’. – Animals 6: 21.

Mettouris, O. et al. 2016. A newt does not change its spots: using pattern mapping for the identification of individuals in large populations of newt species. – Ecol. Res. 31: 483–489.

Millsbaugh, J. J. and Washburn, B. E. 2004. Use of fecal glucocorticoid metabolite measures in conservation biology research: considerations for application and interpretation. – Gen. Comp. Endocrinol. 138: 189–199.

Millsbaugh, J. J. et al. 2001. Fecal glucocorticoid assays and the physiological stress response in elk. – Wildl. Soc. Bull. 29: 899–907.

Mingo, V. et al. 2017. The use of buccal swabs as a minimal-invasive method for detecting effects of pesticide exposure on enzymatic activity in common wall lizards. – Environ. Pollut. 220: 53–62.

Minter, B. A. et al. 2014a. Avoiding (re)extinction. – Science 344: 260–261.

Minter, B. A. et al. 2014b. Specimen collection: plan for the future response. – Science 344: 816–816.

Mitchell, E. P. et al. 2018. A new perspective on the pathogenesis of chronic renal disease in captive cheetahs (Acinonyx jubatus). – PLoS One 13: e0194114.

Monteiro, N. M. et al. 2014. Validating the use of colouration patterns for individual recognition in the worm pipefish using a novel set of microsatellite markers. – Mol. Ecol. Res. 14: 150–156.

Monteiro, P. et al. 2014. Efficiency of hair snares and camera traps to survey mesocarnivore populations. – Eur. J. Wildl. Res. 60: 279–289.

Montiglio, P. O. et al. 2012. Noninvasive monitoring of fecal cortisol metabolites in the eastern chimpanzee (Pan troglodytes): validation and comparison of two enzyme immunoassays. – Physiol. Biochem. Zool. 85: 183–193.

Moreira, D. O. et al. 2018. Determining the numbers of a landscape architect species (Tapirus terrestris), using footprints. – PeerJ 6: e4591.

Morinah, F. et al. 2014. DNA sampling from body swabs of terrestrial slugs (Gastropoda: Pulmonata): a simple and non-invasive method for molecular genetics approaches. – J. Molluscan. Stud. 80: 99–101.

Mucci, N. et al. 2014. Cloacal swab sampling is a reliable and harmless source of DNA for population and forensic genetics in tortoises. – Conserv. Genet. Res. 6: 845–847.

Mulcahy, D. M. and Esler, D. 1999. Surgical and immediate postrelease mortality of harlequin ducks (Histrionicus histrionicus) implanted with abdominal radio transmitters with percutaneous antennae. – J. Zoo Wildl. Med. 30: 397–401.

Muler-Pazmany, M. et al. 2015. Unmanned aircraft systems complement biologging in spatial ecology studies. – Ecol. Evol. 5: 4808–4818.

Munshi-South, J. et al. 2008. Physiological indicators of stress in African forest elephants (Loxodonta africana cyclotis) in relation to petroleum operations in Gabon, Central Africa. – Divers. Distrib. 14: 995–1003.

Nagai, T. et al. 2014. Effectiveness of noninvasive DNA analysis to reveal isolated-forest use by the sable Martes zibellina on eastern Hokkaido, Japan. – Mammal Study 39: 99–104.

Narain, J. V. et al. 2018. An optimized protocol for large-scale in situ sampling and analysis of volatile organic compounds. – Ecol. Evol. 8: 5924–5936.

Nakamura, M. et al. 2017. Evaluating the predictive power of field variables for species and individual molecular identification on wolf noninvasive samples. – Eur. J. Wildl. Res. 63: 53.

Narayan, E. J. 2013. Non-invasive reproductive and stress endocrinology in amphibian conservation physiology. – Conserv. Physiol. 1: cot011.

Narayan, E. et al. 2010. Urinary corticosterone metabolite responses to capture, and annual patterns of urinary corticosterone in wild and captive endangered Fijian ground frogs (Platymantis vitianus). – Aust. J. Zool. 58: 189–197.

NC3Rs 2018. Wildlife research. – <https://www.nc3rs.org.uk/wildlife-research>

Nguyen, H. Q. et al. 2017. Efficient isolation method for high-quality genomic DNA from cicada exuviae. – Ecol. Evol. 7: 8161–8169.

Nichols, R. V. et al. 2012. Browse twig environmental DNA: diagnostic PCR to identify ungulate species. – Mol. Ecol. Res. 12: 983–989.

NORECOPA 2008. Harmonisation of the care and use of animals in field research, Gardermoen, 21–22 May 2008. A consensus document from the participants, Gardermoen, Norway.

Nuvoli, S. et al. 2014. Capture myopathy in a corsican red deer (Cervus elaphus corsicanus) (Ungulata: Cervidae). – Ital. J. Zool. 81: 457–462.

O’Meara, D. B. et al. 2014. Non-invasive multi-species monitoring: real-time PCR detection of small mammal and squirrel prey DNA in pine marten (Martes martes) scats. – Acta Theriol. 59: 111–117.

O’Rorke, R. et al. 2012. PCR enrichment techniques to identify the diet of predators. – Mol. Ecol. Res. 12: 5–17.

O’Rorke, R. et al. 2015. A probing snail to assess how grazing by grazers influences snail communities in geographically structured. – Environ. Microbiol. 17: 1753–1764.
Olah, G. et al. 2016. Validation of non-invasive genetic tagging in two large macaw species (Ara macao and A. chloropterus) of the Peruvian Amazon. – Conserv. Genet. Res. 8: 499–509.

Olivera-Thaiuel, C. et al. 2017. Effect of toe-clipping on the survival of several lizard species. – Herpetol. J. 27: 266–275.

Olson, Z. H. et al. 2012. An eDNA approach to detect eastern hellbenders (Cryptobranchus a. alleganiensis) using samples of water. – Wildl. Res. 39: 629–636.

Osterrieder, S. K. et al. 2015. Whisker spot patterns: a noninvasive method of individual identification of Australian sea lions (Neophoca cinerea). – J. Mammal. 96: 988–997.

Palmer, A. N. S. et al. 2008. Foot mucus is a good source for non-destructive genetic sampling in Polyplacophora. – Conserv. Genet. 9: 229–231.

Panayotova, I. N. and Horth, L. 2018. Modeling the impact of climate change on a rare color morph in fish. – Ecol. Model. 387: 10–16.

Parmenter, C. A. et al. 1998. Small mammal survival and trapability in mark-recapture monitoring programs for hantavirus. – J. Wildl. Dis. 34: 1–12.

Parris, K. M. et al. 2010. Assessing ethical tradeoffs in ecological field studies. – J. Appl. Ecol. 47: 227–234.

Paz, C. R. R. et al. 2015. Fecal cortisol metabolites as indicators of stress in crabeating-fox (Cerdocyon thous) in captivity. – Pesqui. Vet. Bras. 35: 859–862.

Perez, T. et al. 2011. Improving non-invasive genotyping in capercaillie (Tetrao urogallus): redesigning sexing and microsatellite primers to increase efficiency on faeces samples. – Conserv. Gen. Res. 3: 483–487.

Phillips, R. L. et al. 1996. Leg injuries to coyotes captured in three types of foothold traps. – Wildl. Soc. Bull. 24: 260–263.

Pichlmuller, F. et al. 2013. Skin swabbing of amphibian larvae yields sufficient DNA for efficient sequencing and reliable microsatellite genotyping. – Amphib-Reptilia 34: 517–523.

Pimm, S. L. et al. 2015. Emerging technologies to conserve biodiversity. – Trends Ecol. Evol. 30: 685–696.

Pinya, S. and Perez-Mellado, V. 2009. Individual identification and sexual dimorphism in the endangered Balearic midwife toad, Alytes muletensis (Sanchiz and Adrover, 1981). – Amphib-Reptilia 30: 439–443.

Player, D. et al. 2017. An alternative minimally invasive technique for genetic sampling of bats: wing swabs yield species identification. – Wildl. Soc. Bull. 41: 590–596.

Poirier, C. and Bateson, M. 2017. Pacing stereotypes in laboratory rhesus macaques: implications for animal welfare and the validity of neuroscientific findings. – Neurosci. Biobehav. Rev. 83: 508–519.

Pollard, K. A. et al. 2010. Pre-screening acoustic and other natural signatures for use in noninvasive individual identification. – J. Appl. Ecol. 47: 1103–1109.

Pomponon, F. et al. 2012. Who is eating what: diet assessment using extracted DNA from damaged crops. – Ursus 19: 162–167.

Pruhin, J. et al. 2012. Skin swabbing as a new efficient DNA sampling technique in amphibians, and 14 new microsatellite markers in the alpine newt (Ichthyosaura alpestris). – Mol. Ecol. Res. 12: 524–531.

Putman, R. J. 1995. Ethical considerations and animal welfare in ecological field studies. – Biodivers. Conserv. 4: 903–915.

Quinn, J. H. et al. 2010. Complicated association with abdominal surgical implantation of a rado transmitter in an American badger (Taxidea taxus). – J. Zoo Wildl. Med. 41: 174–177.

Ramon-Laca, A. et al. 2018. Extraction of DNA from captive-sourced feces and molted feathers provides a novel method for conservation management of New Zealand kiwi (Apteryx spp.). – Ecol. Evol. 8: 3119–3130.

Rasambainarivo, F. et al. 2017. Interactions between carnivores in Madagascar and the risk of disease transmission. – EcoHealth 14: 691–703.

Rasiulis, A. L. et al. 2014. The effect of radio-collar weight on survival of migratory caribou. – J. Wildl. Manage. 78: 953–956.

Recio, M. R. et al. 2011. Lightweight GPS-tags, one giant leap for wildlife tracking? An assessment approach. – PLoS One 6: e28225.

Reid, S. M. et al. 2012. Validation of buccal swabs for noninvasive DNA sampling of small-bodied imperiled fishes. – J. Appl. Ichthyol. 28: 290–292.

Rendall, A. R. et al. 2014. Camera trapping: a contemporary approach to monitoring invasive rodents in high conservation priority ecosystems. – PLoS One 9: e86592.

Robinson, J. L. and Jones, I. L. 2014. An experimental study measuring the effects of a tarsus-mounted tracking device on the behaviour of a small pursuit-diving seabird. – Behaviour 151: 1799–1826.

Rode, K. D. et al. 2014. Effects of capturing and collaring on polar bears: findings from long-term research on the southern Beaufort Sea population. – Wildl. Res. 41: 311–322.

Rodgers, T. W. et al. 2017. Carion fly-derived DNA metabarcoding is an effective tool for mammal surveys: evidence from a known tropical mammal community. – Mol. Ecol. Res. 17: e133–e145.

Rognan, C. B. et al. 2009. Vocal individuality of great gray owls in the Sierra Nevada. – J. Wildl. Manage. 73: 755–760.

Romano, M. C. et al. 2010. Stress in wildlife species: noninvasive monitoring of glucocorticoids. – Neuroimmunomodulation 17: 209–212.

Romiti, F. et al. 2017. Photographic identification method (PIM) using natural body marks: a simple tool to make a long story short. – Zool. Anz. 266: 156–147.

Roques, J. A. C. et al. 2010. Tailfin clipping, a painful procedure: studies on Nile tilapia and common carp. – Physiol. Behav. 101: 533–540.

Rosner, S. et al. 2014. Noninvasive genetic sampling allows estimation of capercaillie numbers and population structure in the Bohemian Forest. – Eur. J. Wildl. Res. 60: 789–801.

Roy, J. et al. 2014. Challenges in the use of genetic mark-recapture to estimate the population size of Bwindi mountain gorillas (Gorilla beringei beringei). – Biol. Conserv. 180: 249–261.

Rudnick, J. A. et al. 2007. Species identification of birds through genetic analysis of naturally shed feathers. – Mol. Ecol. Notes 7: 757–762.

Russell, W. M. S. and Burch, R. L. 1959. The principles of humane experimental technique. – Methuen, London.

Russo, D. et al. 2017. Collection of voucher specimens for bat experimental technique. – Methuen, London.
Santymire, R. M. et al. 2018. A novel method for the measurement of glucocorticoids in dotal secretions of amphibians. – Conserv. Physiol. 6: coy008.

Sawaya, M. A. et al. 2011. Evaluation of noninvasive genetic sampling methods for cougars in Yellowstone National Park. – J. Wildl. Manage. 75: 612–622.

Scriven, J. J. et al. 2013. Nondestructive DNA sampling from bumblebee faeces. – Mol. Ecol. Res. 13: 225–229.

Shannon, G. et al. 2013. Effects of social disruption in elephants persist decades after culling. – Front. Zool. 10: 62.

Shepherd, G. L. and Somers, C. M. 2012. Adapting the buccal microinvasive cytome assay for use in wild birds: age and sex affect background frequency in pigeons. – Environ. Mol. Mutag. 53: 136–144.

Shyne, A. 2006. Meta-analytic review of the effects of enrichment on stereotypic behavior in zoo mammals. – Zoo Biol. 25: 317–337.

Schell, C. J. et al. 2013. Anthropogenic and physiologically induced stress responses in captive coyotes. – J. Mammal. 94: 1131–1140.

Schmidt, K. and Schwarzkopf, L. 2010. Visible implant elastomer tagging and toe-clipping: effects of marking on locomotor performance of frogs and skins. – Herpetol. J. 20: 99–105.

Schnell, I. B. et al. 2015. iDNA from terrestrial haematophagous leeches as a wildlife surveying and monitoring tool – prospects, pitfalls and avenues to be developed. – Front. Zool. 12: 24.

Schoenecker, K. A. et al. 2015. Estimating bighorn sheep (Ovis canadensis) abundance using noninvasive sampling at a mineral lick within a national park wilderness area. – West. N. Am. Nat. 75: 181–191.

Schulte, U. et al. 2011. Buccal swabs as a reliable non-invasive tissue sampling method for DNA analysis in the lacertid lizard Podarcis muralis. – N.-W. J. Zool. 7: 325–328.

Sint, D. et al. 2015. Sparing spiders: faeces as a non-invasive source of DNA. – Front. Zool. 12: 3.

Sloman, K. A. et al. 2019. Ethical considerations in fish research. – J. Fish Biol. 94: 556–577.

Smith, G. C. and Cheeseman, C. L. 2002. A mathematical model for the control of diseases in wildlife populations: culling, vaccination and fertility control. – Ecol. Model. 150: 45–53.

Sneddon, L. U. 2017. Pain in laboratory animals: a possible confounding factor? – ATLA 45: 161–164.

Sneddon, L. U. et al. 2017. Considering aspects of the 3Rs principle within experimental animal biology. – J. Exp. Biol. 220: 3007–3016.

Sonnie, C. et al. 2012. Tissue healing in two harbor porpoises (Phocoena phocoena) following long-term satellite transmitter attachment. – Mar. Mamm. Sci. 28: E316–E324.

Soto-Gamboa, M. et al. 2013. Validation of a radioimmunoassay for measuring fecal cortisol metabolites in the hystricomorph rodent, Octodon degus. – J. Exp. Zool. 311A: 496–503.

Stetz, J. B. et al. 2011. Genetic monitoring for managers: a new online resource. – J. Fish Wildl. Manage. 2: 216–219.

Stokeld, D. et al. 2015. Multiple cameras required to reliably detect feral cats in northern Australian tropical savanna: an evaluation of sampling design when using camera traps. – Wildl. Res. 42: 642–649.

Stratman, M. R. and Apker, J. A. 2014. Using infrared cameras and skunk lure to monitor swift fox (Vulpes velox). – Southwest. Nat. 59: 500–508.

Strausberger, B. M. and Ashley, M. V. 2001. Eggs yield nuclear DNA from egg-laying female cowbirds, their embryos and offspring. – Conserv. Genet. 2: 385–390.

Sukalo, G. et al. 2013. Novel, non-invasive method for distinguishing the individuals of the fire salamander (Salamandra salamandra) in capture–mark–recapture studies. – Acta Herpetol. 8: 41–45.

Sutherland, W. J. et al. 2004. Bird ecology and conservation: a handbook of techniques. – Oxford Univ. Press.

Sver, L. et al. 2016. Camera traps on wildlife crossing structures as a tool in gray wolf (Canis lupus) management: five-year monitoring of wolf abundance trends in Croatia. – PLoS One 11: e0156748.

Swanson, B. J. et al. 2006. Shed skin as a source of DNA for genotyping seals. – Mol. Ecol. Notes 6: 1096–1099.

Sykes, A. V. et al. 2017. Refining tools for studying cuttlefish (Sepia officinalis) reproduction in captivity: in vivo sexual determination, tagging and DNA collection. – Aquaculture 479: 13–16.

Taslima, K. et al. 2016. DNA sampling from mucus in the Nile tilapia, Oreochromis niloticus: minimally invasive sampling for aquaculture-related genetics research. – Aquat. Res. 47: 4032–4037.

Terio, K. A. et al. 2004. Evidence for chronic stress in captive but not free-ranging cheetahs (Acinonyx jubatus) based on adrenal morphology and function. – J. Wildl. Dis. 40: 259–266.

Terry, A. M. R. et al. 2005. The role of vocal individuality in conservation. – Front. Zool. 2: 10–10.

Tete, N. et al. 2014. Hair as a noninvasive tool for risk assessment: do the concentrations of cadmium and lead in the hair of wood mice (Apodemus sylvaticus) reflect internal concentrations? – Ecotoxicol. Environ. Saf. 108: 233–241.

Thaxter, C. B. et al. 2017. Sample size required to characterize area use of tracked seabirds. – J. Wildl. Manage. 81: 1098–1109.

Thompson, D. P. et al. 2018. Vaginal implant transmitters for continuous body temperature measurement in moose. – Wildl. Soc. Bull. 42: 321–327.

Thornton, D. H. and Pekins, C. E. 2015. Spatially explicit capture-recapture analysis of bobcat (Lynx rufus) density: implications for mesocarnivore monitoring. – Wildl. Res. 42: 394–404.

Treffy, S. A. et al. 2013. Wing marker woes: a case study and meta-analysis of the impacts of wing and patagial tags. – J. Ornithol. 154: 1–11.

Uher-Koch, B. D. et al. 2015. Nest visits and capture events affect breeding success of yellow-billed and Pacific loons. – Condor 117: 121–129.

Vallant, S. et al. 2018. Increased DNA typing success for feces and feathers of capercaillie (Tetrao urogallus) and black grouse (Tetrao tetrix). – Ecol. Evol. 8: 3941–3951.

Vargas, M. L. et al. 2009. Noninvasive recovery and detection of possum Trichosurus vulpecula DNA from bitten bait interference devices (WaxTags). – Mol. Ecol. Res. 9: 505–515.

Vethim, I. et al. 2015. Assessing capture and tagging methods for brolgas, Antigone rubicunda (Gruidae). – Wildl. Res. 42: 373–381.

Vermeulen, F. et al. 2009. Relevance of hair and spines of the European hedgehog (Erinaceus europaeus) as biomonitoring tissues for arsenic and metals in relation to blood. – Sci. Total. Environ. 407: 1775–1783.

Vilstrup, J. T. et al. 2018. A simplified field protocol for genetic sampling of birds using buccal swabs. – Wilson. J. Ornithol. 130: 326–334.

Vincent, C. et al. 2001. Photo-identification in grey seals: legibility and stability of natural markings. – Mammalia 65: 363–372.

Voigt, C. C. et al. 2005. Blood-sucking bugs as a gentle method for blood-collection in water budget studies using doubly labelled water. – Compar. Biochem. Physiol. 142: 318–324.

Vucetich, J. A. and Nelson, M. P. 2007. What are 60 warblers worth? Killing in the name of conservation. – Oikos 116: 1267–1278.

Waits, L. P. and Paetkau, D. 2005. Noninvasive genetic sampling tools for wildlife biologists: a review of applications and recommendations for accurate data collection. – J. Wildl. Manage. 69: 1419–1433.
Walker, K. A. et al. 2010. Behavioural responses of juvenile Steller sea lions to hot-iron branding. – Appl. Anim. Behav. Sci. 122: 58–62.

Washburn, B. E. et al. 2003. Using fecal glucocorticoids for stress assessment in mourning doves. – Condor 105: 696–706.

Waugh, C. A. and Monamy, V. 2016. Opposing lethal wildlife research when nonlethal methods exist: scientific whaling as a case study. – J. Fish Wildl. Manage. 7: 231–236.

Wechsler, B. 1991. Stereotypies in polar bears. – Zoo. Biol. 10: 177–188.

Weiss, B. M. et al. 2018a. Chemical composition of axillary odorants reflects social and individual attributes in rhesus macaques. – Behav. Ecol. Sociobiol. 72: 65.

Weiss, B. M. et al. 2018b. A non-invasive method for sampling the body odour of mammals. – Methods Ecol. Evol. 9: 420–429.

Welbourne, D. J. et al. 2015. The effectiveness and cost of camera traps for surveying small reptiles and critical weight range mammals: a comparison with labour-intensive complementary methods. – Wildl. Res. 42: 414–425.

Wey, T. W. et al. 2015. Stress hormone metabolites predict overwinter survival in yellow-bellied marmots. – Acta Ethol. 18: 181–185.

Wheat, R. E. et al. 2016. Environmental DNA from residual saliva for efficient noninvasive genetic monitoring of brown bears (Ursus arctos). – PLoS One 11: e0165259.

Wilbert, T. R. et al. 2015. Non-invasive baseline genetic monitoring of the endangered San Joaquin kit fox on a photovoltaic solar facility. – Endanger. Species Res. 27: 31–41.

Wilkening, J. L. et al. 2016. When can we measure stress noninvasively? Postdeposition effects on a fecal stress metric confound a multiregional assessment. – Ecol. Evol. 6: 502–513.

Wilkie, S. C. et al. 2018. Trapped river otters (Lontra canadensis) from central Saskatchewan differ in total and organic mercury concentrations by sex and geographic location. – Facets 3: 139–154.

Williams, E. et al. 2018a. A review of current indicators of welfare in captive elephants (Loxodonta africana and Elephas maximus). – Anim. Welf. 27: 235–249.

Williams, K. E. et al. 2018b. Detection and persistence of environmental DNA from an invasive, terrestrial mammal. – Ecol. Evol. 8: 688–695.

Wilson, R. P. and McMahon, C. R. 2006. Measuring devices on wild animals: what constitutes acceptable practice? – Front. Ecol. Environ. 4: 147–154.

Wilson, R. P. et al. 2015. Pushed to the limit: food abundance determines tag-induced harm in penguins. – Anim. Welf. 24: 37–44.

Xing, H. et al. 2019. Identification of signal pathways for immunotoxicity in the spleen of common carp exposed to chlorpyrifos. – Ecotoxicol. Environ. Saf. 182.

Xu, C. C. Y. et al. 2015. Spider web DNA: a new spin on noninvasive genetics of predator and prey. – PLoS One 10: e0142503.

Yeruham, I. et al. 1996. Skin tumours in cattle and sheep after freeze- or heat-branding. – J. Comp. Pathol. 114: 101–106.

Yin, D. Y. and He, F. L. 2014. A simple method for estimating species abundance from occurrence maps. – Methods Ecol. Evol. 5: 336–343.

Yu, X. J. et al. 2011. Non-invasive determination of fecal steroid hormones relating to conservation practice in giant panda (Ailuropoda melanoleuca). – Anim. Biol. 61: 335–347.

Zang, L. Q. et al. 2013. A novel, reliable method for repeated blood collection from aquarium fish. – Zebrafish 10: 425–432.

Zemanova, M. A. 2017. More training in animal ethics needed for European biologists. – Bioscience 67: 301–305.

Zemanova, M. A. 2019. Poor implementation of non-invasive sampling in wildlife genetics studies. – Rethink. Ecol. 4: 119–132.

Zemanova, M. A. et al. 2018. Slimy invasion: climatic niche and current and future biogeography of Arion slug invaders. – Divers. Distrib. 24: 1627–1640.

Zheng, X. et al. 2016. Individual identification of wild giant pandas from camera trap photos – a systematic and hierarchical approach. – J. Zool. 300: 247–256.