Conservation Biology

Diversity

Defining Noninvasive Approaches for Sampling of Vertebrates

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Introduction

Conservation biologists are increasingly using novel techniques to sample free-ranging vertebrates. In particular, noninvasive approaches to sampling have become a major component of wildlife research (Garshelis 2006; MacKay et al. 2008). Originally these approaches were applied to "remote" tissue collection (e.g., feather, hair, feces) for DNA-based analyses, but they now include other techniques (e.g., stable isotopes, remote sensing, hormone analyses). Because noninvasive approaches generally imply that animals are sampled without capture or handling, they offer clear practical benefits. Noninvasive sampling often increases safety for both animal subjects and researchers, minimizes disturbance to animals, and, therefore, increases the accuracy of data while allowing larger sample sizes at a lower cost. For small populations, particularly of threatened and endangered species, minimizing risk to study animals is highly desirable and, in many cases, legally mandated.

Noninvasive sampling approaches also offer ethical advantages; as a result, the term *noninvasive* is appealing to funding agencies, institutional animal use and care committees, and the general public. Indeed, noninvasive approaches originated in the United States following policy changes of the 1980s that instituted greater oversight of animal-based research, changes which resulted from public concern over the treatment of animals (Orlans 1988). Conservation biologists have a responsibility to minimize stress and disturbance to their study animals and, therefore, are obliged to use noninvasive techniques where appropriate. In some instances noninvasive sampling is not practical because it will not provide the desired data. Nevertheless, we welcome the increasing reliance on such approaches and hope that technological advancement and public interest will continue to encourage their role in field-based studies involving vertebrates.

Defining Noninvasive Sampling

Presumably because of the benefits of noninvasive sampling, the number of wildlife-related articles in which the term *noninvasive* is used has grown rapidly in the last 2 decades (Fig. 1). Noninvasive sampling has even received recent public attention in the media as a novel and powerful approach to better understand vertebrate populations (Robbins 2009). But, when researchers claim to use noninvasive sampling, do their fellow biologists and the public have a common perception of the interaction between a researcher and a subject? Because of the growing use and importance of noninvasive methods, a clear definition of the term is needed.

Although unresolved debate surrounding some scientific terminology is useful because it exposes the complexities of broad concepts and provides flexibility for the inclusion of new findings (Hodges 2008), the term *noninvasive* requires an unambiguous definition. As a sampling procedure, noninvasive techniques have the specific, circumscribed goal of reducing stress and risk to study animals. We have found, however, that as currently used, the term *noninvasive* has little meaning and is indistinguishable from other sampling terminology. Thus, we propose new definitions for sampling methods that are based on the perspective of and level of risk to the study animal (Table 1). Our definitions avoid some inconsistencies that limited previous ones, account for predicted consequence to the animal and the level of risk inherent to the sampling procedure, and enable researchers to design, evaluate, and replicate sampling

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Paper submitted March 16, 2009; revised manuscript accepted April 19, 2009.

Conservation Biology, Volume 24, No. 1, 349–352

DOI: 10.1111/j.1523-1739.2009.01298.x

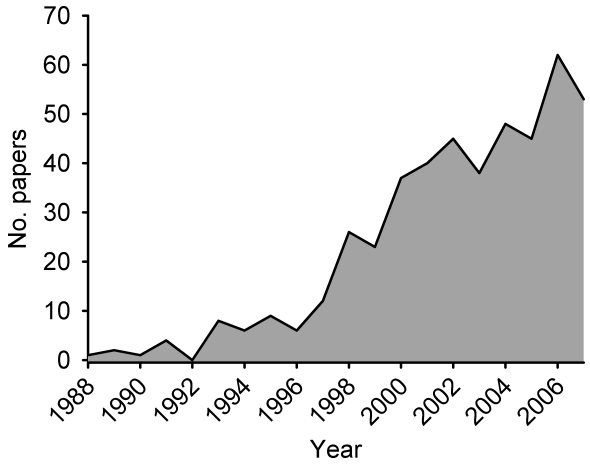

Figure 1. Frequency of the term noninvasive found as a keyword in peer-reviewed literature (1988–2007). Data obtained from Wildlife Ecology Studies Worldwide (accessed November 2008).

methodologies consistently. Clear definitions are particularly important as noninvasive sampling becomes more central to conservation biology and as sampling methods continue to evolve. In our definition noninvasive sampling techniques are either unperceived by an animal subject or are perceived by an animal but do not elicit a chronic-stress response or a reduction in fitness (Table 1).

Etymology and Use of Noninvasive Sampling

Persistent confusion over *noninvasive* may stem from confounding biomedical and colloquial origins of the word. Scientific use of *noninvasive* began in a biomedical context, in which any procedure not requiring the "insertion of an instrument or device through the skin or a body orifice" is considered noninvasive (Stedman's Medical Dictionary 2008). In field-based studies in which animals are captured or restrained, often without an instrument penetrating the body, such a definition was impractical and, in the extreme, could incentivize inappropriate sampling. For example, rather than chemically immobilizing animals during capture, researchers could be motivated to sample via physical restraint alone, even if the chance of capture-related injuries is increased. In such a case, adherence to a medically noninvasive protocol would be more injurious to the animal than using medically invasive methods.

Presumably because of the unsuitability of the medical definition, conservation biologists have variously defined sampling as noninvasive when researchers avoided contact with study animals (Schwartz et al. 1998) or when study animals were not "directly observed or handled by the surveyor" (MacKay et al. 2008:1). Nevertheless, contradictions within these definitions are evident.

Table 1. Definitions for sampling approaches used by conservation biologists.

Term	*Definition*	*Examples*
Noninvasive[a]	animals are unaware of sampling and, therefore, are unaffected by it (unperceived) or animals are unrestrained and do not exhibit a chronic or severe stress response or experience reduction in survival or reproduction (perceived)	aerial photography, biopsy darts, camera traps, hair traps, harpoon-insertion data loggers, scat collection, sign indices, spotlight surveys, tracking, track plates, visual and acoustic observation
Nonlethal	animals experience capture or restraint, and samples or measurements are collected from the animal prior to release	immobilization, live capture
Lethal	animals are immediately euthanized in the field or euthanized following any of the above techniques	captive trials, culling experiments, specimen collection
Postlethal	animal samples or measurements are obtained from those previously killed by hunters, trappers, museum collectors, vehicles or found in the field	demography, diet analysis, DNA analyses, morphometrics

[a] *This is our definition of* noninvasive. *Techniques can be unperceived or perceived by an animal, depending on the biology of the species and implementation of methodology.*

Observing an animal from a blind is invasive, whereas some large-scale manipulative studies (e.g., prescribed burns) that displace or kill animals are not. Perhaps most importantly, these definitions arise from the perspective of the researcher, rather than the animal. Noninvasive sampling has also been defined as a genetic sampling approach in which animals deposit samples without being caught or disturbed by the researcher (Taberlet et al. 1999). Nevertheless, the definition of *disturbed* is unclear, and this definition of *noninvasive sampling* is limited to those studies collecting samples for DNA-based analyses. Conservation biologists also seem to have been influenced by the common understanding of *invasive* as actions or sensations that tend to intrude on one's thoughts or privacy (The American Heritage Dictionary of the English Language 2004), with invasiveness relating to intrusion on the perceptions or behaviors of animal subjects.

To illustrate the current inconsistent use of *noninvasive* in contemporary literature, we searched the Wildlife and Ecology Studies Worldwide database for the following terms: "noninvasive," "non-invasive," and "non invasive," for the year 2007. We found 53 studies. We excluded 13 papers because they were not suitable for our review (see Supporting Information). In 27% (11/40) of remaining papers, authors described procedures as noninvasive even when they involved methods that caused substantial stress and risk to study animals, such as the capture, restraint, and temporary or prolonged captivity of animals. For example, Gustine et al. (2007) captured woodland caribou (*Rangifer tarandus*) via helicopter net-gunning and used ultrasound to measure rump fat for estimation of body condition. Although ultrasound allows precise fat-depth measurement without surgery, the procedure requires animal capture. Furthermore, helicopter capture of ungulates induces stress not only in the target animal, but also in nearby conspecifics. There were less clear-cut cases in which animals were not captured or handled, but we classified the research as invasive. Arlettaz et al. (2007) collected feces from grouse (*Tetrao tetrix*) in a manner characterized as noninvasive, but some samples were collected after repeatedly flushing birds from their snow dens, and these individuals exhibited a clear physiological stress response. Our definition of *noninvasive* was applied in most studies (73% or 29/40). Among these studies, the most common techniques were collection of feces ($n = 15$) and shed hair or feathers ($n = 3$) for DNA and hormonal analyses.

Even among chapters in a textbook (*Techniques for Wildlife Investigations and Management*), what constitutes noninvasive is unclear. From a mark-recapture perspective, Lancia et al. (2005:131) state that noninvasive studies are those that do not "...physically capture an animal if it can be uniquely identified without doing so." Subsequently, Silvy et al. (2005) discuss noninvasive marking of wildlife as that which avoids inserting implants (such as PIT tags) or marking tissue (such as tattoos), regardless of whether an animal is captured and handled. Finally, Oyler-McCance and Leberg (2005:645) restrict noninvasive approaches to material collected for DNA-based analyses when they state that "...biologists have non-invasive ways (through hair snags or from collecting feathers, feces, or frozen urine) to sample individuals that have been difficult to sample in the past."

Conclusions

Various terms in ecology and conservation biology have been evaluated to improve clarity and effectiveness in communication (e.g., Adams et al. 1997). Although strict definitions are not always necessary or even desired (Hodges 2008), the benefits of precise language for scientific procedures are clear; it increases precision and avoids misconception when describing a study. Particularly for noninvasive sampling, which has a specific goal to reduce stress and risk relative to study animals, a clear definition will help avoid miscommunication about sampling procedures. Currently, the multiple and vague definitions that have been applied to *noninvasive* have created a term largely without meaning and, as currently used, indistinguishable from other sampling approaches.

Garshelis (2006) first recognized the inconsistency of use of *noninvasive* and called for a standardized definition for such approaches. He argued that because *noninvasive* denotes a sort of ethical hierarchy of sampling techniques the term *remote* should be used instead. However, as MacKay et al. (2008) identified, *noninvasive* is already so common that switching to a previously defined term (i.e., remote sensing) would further obfuscate meanings. Furthermore, we believe noninvasive sampling does provide ethical advantages over traditional sampling procedures. Thus, rather than adding new and potentially confusing terminology, we have clearly defined *noninvasive* by the amount of disturbance and level of risk the procedure has for study animals and have placed it in context with other sampling approaches (Table 1). In many instances, live capture and immobilization (i.e., invasive approaches) are necessary; however, conservation biologists should strive to minimize impact on animals by using the least invasive techniques possible, a goal formally recognized in our definitions. Inevitably, almost all sampling involves risk to study animals. Under our definition of *noninvasive*, the probability of animal mortality and injury from sampling should be virtually nil. The descriptive definitions we provide in Table 1 can be used by conservation biologists to clarify the meaning and intent of various sampling procedures, including the increasingly important noninvasive ones.

Acknowledgments

This paper originated from in-class discussions with professors S.T. Jackson and J.A. Lockwood. G.D. Hayward, S.W. Buskirk, and D. Rule provided helpful comments on earlier drafts. The authors were supported by the U.S. Forest Service, Pacific Northwest Research Station, Juneau Forestry Sciences Laboratory (J.N.P.) and the Program in Ecology (PiE; J.P.W. and A.D.M.) at the University of Wyoming. Publication costs were paid for by PiE.

Supporting Information

A complete list of articles reviewed and our classification of the sampling approach for each are available as part of the on-line article (Appendix S1). The author is

responsible for the content and functionality of these materials. Queries (other than absence of the material) should be directed to the corresponding author.

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