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Noninvasive Biological Sampling of Free-Ranging Wildlife

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Noninvasive biological sampling of wildlife aims to support wildlife population management while reducing the potential pain and distress associated with invasive or destructive sampling. Visual and acoustic surveillance, collection of discarded biological materials (e.g., skin, hair, feces, saliva), and environmental DNA analysis are techniques that don't necessitate animal capture and still provide important information about wildlife movement, genetics, and diseases.

Keywords
eDNA, health assessment, nondestructive sampling, nonlethal sampling, population genetics, wildlife population management

What Is Noninvasive Sampling?

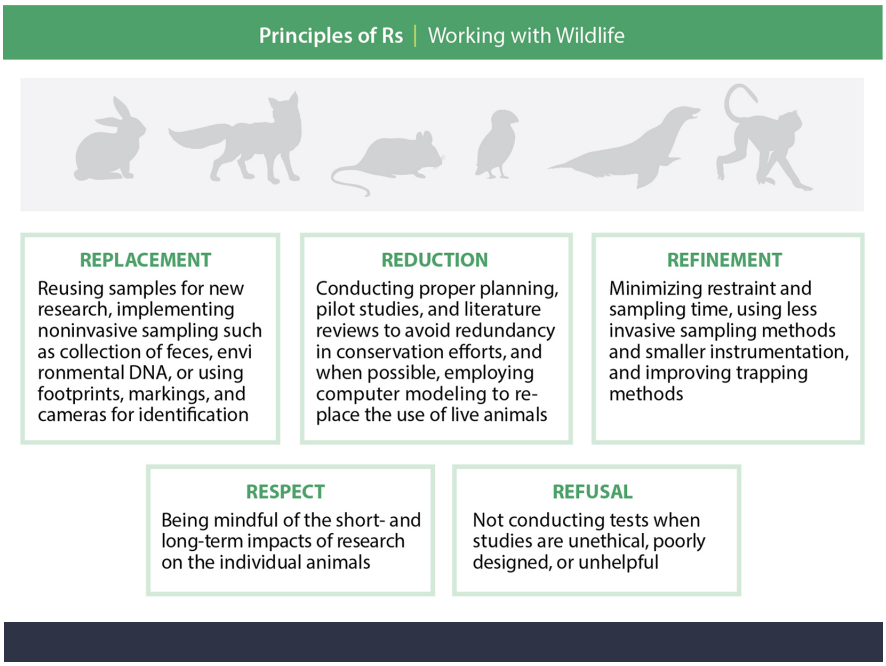
Due to the threatened and endangered status of many animals around the globe, conservation efforts are critical and depend on information gained from research projects regarding population size, species distribution, disease prevalence, and impacts of a rapidly changing environment (Zemanova, 2020). While sample collection from wildlife is necessary to improve conservation and population recovery, it is imperative that sampling procedures reduce risk and impacts to animal welfare (Pauli et al., 2009; Zemanova, 2020). These risk-minimization

strategies are often legally required and typically serve to satisfy the public by minimizing the pain and distress of animals used in research (Pauli et al., 2009; Zemanova, 2019). The term “noninvasive sampling” was introduced by Taberlet et al. (1999), whereby samples (e.g., hair or feces), typically required for DNA analysis, are collected from biological materials left behind by animals, eliminating the need to catch and disturb them. Confusion often arises with this term because, in medicine, “noninvasive” refers to procedures where animals are sampled without pain; however, a stress response still occurs due to capture and restraint (Garshelis, 2006). This is particularly true for wild animals, which often have an instinctive fear of humans (Clinchy et al., 2016). Therefore, scientists should be clear about which definition of noninvasive sampling is used in their research projects (Pauli et al., 2009; Lefort et al., 2019). “Noninvasive monitoring” refers to using noninvasive sampling methods to track animal movement patterns, population genetics and demographics, habitat utilization, and health. Other types of sampling of wild animals include “nondestructive sampling,” which occurs when an animal is captured and sampled using invasive or minimally invasive methods (e.g., blood sampling); however, animal capture is not always required (e.g., biopsy dart guns; Taberlet et al., 1999). “Destructive sampling,” when animals are killed to facilitate sample collection, is archaic and should be avoided (Taberlet et al., 1999).

A zoologist setting a camera trap to observe animals is an example of noninvasive sampling.
Photo courtesy Getty Images.

Truly noninvasive studies were first implemented thirty years ago with the collection of feces of Eurasian brown bears (*Ursus arctos arctos*) and the shed hair

of chimpanzees (*Pan troglodytes*) (Hoss et al., 1992; Morin et al., 1993). Although a potential pitfall is lack of awareness among researchers about the advantages of prioritizing these techniques over invasive methods whenever possible, noninvasive sampling is often associated with improved data accuracy, reduced fieldwork costs, and improved safety for researchers, in addition to enhanced animal welfare (Waits & Paetkau, 2005; Pauli et al., 2009; Zemanova, 2019). Pain and distress can induce an acute stress response in animals, potentially influencing the quality of any physiological data obtained. The stress of animal capture and handling can also lead to predation, physiological/behavioral changes, injury, or death of the captured animal(s). Therefore, the most important aspect of noninvasive sampling is the benefit to the animal by the elimination of capture stress and pain, as fitness (i.e., physiology and behavior) goes unchanged and the chance of injury or mortality is reduced to nearly zero (Russell & Burch, 1959; Pauli et al., 2009; Lefort et al., 2019; Zemanova, 2020). Importantly, noninvasive sampling approaches can significantly increase sample sizes, a limiting factor in many studies of free-ranging wildlife (Ryser-Degiorgis et al., 2013).



When designing studies using wild animals, researchers should determine *a priori* if the research objectives can be addressed using noninvasive sampling techniques (Taberlet et al., 1999). Pilot studies and a cost-benefit analysis are encouraged to develop a proof-of-concept for noninvasive sampling prior to commencing sample collection on numerous individuals or a population (Taberlet et al., 1999; Zemanova, 2020). Researchers working with wildlife should always seek to minimize pain and distress through the principles of the Three Rs: Replacement,

Reduction, and Refinement (Russell & Burch, 1959; Lefort et al., 2019; Zemanova, 2020). A fourth R, Refusal, has been proposed (Curzer et al., 2013), and we propose a fifth R, Respect, to improve the scientific benefit while minimizing harm to animals. Replacement is recycling archived samples for new research projects or using noninvasive sampling, including the collection of feces, environmental DNA, or using footprints, markings, and cameras for identification instead of permanent internal or external tags. Reduction involves proper planning, pilot studies, literature reviews to avoid redundancy in conservation efforts, power analysis to determine sample sizes, and computer modeling to replace the use of live animals. Refinement minimizes restraint and sampling time, uses less invasive sampling methods and smaller instrumentation, and improves trapping methods (Zemanova, 2020). Refusal should occur when studies are unethical, poorly designed, offer little in terms of conservation efforts, or when the risk outweighs the benefit (Curzer et al., 2013). Researchers working with animals should maintain respect for their research animals and be aware of the short- and long-term impacts of their research on the individual animals.

Types of Noninvasive Monitoring and Sampling

Visual and Acoustic Methods

On a population level, visually observing animals can enable biodiversity assessments through species abundance estimates and monitoring animal movement (Ksiazkiewicz-Parulska & Goldyn, 2017). Visually observing individual animals allows researchers to assess body condition and any external lesions or developmental abnormalities. Documenting images of free-ranging animals can be achieved using high-quality serial photography and/or videography conducted with handheld cameras, camera traps, and thermal imaging. Aerial photography using drones, and terrestrial and/or underwater photography using remotely operated vehicles (ROVs), can visualize animals but would not technically qualify as noninvasive when the presence of the drone/ROV causes stress or distress in animals. Individual animals can be visually identified by footprints or natural markings instead of more invasive tagging methods that require capture and tag attachment (Zemanova, 2020). Acoustic methods, such as vocalizations, can also be used to distinguish individuals of certain species (Terry et al., 2005). Integration

of machine learning using computers to automate monitoring technologies enhances the ability of researchers to detect and identify individual animals among recorded surveillance data (Norouzzadeh et al., 2018; Francisco et al., 2020). Citizen science initiatives incorporated into camera-based research are an increasingly common resource for developing large visual monitoring datasets for wildlife species.

Alternatives to Invasive Blood and Tissue Sampling of Captured Live Animals

The collection of biological samples from free-ranging wild animals is a valuable approach for addressing important questions about wildlife population management and conservation strategies. In particular, genetic monitoring using animal DNA can identify and distinguish between cryptic species and estimate biological parameters of interest, including demographic parameters (e.g., abundance, vital rates, occupancy, sex identification, hybridization, disease status); population genetic parameters (e.g., assessment of phylogenetic relationships between organisms to evaluate genetic diversity and structure, and the effective population size); and responses to selective pressures (e.g., exploitation, climate change) (Lefort et al., 2019; Carroll et al., 2018).

Noninvasive sampling includes the collection of various sample types, including shed materials and samples from carcasses such as feces, urine, hair, feathers, and bone (see Table 1). Wild animal health information can be collected using various methods, including evaluating data from animals that have been hunted, trapped, and/or slaughtered for food, animals struck by cars (roadkill), surveillance of diseased animals admitted to wildlife rehabilitation facilities, performing clinical examinations within the context of animal translocation, utilizing museum specimens, and questionnaires and interviews with wildlife managers and hunters to obtain information on disease occurrence (Ryser-Degiorgis et al., 2013). Such data, in combination with spatial data analysis using geographic information systems (GIS) technology, allow us to understand and predict wildlife disease exposure risks (Bechert, 2012). Less commonly used methods of DNA collection include the collection of body odor or scent marks, snow footprints, insect exuviae, blood-sucking parasites, spider webs, antlers, or shed skin, and the collection of saliva from mineral licks or damaged crops.

Sample Type*	Possible Analyses				
	Population Genetics	Ecotoxicology	Endocrinology	Microbiology	Infectious Disease Training
Carcasses	X	X	X	X	X
Feces	X	X	X	X	X
Hair	X	X	X		
Feathers	X	X	X		
Bone	X	X			
Eggshells	X	X		X	
Fish Scales	X				
Sputum/Saliva	X	X	X	X	X
Urine	X	X	X		
Environmental DNA (eDNA) from Water	X			X	

**Samples that do not necessitate animal capture (i.e., noninvasive)*

Table 1.

Types of biological samples that can be collected from wildlife specimens using noninvasive methods and possible analyses for which the samples can be used.

Unique Challenges and Adaptations for Noninvasive Sampling of Aquatic Species

Free-ranging aquatic species present unique challenges and opportunities for observation and noninvasive sampling. Thus, traditional approaches for studying animal physiology, behavior, movement, and health in terrestrial animals are often impractical for free-swimming aquatic species. This is particularly true for large-bodied marine organisms such as whales, which routinely migrate long distances, are difficult and/or dangerous to observe and approach, are not easily kept in captivity, and often cannot be sampled without killing the animal or encountering it dead (Hunt et al., 2013). Several noninvasive sampling techniques for studying aquatic species such as whales were designed to circumvent these challenges, including adapting visual and acoustic surveys to aquatic environments and collecting and analyzing fecal and respiratory vapor samples (Hunt et al., 2013; Nowacek et al., 2016). Both passive and active surveillance of aquatic species can be conducted using remotely operated camera equipment and acoustic and/or satellite tracking systems, which provide critically needed information on population densities, habitat use, movement of individual animals, and behavior (Nowacek et al., 2016). Fecal samples can be collected from the water either opportunistically or via focal follows of free-ranging aquatic species. Respiratory

vapor samples (from exhalations or “blows”) can be sampled using either handheld or drone-assisted collection techniques as marine mammals breathe at the surface (Acevedo-Whitehouse et al., 2010; Appril et al., 2017).

The aquatic environment, as well as the morphological and physiological adaptations that enable aquatic species to survive, often preclude the direct collection of shed biological samples that can be collected from terrestrial wildlife, such as shed skin, hair, and saliva. Aquatic species do, however, shed their DNA into the aquatic environment via feces, sloughed tissue cells, and/or gametes, which can be collected as environmental DNA (eDNA) without animal capture or visual surveys. An eDNA sample consists of the genetic substances contained in a state of free-floating DNA within cell fragments, skin cells, saliva, feces, and urine. Once extracted from water samples, eDNA can be analyzed using genomic sequencing technologies (Rees et al., 2019; Tsuji et al., 2019; Bessey et al., 2021). Metabarcoding of eDNA is a sensitive, universal monitoring tool that allows researchers to inventory and monitor aquatic species abundance and biodiversity, including the detection of invasive species (Taberlet et al., 2012; Bessey et al., 2021). In turn, these data can estimate species richness and characterize community structure and trophic relationships (Bessey et al., 2021). Further refinement of eDNA methodologies is needed because detection/nondetection of species-specific DNA fragments in a water sample does not necessarily confirm presence or absence. Varying environmental conditions (e.g., UV-B light, temperature, pH) influence eDNA persistence in aquatic environments, and the detection of aquatic invertebrates remains weakly documented to date (Barnes et al., 2014; Roussel et al., 2015; Strickler et al., 2015).

While noninvasive sampling of aquatic species can be difficult, eDNA by way of cell fragments, saliva, feces, and urine can be extracted from water samples. Photo courtesy Getty Images.

Conclusions

Noninvasive sampling provides clear benefits, including the reduction of stress in animal subjects, and provides physiological measurements more accurately representative of baseline health (Pauli et al., 2009). While not all research can utilize these methods, thorough literature reviews, proper planning, and education on new techniques will help improve data quality and reduce impacts to wildlife species and their ecosystems (Schwartz et al., 2011; Zemanova, 2020). Biologists and managers who aim to improve species conservation and preserve natural ecosystems will benefit from adopting noninvasive sampling techniques as part of routine wildlife population assessments and research (Schwartz et al., 2011). Often, noninvasive sampling is the superior methodology for determining population size and the presence of rare species (Waits & Paetkau, 2005). Although currently there is less oversight for the sampling of invertebrates (other than cephalopods) compared to the sampling of vertebrates, many validated noninvasive sampling methods now exist for invertebrate sampling and should be implemented (Zemanova, 2019). Noninvasive sampling methods are becoming increasingly popular as wildlife populations around the world experience unprecedented anthropogenic pressures, and conservationists strive to reduce our impacts on

already imperiled ecosystems.

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