Wildlife Research Techniques in Rugged Mountainous Asian Landscapes

Edited by
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INTRODUCTION

Genesis of "Wildlife Research Techniques in Rugged Mountainous Landscapes"

One of Bhutan's greatest treasures, spanning from the high Himalayas to low elevation subtropical forests, is its remarkable biodiversity. Bhutan's biodiversity—including its charismatic species like tigers (*Panthera tigris*), Asian elephants (*Elephas maximus*), and snow leopards (*Panthera uncia*)—are largely intact, due to low developmental impacts and a strong commitment to conservation emerging from the culture and the leadership of their Kings; environmental integrity sits as one of the pillars of the country's "Gross National Happiness" philosophy.

We treasure Bhutan's rich natural heritage and take great pride in conservation of it, while also being committed to sustainable socio-economic development of the local people. In most places around the world these parallel goals of conservation and economic development often lead to disagreements among different players. Increasingly conservation agencies and organizations are viewed as antagonists for development. As Bhutan is undergoing unprecedented development with a larger and larger section of the society viewing these changes as favorable and desirable, direct opposition and attempting to stop it is not a card that conservation agencies and organizations can play.

Because socio-economic development is inevitable, the issue is not a matter of yes or no to development, but rather "how", "where", and "how much"? All development activities are not necessarily detrimental to wildlife nor are all areas affected the same; there are ways and means to minimize the impact if conscious and committed efforts are made. For this to happen, a sound understanding of species and their responses to change is needed. This information and knowledge can be only generated through rigorous scientific studies. The lack of capacity has been a major setback for us to generate scientific knowledge, so we have had to depend on external scientists to conduct even simple wildlife surveys. The dependence on external scientists further affects the development of local capacity. We have very enthusiastic biologists in the field, yet their scope of research has been limited to collecting data and storing them on computers.

Realizing the lack of research training as a major impediment for conducting scientific studies on wildlife, UWICE assembled in 2010 an international workshop on conducting research in rugged mountainous landscapes like Bhutan. The objectives of the workshop were to provide hands-on training, and to provide a manual on wildlife research techniques in our landscapes. Such wildlife manuals have been lacking in both relevancy and availability, yet remain much needed by field staff. This practical book emerged as a direct result of the 2010 workshop conducted in Bhutan by UWICE in collaboration with University of Montana. Together with invited professors from different universities from the US and Europe, we felt the need to produce a handbook/techniques manual for field researchers applicable to our local conditions. To our delight, the invited instructors not only agreed to train local researchers and biologists in the region, but also to write book chapters in collaboration with local biologists. This book is a direct outcome of the workshop.

The scientists we invited as workshop instructors and chapter authors were chosen to help us customize the rigor and applicability of this book to the challenges in remote mountainous landscapes. The invited participants are not only leaders in their field with cutting edge scientific techniques; they also have worked in similar landscapes in Asia and other rugged regions.

Therefore this book has a unique focus to fill the need in mountainous regions for insights into rigorous yet applicable wildlife biology research techniques. The need to have such a manual has been felt by the people in the field. Our earlier experience was that providing one-time training does not necessarily lead to building capacity of field staff. We realize that due to sheer remoteness and the far-flung locations of most field offices, the access to resources is extremely limited and reliable training manuals are non-existent in the field. So, after training, our field staff return to their bases and conduct research that can neither corroborate other methods nor be used for other purposes, thus undermining the objective of trainings. It is for this purpose that we decided a manual on research techniques is crucial for our field staff, so that whatever they learned in this training, they can revisit in the future. Within Bhutan, increasingly we see our field staffs and other personnel drawn towards scientific questions and science-based knowledge. The techniques applicable for landscapes like Bhutan are challenging and for aspiring field researchers and upcoming future conservationists, it is very important to have focused guidance. It is with these noble reasons that we embarked on writing this book. We are very grateful to our contributing authors for consenting to write chapters on the topics they provided training on during the workshop.

Although our initial focus was on wildlife research techniques in Bhutan, the book targets concepts broadly applicable to wildlife research in mountainous landscapes. This book has 7 main chapters. In the first chapter, we identify some of the broad conservation research priority areas that warrant our focus and attention for the immediate future. However, this is not to de-emphasize the importance of other areas that other institutions are currently engaged in that are not included here. The second chapter deals with monitoring wildlife populations: presence, abundance, and trends with special reference to our landscapes. This chapter provides an overview of the "what, how, where, and why" to monitor wildlife with explicit examples and figures. Chapter 3 covers the use of cutting-edge scientific techniques for monitoring wildlife through non-invasive genetic sampling, including not only general aspects of applicability of genetics in wildlife monitoring but also basic protocols to collect and assay DNA samples in the lab. Chapter 4 is on use of remote cameras in wildlife research, another technique that has been widely used for monitoring wildlife species. Chapter 5 covers the use of radiotelemetry in wildlife research, describing critical concepts for obtaining key insights on survival, reproduction, habitat use, and movement. Chapter 6 is on techniques for diet analysis, describing succinctly how to conduct diet analysis for predators and other species. The last chapter describes methods for aquatic and freshwater monitoring. Bhutan has abundant freshwater and streams, yet little effort has been made to study the diversity of aquatic life. With huge hydropower dams under construction, how this development will impact aquatic diversity is an important question.

We hope this book will help in building local capacity to conduct wildlife research and studies that are based on rigorous scientific methods.

CHAPTER 1

Conservation Biology Research Priorities For Rugged Mountainous Landscapes

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Background

Mountain landscapes contain some of the world's most diverse and intact biological ecosystems. To that end, we begin this book with a short overview of some of the most pressing research needs for conservation biology in this ecoregion.

The research needs described in this chapter focus on biological aspects of conservation biology. Although sociological/ human dimensions/ community conservation topics are of great importance for developing conservation biology strategies, they are not the focus of this chapter, nor of this book. We underscore the old saying that "wildlife management is really people management", and stress that human needs must be accounted for in developing conservation strategies. However, we wish to focus here in specific ways on understanding status, trends, and responses of wildlife populations in mountainous landscapes. Our emphasis is on the biological science necessary to understand distribution, numbers, trends, and interactions among wildlife species. With our tight focus on wildlife research techniques, we hope to provide the means to obtain biological insights that will complement sociological, political, and economic components in conservation decision-making.

Although most of the research priorities in this chapter are relevant to any wildlife species and even to plants, the focus for our examples and applications will be biased towards vertebrate species. Philosophically we take a broad view of wildlife as including all non-human and non-domesticated animals. But we also acknowledge the strong public interest and support for vertebrate conservation, especially mammals (see "Focal Species" section below). Therefore, our examples

focus primarily on vertebrates, with less mention of sampling or studying other animal groups such as arthropods and other invertebrates (although the final chapter provides an important overview of aquatic sampling including invertebrates).

Finally, we certainly do not strive to make this chapter comprehensive in its assessment of research priorities in conservation biology. Other books (e.g., Soule and Orians 2001) and articles have amassed useful compilations of important research priorities. We note for example a special section on "Priorities for Policy-Relevant Conservation Research" for SCB Regional Sections, including Asia, Africa, Austral and Neotropical America, Europe, North America, Oceania, and marine sections (Rodriguez 2009). Our research priorities are different from these efforts in two ways. First, ours are oriented around topics most relevant to sampling wildlife in rugged mountainous Asian landscapes. Second, ours are much more tightly focused on specific wildlife conservation research topics that could be accomplished immediately with sufficient training and funding. This chapter, and this volume, will be much more specific, for example, than the following important topics listed as "major policy challenges" for conservation biology in Asia by McNeely et al. (2009):

- Funding for forest conservation
- Identifying potential impacts of energy alternatives on conservation of biodiversity
- Curbing the trade in endangered species of plants and animals
- Conservation of mountain diversity
- Expanding ecological, biological, economic and social assessments of ecosystems
- Enhancing scientific input into the convention on biological diversity on the issue of invasive alien species
- Enhancing conservation biology support of the Global Environment Facility
- Using conservation biology to build a better understanding of zoonotic diseases
- Using conservation biology to help address human-animal conflict
- Enhancing community-based conservation
- Using conservation biology to help address water-deficit problems

Although some of our research priorities will overlap with some of these, ours will be notably more focused on wildlife conservation in rugged mountainous landscapes. We see this chapter as a snapshot view of current, urgent, pressing needs for immediate wildlife conservation research in Bhutan and neighboring countries at the dawn of a new era of development and stresses on wildlife populations.

Not accidentally, the chapters following this one provide major introductions to the scientific techniques necessary for in-country field and laboratory researchers to accomplish these research priorities. In other words, this chapter describes some research priorities, and the rest of the book describes the methods, approaches, and techniques to carry out these research priorities.

Next we describe our 8 research priorities:

I. Select Focal Species

This task is not so much a research priority as it is a critical discussion to initiate between researchers, policy makers, and planners. Focal species have proven to be an effective way to link large-scale objectives such as "ecosystem health" and "integrity" to practical tools for monitoring and assessing impacts (Mills 2013). The key is to pick a suite of focal species that serve various roles in linking management objectives to community and ecosystem structure and function. Examples of types of focal species that may be chosen include: a) flagship species that are iconic and mobilize the public's attention; b) indicator species that may be most vulnerable to a particular stressor, for example water temperature or loss of snowpack; c) umbrella species whose wide-ranging movements define landscapes for conservation focus; d) keystone species that are particularly strong interactors, and whose loss might be expected to lead to an unraveling of the ecosystem; e) endangered, threatened, or otherwise at-risk species of concern; f) species with long-term datasets that are relatively easy to sample, leading to high statistical power to detect changes. The optimal approach would choose a suite of focal species across these categories, and across taxonomies (e.g., from mammals to insects).

Some example focal species in various regions across mountainous Asian landscapes include takin, tiger, snow leopard, hornbills, elephants, red pandas, and several primate species. All of these would be considered "flagship" species, but several would also qualify as indicator, keystone, umbrella, or species at risk.

II. Plant and Animal Inventory

This group of research topics includes a baseline inventory of plants and animals in National Parks, other government, and private lands. All conservation actions require a basic knowledge of species distribution. Such assessments should be rigorous, accounting for the fact that species may be in an area but not detected in one or more surveys. Species inventories, or distribution surveys, should also be stratified by land ownership, vegetation type, elevation, and bioclimatic zones.

The overall methodology of choice for distribution presence or absence surveys would be occupancy sampling (McKenzie et al. 2006; Chapter 2 this volume). In the field, occupancy sampling may occur through a variety of techniques including non-invasive sampling (e.g., camera trap [Chapter 4] and genetic samples [Chapter 3]), live trapping (especially for small mammals) (See UWICE publication: Foresman et al. 2010), and sighting transects. Note that inventory or distribution surveys must be linked to pre-identified focal taxa because the appropriate sampling tools, sampling scale, and study design will be specific to certain species or groups of species.

For example, grouping roughly by taxonomy and by the field techniques necessary to do the sampling, distribution inventories may target:

- Carnivores (e.g., felids, canids, ursids, large mustelids)
- Ungulates
- Birds
- Insects (especially butterflies)
- Aquatic vertebrates, and invertebrates that may serve as sentinels for water quality
- Plants (forest plant surveys)

III. Monitoring, or Tracking Species "Health"

For selected focal species, monitoring programs should be established as barometers of conservation success or risk. This topic is examined thoroughly in Chapter 3 of this volume.

IV. Evaluating Response to Human-Caused Perturbations, or Stressors

Suspected negative stressors on wildlife populations can be evaluated by specific experiments or directed studies, coupled to hypothesis-driven monitoring projects. Careful experimental design and study execution is critical to maximize the ability

to detect effects that are present (statistical power). Some examples of stressors to study may include:

- Hydropower effects on aquatic organisms
- Mining (including sand and gravel) effects on shorebirds and aquatic organisms
- Logging
- Harvest/poaching (e.g., cordyceps and elephants)
- Ecotourism
- Habitat loss due to development
- Fire/fire exclusion (including effects of potential encroachment of forests)
- Firewood collection (especially on small mammals)
- Grazing
- Feral/domestic dogs
- Cordyceps collection
- (Climate change is given its own category in the next section)

V. Effects of Climate Change on Wildlife

The physical science documenting human-caused changes in climate variables has matured to mainstream status among both scientists and the general public. But how will these rapidly changing and globally distributed physical changes translate into effects on biotic communities? Biologists do not have a cohesive answer to this question, making the issue of "adaptation" to climate change an issue of cutting-edge interest for both scientists and policymakers (e.g., Bell and Collins 2008, Hendry 2008, Kintisch 2008, Svenning and Condit 2008, Visser 2008).

In the Himalayan region, climate change effects are expected to be as extreme as anywhere else on the planet. How will plants and animals be affected? Based on biological first principles, we know that some species will move (shift geographic range), some will adapt in place through phenotypic plasticity or evolutionary change, while still others will be unable to move or adapt, and so will decline (Running and Mills 2009).

Changes in hydrologic flow and snowpack are two of the physical changes expected to change in the most dramatic ways in temperate mountain regions. Therefore, some of the most pressing questions include:

- How would changes in hydrologic flow affect aquatic organisms?
- How would changes in snowpack affect terrestrial focal species such as:
    - Marmots, pika, blue sheep, snow leopard
    - Snow leopard/common leopard interactions

VI. Connectivity and Utility of Corridors

Connectivity among wildlife populations is important both for retaining genetic variation and for sustaining multiple populations through fluctuating environmental stressors (Mills 2013, 2012, Crooks and Sanjayan 2006). Recent breakthroughs in GPS-based radiotelemetry and in genetic analysis offer remarkable tools for addressing connectivity (see Chapters 3, 4, and 5 in this volume). A key set of questions addresses the extent to which designated linkage zones and corridors actually facilitate biologically relevant levels of movement for focal species.

Specific questions might include:

- Do focal species found in Parks connected by corridors show higher levels of connectivity than those not connected by corridors?
- What species use corridors as extant habitat (extensions of this question could address the extent to which species interact, as in source-sink dynamics whereby corridors have negative effects on certain species because predators or competitors are drawn in)?
- Are there areas beneficial for connectivity *outside* the designated corridor?

VII. Taxonomic Distinctiveness and Potential Isolation of Wildlife Populations

The formidable topography in rugged mountainous landscapes may be expected to subdivide many wildlife populations from others. In addition, severe population and developmental pressure has in many cases decimated potential movement pathways for many species. Thus, it is likely that there are subspecies or evolutionarily distinct populations that deserve special conservation attention.

A high-profile example is the takin, found in China, Myanmar, India, and Bhutan. Three subspecies of takin in China were shown to show distinctly different DNA profiles, and to exhibit low genetic variation (Li et al. 2003). Takin in some less developed areas, such as Bhutan, might be expected to show even greater genetic differentiation.

Geographic isolation and taxonomic distinctiveness can be evaluated using both morphologic features and genetic analyses, both of which benefit from established in-country DNA laboratories and natural history museums (see also Chapter 3).

VIII. Human-Wildlife Conflicts

Conflicts between humans and wildlife can undermine local support for wildlife conservation. Therefore, research should focus both on ways of minimizing conflicts with individual species (e.g., through fencing, husbandry, regulations on cattle grazing areas) and on interactions among species. The latter category can lead to non-intuitive yet fundamentally important management insights. For example, the poisoning campaign to reduce wild dhole numbers in Bhutan is thought to have led to an increase in numbers of one of their prey, wild boar. If true, this means that the management action to reduce a conflict for cattle farmers (who lose cattle to dhole) may have actually made the conflict worse for crop farmers (who lose crops to boar). To date, this potentially important "trophic cascade" has not yet been studied. Another area where multiple species dynamics may be important is in the Himalayas, where predation by native predators (snow leopards) on domestic livestock (yak, cattle) might be influenced by presence and abundance of blue sheep or other prey.

In extreme cases of conflict with a wildlife species, research may need to investigate population dynamics of overabundant species in order to determine the most efficient ways to reduce their numbers (for example, through sterilization of males or females or possibly through lethal control). Population modeling can help direct such direct control measures to accomplish the largest reduction in population growth with the least cost or number of animals killed or sterilized (see Mills 2013).

Conclusion

We hope that these eight suggested priorities help to catalyze discussion about specific wildlife research directions in mountainous landscapes. The special challenges of terrain and logistics in mountainous landscapes underscore the importance of research being well thought-out and collaborative whenever possible, to maximize efficiency and strength of inference. This is especially true in Asian landscapes where wildlife conservation research dollars are especially scarce.

Research stations and research groups are efficient ways to pool expertise and increase synergy across topics in wildlife research. As just one possible example, the incredibly biologically rich region along the rugged Bhutan-India border contains National Parks in both countries (Royal Manas National Park on the

Bhutan side and Manas Tiger Reserve on the Indian side). Although professional and personal interactions among biologists are good, research efforts tend to be idiosyncratic both within and between the Parks. This would be an excellent place where formal Biological Research Stations could be established to coordinate research that spanned both the international borders and disciplinary divides. A Research Station (or one on each side of the border) would foster collaborations, support logistical needs of researchers (e.g., housing, food, basic research supplies), provide a formal framework for prioritizing limited research dollars, and provide the infrastructure to leverage cross-cutting grant funding initiatives. Such efforts will help define the critical wildlife research priorities and provide the critical mass of researchers to tackle them. We hope that the wildlife research techniques described in the rest of this book are useful for taking the next step of actually implementing the studies.

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CHAPTER 2

Monitoring Wildlife Populations

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Background

A fundamental property of wildlife populations is that they change in size and geographic distribution over time in response to naturally occurring or anthropogenically-induced perturbations to the environment. Wildlife monitoring—a collection of tools and techniques for detecting and quantifying such changes—is central to effective wildlife management programs because monitoring allows managers to detect direction and extent of such changes and adjust management activities accordingly. In this chapter, we will consider techniques that can be used to monitor distribution and abundance of wildlife populations.

The online dictionary Wikipedia (http://en.wikipedia.org/wiki/) defines monitoring as being "aware of the state of a system". Likewise, Yoccoz et al. (2001) defined monitoring as "the process of gathering information about some system state variable(s) at different points in time for the purpose of assessing system state and drawing inferences about changes in state over time." In wildlife population studies, state of a system is typically characterized by presence, distribution, size or growth rate of a population, and wildlife monitoring programs typically focus on monitoring presence, population abundance, or growth rate over time and space. In other words, with a monitoring program we are attempting to answer the simple questions (McComb et al. 2010): How is the population of interest doing? Is it increasing or declining? Indeed, a majority of monitoring programs focus on detecting trends in state variables (usually a population parameter (Marsh and Trenham 2008)). If it is declining in size or geographic range (or if it has already declined to low numbers), one might consider management actions aimed at reversing the trend. If, on the other hand, the population of interest is a pest species and is increasing rapidly, one might consider approaches to reduce the size or growth rate of the population. However, specific methods to be employed depend on factors such as study species, goals, or objectives of the monitoring program, spatial and temporal scales, and availability of time and resources.

An important question that must be addressed before devising or implementing a monitoring program is: why monitor? Specific objectives of monitoring programs can vary widely, but they can be placed into one of two broad categories (Gibbs et al. 1999, Yoccoz et al. 2001, Nichols and Williams 2006): scientific objectives and management objectives. Populations of some species may be monitored with the idea that, by monitoring abundance (or some other state variables and perhaps associated covariates) over a long period of time, we might improve our understanding of the dynamics and persistence of the population or about factors influencing population dynamics or persistence. Monitoring programs focus on improving our understanding of the system behavior; such a program may provide tests of hypotheses or contribute to development of new hypotheses or theories, but they may or may not provide useful information for management (Yoccoz et al. 2001). Management objectives, on the other hand, focus on providing information useful for management. Yoccoz et al. (2001) suggest that monitoring programs focusing on management objectives serve two purposes: identifying the system state and providing information on system response to management actions. These two classes of objectives need not be mutually exclusive (some monitoring programs may have both scientific and management objectives), but do point to the fact that the choice of the state variable(s) and design or implementation of monitoring programs depend on whether the species is being monitored primarily with the scientific or management objectives in mind.

Wildlife monitoring programs are typically implemented to monitor status and trend of species with high economic, recreational, conservation, scientific or socio-cultural values; as a part of a resource or protected area planning process; in response to a crisis (e.g., high risk of extinction); in response to legal challenges; or as part of an adaptive management program (McComb et al. 2010). For example, white-tailed deer (*Odocoileus virginianus*) and turkey *(Meleagris gallopavo)* are popular game species in the USA, and state wildlife agencies in many states monitor abundance of these species. The monitoring programs for the northern spotted owl (*Strix occidentalis caurina*) in the pacific northwestern USA (Anthony et al. 2004) and the Royal Bengal tiger (*Panthera tigris*) (Karanth and Nichols 2002) were primarily a response to a crisis of projected demise of these species. Likewise, many bird monitoring programs (e.g., North American Breeding Bird Survey; NABBS) were initiated to monitor status and trend of North American avian populations during the 1950's and 1960's when pesticides were suspected to be a major threat to avian populations (http://www.pwrc.usgs.gov/bbs/).

Various facets of animal population monitoring have been thoroughly examined (McComb et al. 2010, Williams et al. 2001, Wilson et al. 1996). Here, we focus on

some basic concepts as they apply to mountainous landscapes where sampling of wildlife populations can be particularly challenging.

What (and Where) to Monitor?

This is an easy question to answer if a monitoring program is legally mandated or if such a program is implemented in response to a crisis because legal mandates frequently specify the species (or habitats/ecosystems) and a general monitoring approach. Likewise, if a monitoring program is to be implemented in response to a crisis of imminent demise of a species, there is little confusion regarding what to monitor (species in danger and/or its habitat, prey, etc.). In most other cases, however, monitoring programs tend to focus on species that are highly ranked based on perceived economic, recreational, scientific, or socio-cultural values. A species may be valued differently in societies that are culturally different. Generally speaking, rare or endangered species, high profile, charismatic megafauna and species of economic or recreational value tend to be favored by monitoring programs. The monitoring of Royal Bengal tiger (*Panthera tigris*) and the greater one-horned rhinoceros (*Rhinoceros unicornis*) are monitored in Nepal because of their conservation status, and also because of their high profile as charismatic megafauna that attract attention of governments, conservation organizations, and the general public alike. Most state wildlife agencies in the USA monitor populations of popular game species, because of their recreational, cultural, and economic values. Populations of commercially harvested species of fish are monitored because of their economic values. McComb et al. (2010) offered the following criteria for selecting species to be monitored: level of risk, regulatory status, government rare species classification, restricted to specific seral stages, sensitivity to environmental change, ecological function, keystone species, umbrella species, link species, game species, species with limited data or knowledge, and species with public interest (see also Chapter 1 Discussion on 'Focal Species'). A recent survey revealed that a vast majority of monitoring programs focus on species of conservation concern (62%); other species monitored included indicator (18%) or invasive (8%) species (Marsh and Trenham 2008).

Next, the state variable(s) to be monitored must be identified. The choice of state variable(s) depends on a variety of factors, including the monitoring objectives (scientific or management), specific needs or legal mandate, species, and time and resources at one's disposal. For example, species such as the yellow-bellied marmots (*Marmota flaviventris*) hibernate for about 8 months every year. When marmots emerge from hibernation, they can be live-trapped and marked. Using these mark-recapture methods, in conjunction with visual observations, allows researchers to monitor a suite of state variables including abundance, survival, and

reproductive output (Armitage 1991, Armitage 1996, Ozgul et al. 2006, Ozgul et al. 2009, Ozgul et al. 2010). Simultaneous monitoring of abundance, survival, and reproductive rates would not be possible for most species of mammals or birds that are rare or elusive, and those that are difficult to capture or observe. An excellent example of monitoring with management objectives is the monitoring of North American mallards (*Anas platyrhynchos*), a species popular among waterfowl hunters. In addition to abundance, other variables being monitored include harvest and the number of wetlands in the primary breeding areas (Johnson et al. 1997, Yoccoz et al. 2001).

Once the species to be monitored has been identified, it is often necessary to determine where the species of question is to be monitored. If the focal species is endemic to a specific location or otherwise has limited distribution or if a monitoring program is legally mandated, there would be little confusion regarding where the focal species is to be monitored. In most cases, however, it is frequently not possible to monitor a species in its entire range of geographic distribution. In such cases, location and number of sites to be monitored is dictated by the species being monitored, logistic challenges, and available time and resources.

How to Monitor?

The choice of monitoring method(s) depends on the specific objectives, focal species, and state variable(s) to be monitored, and time and resources available for monitoring purposes. Most monitoring programs are designed to answer one or more of the following questions: (1) *Is the species there*? This seemingly simple question also is very important because monitoring methods or conservation measures are not useful if the species is not present. A related question is whether the occupied range is increasing or decreasing. Patch occupancy estimation and modeling approaches focus on addressing these questions; (2) *How many are there?* If a species is present, it is natural to ask how many are there because the answer to this question may determine whether or not the species needs monitoring or conservation action. A rare species with restricted geographic range would generally be in need of conservation action and monitoring, whereas a widely distributed, abundant species may be low in the priority list for monitoring. Methods for estimating absolute or relative abundance (or other appropriate state variables) focus on addressing this question; (3) *Are they increasing or decreasing*? An important goal of monitoring programs is to collect information on the chosen state variable(s) over time and/or space to assess the state of the system. Addressing this question necessitates repeated application of an appropriate monitoring method over time and/or space.

Marsh and Trenham (2008) found that monitoring methods that were commonly used by surveyed researchers included presence/absence or occupancy surveys, and methods that focused on estimating relative or absolute abundance.

Patch Occupancy Estimation and Modeling

Presence or absence of a species at a given site is the simplest form of information that is easy to collect, but also very helpful for determining a species' presence, status, and geographic range (MacKenzie et al. 2002, MacKenzie et al. 2004, MacKenzie et al. 2005, MacKenzie and Royle 2005, MacKenzie et al. 2006). If the objective of a monitoring program is to detect changes in presence of a species or proportion of sites (or area) occupied, patch occupancy modeling approaches are the methods of choice. Given the relative ease and low cost of data collection, it is not surprising that presence/absence or patch occupancy methodologies have become one of the most commonly used monitoring methods currently in use, and one that is increasing in popularity (Marsh and Trenham 2008). Patch occupancy modeling approaches have been discussed thoroughly elsewhere (MacKenzie et al. 2004, MacKenzie et al. 2005, MacKenzie and Royle 2005, MacKenzie et al. 2006). Here, we briefly review basic concepts and approaches to estimating and modeling patch occupancy.

Data collection for occupancy surveys typically involves searching for evidence of presence in sampling units. Method of detection can vary widely, ranging from visual observations, photographs, and indirect evidence of presence such as tracks, hairs, dung piles, scent marks, or scrapes (as long as the species identity of the indirect evidence is definitive).

Sampling units may include artificially created sampling units (e.g., grid cells of given size) or natural discrete sampling units such as ponds, lakes, remnant forest fragments, or alpine meadows interspersed within coniferous forests (Figure 2.1) (MacKenzie et al. 2004).

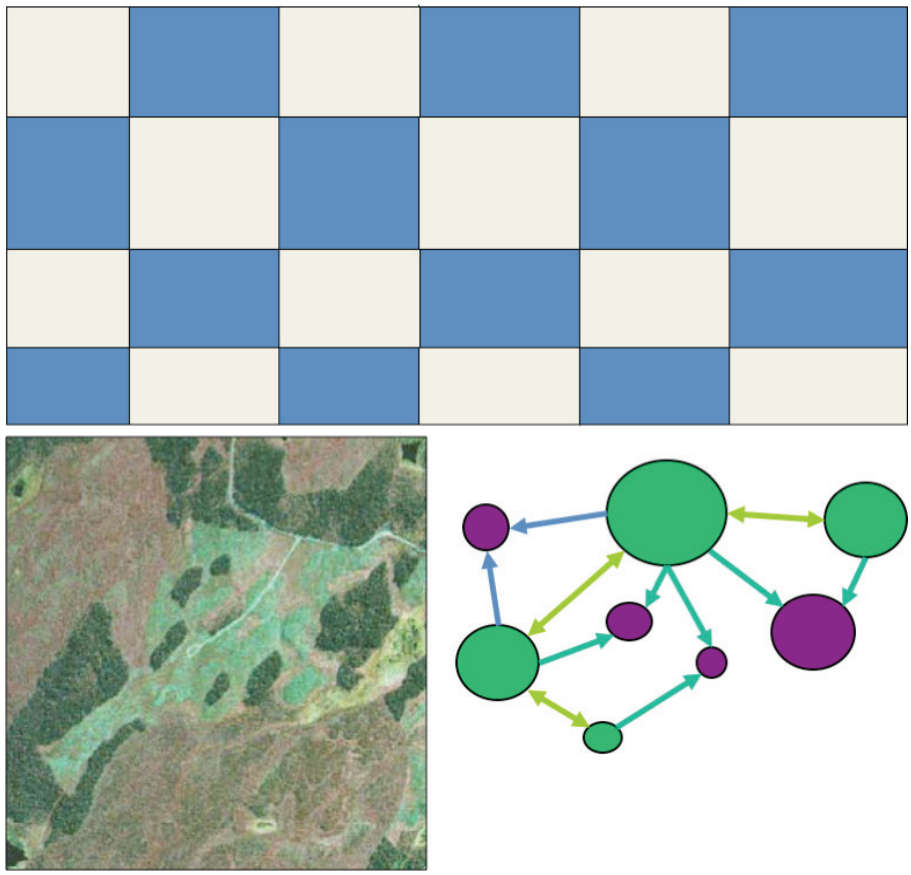


Figure 2.1. Sampling units in patch occupancy studies can consist of artificially created units such as grid cells of given size (top), habitat fragments (bottom left) or naturally-occurring discrete habitat patches that compose a metapopulation (bottom right).

Surveys (for the single season occupancy surveys; see below) are repeated over a short period of time such that occupancy status does not change during the sampling period, but the repeat visits should be far enough apart such that they are independent. For each sampling unit, results of occupancy surveys can be indicated by a string of 1's (indicating the species was detected) or 0's (indicating that the species was not detected). For example, a detection history of (1011) indicates that the species was detected at that sampling unit on sampling occasions 1, 3 and 4 but it was not detected on occasion 2. Detection histories can be similarly compiled for each sampling unit.

If a species is detected in a sampling unit during a particular survey, this conclusively proves that the species is present and there is no confusion. However, a non-detection can arise from two possible reasons. First, the species may have really not been there during the survey and therefore was not available to be detected. Alternatively, the species was present but was not detected for some

other reason (e.g., inability to survey the entire sampling unit thoroughly, elusive nature of the species). Because the probability of detecting a species when present is typically less than 1.0 this must be appropriately accounted for while estimating occupancy rate or proportion of sites occupied. Patch occupancy modeling approaches focus on estimating and modeling occupancy rates when the species is detected imperfectly (MacKenzie et al. 2002, MacKenzie et al. 2006).

We will begin with the so called "single season" occupancy surveys, where sampling units are surveyed repeatedly during a "season"; season is defined as a period during which the occupancy status does not change (i.e., the site is assumed closed to occupancy changes during the sampling period; hereafter closure assumption). Repeat visits are planned such that each visit is independent but that visits are close enough so as not to violate the closure assumption. When the survey is completed, each sampling unit *i* will have a detection history (e.g., h_i = 1011). Next, we ask: what is the probability of observing a detection history h_i? There are two stochastic processes that influence whether or not a species is detected at a site (MacKenzie et al. 2006). The site may be occupied by the focal species with probability ψ or unoccupied with probability (1 - ψ). If the site is occupied during a survey *j*, it may be detected with probability p_j or not detected with the probability (1- p_j). With this in mind, we can write an expression for the probability of observing a detection history. For example,

$$\Pr(h_i = 1011) = \psi\left[p_1(1-p_2)p_3p_4\right]$$

$$\Pr(h_i = 1001) = \psi_i\left[p_1\left(1-p_2\right)\left(1-p_3\right)p_4\right].$$

If the species is not detected at site *i* during the entire survey, we must consider two distinct possibilities. The first possibility is that the site was not occupied and thus, the species was not available to be detected. The second possibility is that the site was occupied but it was not detected. For example,

$$\Pr(h_i=0000) = \psi_k\prod_{j=1}^{4}\left(1-p_{kj}\right)+\left(1-\psi_k\right).$$

The first term of the above equation is the probability that the site was occupied by the focal species but it was not detected, and the second term is the probability that the site was not occupied during the survey period; the probability of observing the capture history consisting of 4 non-detections is the sum of these two pieces.

Once the probability of each detection history is compiled, the model likelihood given the capture histories is then the product of the probability of observing each detection history:

$$L(\psi, p \mid h_1, h_2, \ldots h_U) = \prod_{i=1}^{S} \Pr(h_i)$$

The model outlined above assumes that the probability of site occupancy and probability of detecting the species in a site are equal across all sites. This assumption is unlikely to hold in many situations because characteristics of the sites can influence occupancy probability as well detection probability (MacKenzie et al. 2006). Such heterogeneities in capture or occupancy probabilities can be modeled using site-specific covariates that can potentially influence the occupancy and detection probabilities. Using a logit-link function and site-specific covariates, ψ can be modeled as:

$$\psi_i = \frac{e^{\beta_0 + \beta_1 x_i + \beta_2 y_{ij}}}{1 + e^{\beta_0 + \beta_1 x_i + \beta_2 y_{ij}}},$$

where x_i's are site-specific (season-constant) covariates. Likewise, the probability of detection can be modeled as a function of site-specific season-constant and site-specific time-varying covariates (MacKenzie et al. 2006). Parameter estimation (using the maximum-likelihood method) and covariate modeling can be achieved using specialized software such as PRESENCE (http://www.mbr-pwrc.usgs.gov/software/presence.shtml) or MARK (http://warnercnr.colostate.edu/ ~gwhite/mark/mark.htm). Information theoretic approaches can be used for model selection and statistical inference (Burnham and Anderson 2002).

The single season occupancy model outlined above assumed that a site is closed for occupancy during the survey season. That is, each site is either occupied or not during the entire sampling season. This is a useful approach if the goal is simply to estimate the probability of occupancy or the proportion of sites/area occupied during a short period of time.

But frequently, the interest is to understand how occupancy changes over time, and to estimate probability of colonization (i.e., the probability that an unoccupied site becomes occupied during a time interval) and extinction (the probability that an occupied site becomes unoccupied), and factors influencing these probabilities. This can be achieved by multiple season patch occupancy surveys, whereby patch

occupancy probability is treated as a state variable, and occupancy dynamics is modeled over time (MacKenzie et al. 2006).

As in single season occupancy surveys, *s* sites are selected for survey. Surveys are conducted as in single season surveys except that we now consider two temporal scales (Figure 2.2) (MacKenzie et al. 2006). The occupancy status of the site can change (i.e., occupied sites can become unoccupied, and vice versa) among seasons (the larger temporal scale). Within season (labeled "Surveys" in Figure 2.2; smaller temporal scale), however, occupancy status is presumed closed. This sampling scheme is similar to Pollock's robust design, which was originally developed for mark-recapture studies (Pollock 1982). Within seasons, sites are surveyed for the presence of the focal species, and scored 1 if detected and 0 otherwise. From these data, a detection history can be constructed for each site that contains information on detection history h_i for each survey and season. For example, detection history of site *i*, h_i = 100 111 101 indicates that there were 3 seasons, and 3 surveys within each season; these detection histories are interpreted as in single season surveys.

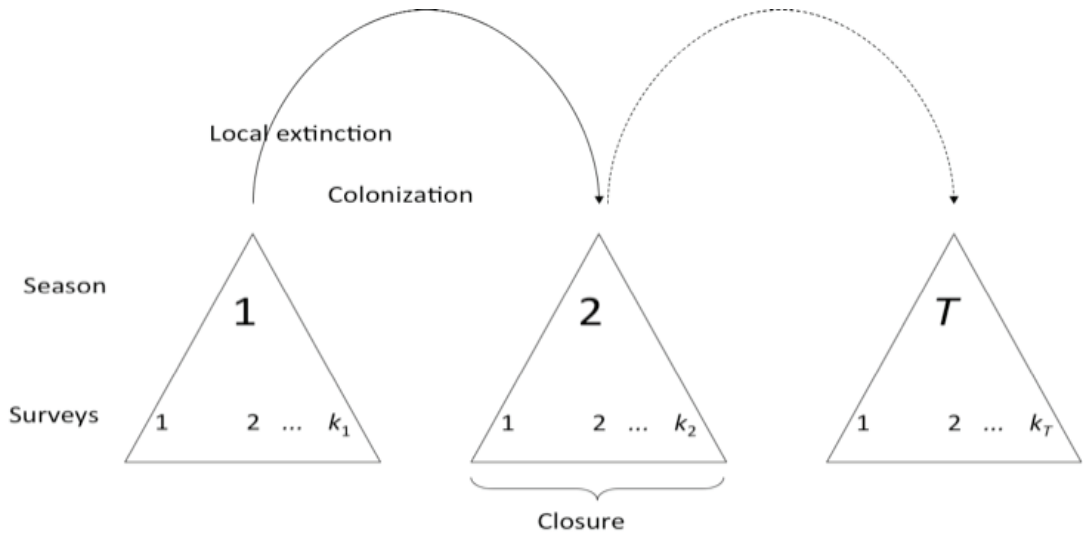


Figure 2.2. Pollock's robust design sampling scheme for multiple season patch occupancy surveys. Each triangle represents a season (1, 2, …, T) during which occupancy of sites does not change. Within each season, multiple samples (1, 2, 3, …, k_i) are taken. Sites are closed within a season but open among seasons to occupancy changes due to colonization of previously unoccupied sites or extinction of previously occupied sites (from MacKenzie et al. 2006).

Detection histories within each season, during which occupancy status is assumed not to change, provide information for estimation of occupancy and detection probabilities as discussed for the single season surveys. However, occupancy status can change among seasons such that occupied sites can be empty the next time

step due to extinction, or unoccupied sites may become occupied due to colonization of the empty sites (MacKenzie et al. 2006). An important feature of multiple season occupancy models that make these models valuable for monitoring purposes is that we can now estimate and model colonization (γ_t) and extinction (ε_t) probabilities, and rate of change in occupancy probabilities (λ_t) over time, defined as follows (MacKenzie et al. 2006):

$\lambda_t = \psi_{t+1} / \psi_t$ = rate of change in occupancy
ε_t = Pr(absence at time t+1 | presence at t) = patch extinction probability
γ_t = Pr(presence at t+1 | absence at t) = patch colonization probability

As for single season surveys, parameter estimation (using the maximum-likelihood method) and covariate modeling can be achieved using software such as PRESENCE or MARK.

Estimating Abundance: Conceptual Issues

Perhaps the most important question in wildlife management and conservation biology is: how many individuals are there? The answer to this question often dictates appropriate management actions. An estimate of abundance is often a prerequisite for conservation or management of wildlife populations. In fact, many monitoring programs are designed to track population abundance over time, and the state variable in such programs is usually abundance (population size or density). Methods of abundance estimation have been examined thoroughly by several authors, and we refer the reader to those sources for details (Seber 1982, Pollock et al. 1990b, Lancia et al. 1996, Nichols and Conroy 1996, Krebs 1999, Williams et al. 2001, Conroy and Carrol 2009, Mills 2013). Various issues relevant to using abundance as a state variable in a monitoring program are discussed by Ganey (2004). Here, we briefly review a conceptual framework for abundance estimation, and some abundance estimation methods that are commonly used for monitoring purposes.

All methods of abundance estimation depend on some kind of “count statistic”. The count statistic may be the number of tortoise burrows detected, birds heard, voles trapped, or tigers detected using camera traps, just to name a few. Counts are necessary but not sufficient for estimating abundance because there are two important issues that must be addressed before abundance can be estimated: *observability* (also known as detectability or detection probability) and *spatial sampling* (Williams et al. 2001).

Most sampling methods do not result in count (or capture) of all animals present in the study area, and counts typically represent an unknown fraction of the target population. This arises because the probability of observing or capturing an animal if it is present (β) is usually less than 1. Thus, we need information on β for estimating abundance. If we know β , we can write the relationship between a count statistic (*C*) and the "true" population size (*N*) as: E(*C*) = β **N*. If we have an estimate of β , we can estimate population size as (Williams et al. 2001):

$$\hat{N} = \frac{C}{\beta}$$

As an example, suppose we detect 20 tigers using remote cameras (Karanth and Nichols 1998, 2002) in a national park. Suppose further that the number of tigers detected represents 25% of the total tigers present in that study site. So, we have

$$C = 20,\ \beta = 0.25, \text{ and } \hat{N} = \frac{C}{\beta} = \frac{20}{0.25} = 80 \text{ tigers}$$

The *spatial sampling* issue refers to the fact that time and resource limitations typically preclude thorough sampling of the entire study site(s). Consequently, only a fraction α of the study site is typically sampled. Assuming β = 1 and that we have an estimate of α, population size in the entire study site can be estimated as:

$$\hat{N} = \frac{C}{\alpha}$$

If the study site represented 10% of a National Park and *C* = 20 tigers, the estimated number of tigers in the entire National Park is

$$\hat{N} = \frac{C}{\beta} = \frac{20}{0.10} = 200 \text{ tigers}$$

Finally, considering both observability and spatial sampling issues, estimates of population size is given by (Williams et al. 2001):

$$\hat{N} = \frac{C}{\beta * \alpha}$$

For example, if *C* = 20, β = 0.25 and α = 0.10, we have

$$\hat{N} = \frac{C}{\beta * \alpha} = \frac{20}{0.25 * 0.10} = 800 \text{ tigers}.$$

The approximate variance of the estimated population size is given by (details in Williams et al. 2001):

$$\mathrm{var}(\hat{N}) \approx \hat{N}^2 \left[\frac{\mathrm{var}(C)}{E(C)^2} + \frac{\mathrm{var}(\hat{\beta})}{\hat{\beta}^2} \right].$$

In the above examples, we pretended like we knew exact values of β and α. In rare cases, a complete census of a species of interest in the entire study site may be possible, especially when the site to be surveyed is reasonably small, the focal species is easy to detect, and if unlimited time and resources are available. In most cases, however, the entire study site can rarely be sampled thoroughly, and logistic difficulties and the elusive nature of many wildlife species make it impossible to detect 100% of the individuals that are present in the study area during a survey. Thus, values of β and α are almost never known. One must, therefore, rely on statistical models to *estimate* these parameters and associated variances. Virtually all abundance estimation methods focus on estimating β and/or α so that population size and associated variance can be estimated.

Estimating Abundance: A Survey of Commonly Used Methods

A thorough review of methods for estimating animal abundance are given by, among others, Lancia (1996), Krebs (1999), Conroy (2009), Pollock (1990a) and Williams (2001), and will not be repeated here. Briefly, abundance estimation methods can be placed into one of the following 3 broad categories (Figure 2.3; [Lancia et al. 1996, Williams et al. 2001]): (1) Capture-mark-recapture (CMR), (2) Distance sampling, and (3) Count-based methods.

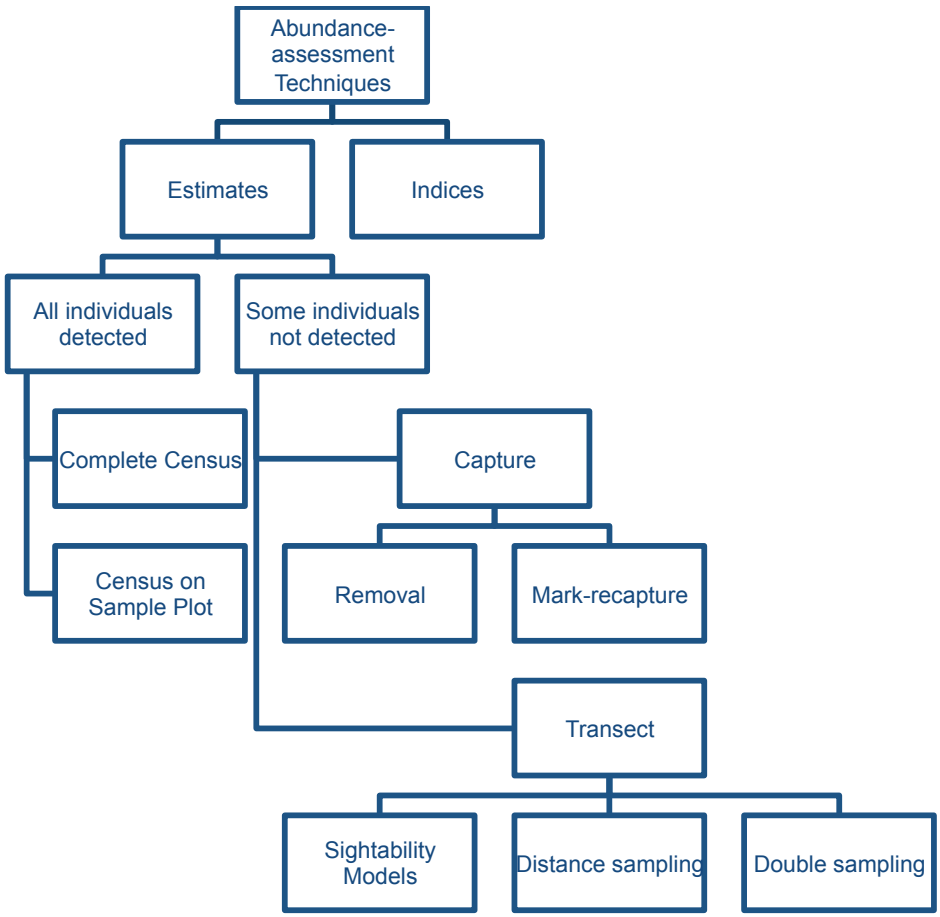


Figure 2.3. A schematic representation of methods for estimating abundance (modified from Mills 2013)

Capture-mark-recapture (CMR) methods

CMR methods are some of the most rigorous methods for estimating animal abundance. A sample of the study population is captured, marked, and returned to the population. This process is repeated on two or more occasions. Capture and marking methods vary widely, and typically depend on study species and objectives and availability of time and resources. Commonly used capture-recapture methods include leg snares/leg-hold traps (e.g., bears, snow leopards); drift-fence and pit/bucket traps (e.g., amphibians and reptiles); cage or box traps (e.g., leopards, small mammals, birds); mist nets (e.g., birds, bats); drive-nets (deer, wild goats, and sheep); nest boxes (e.g., wood ducks, house finches); net guns (e.g., deer, snail kite, turkeys); and various non-invasive methods such as hair snares (hair “capture”; black and brown bears); “visual” recapture using photographic records (e.g., manatees, whales) or camera traps (e.g. tigers, snow leopards); genetic capture using fecal samples (e.g., many species of mammals); and camera traps (e.g., tigers, snow leopards). Likewise, marking methods vary widely and may include radio

collars/transmitters (e.g., many species of birds and mammals); numbered or color-coded collars, ear-tags, or leg bands (many species of birds and mammals); fur dyes (e.g., marmots and other ground squirrels); passive infrared transponders (PIT tag; e.g., most vertebrates); web-tags (e.g., waterfowls); "natural" marks (e.g., scars on manatees, stripe patterns on tigers) (Chapter 4); DNA-tagging (hair, tissue or fecal samples; e.g., many species of mammals) (Chapter 3); and toe-clipping (e.g., small mammals, amphibians and reptiles).

Under certain assumptions population size and other relevant parameters can be estimated using CMR data. CMR methods for estimating abundance can be divided into two broad categories. *Closed population* CMR models assume that population size does not change during the sampling period (closure assumption; i.e., no birth, death, immigration or emigration). This assumption implies that all samples are taken within a short enough period of time such that the closure assumption is not violated.

The simplest closed population CMR model is the classic Lincoln-Peterson method, which requires a single episode of marking, and a single episode of recapturing individuals. A random sample of the population is captured, marked, and released back to the population. After a complete intermixing, a second random sample is taken. If assumptions of the models are approximated, then the population size can be estimated as

$$\hat{N} = \frac{M * C}{R},$$

where

M = number of animals marked and released in the 1st sample
N = population size (as yet unknown)
R = number of marked animals recaptured in the 2nd sample
C = total number of animals captured in the 2nd sample

The above estimator is biased, and tends to overestimate population size. An unbiased estimator is given by Seber (1982):

$$\widehat{N} = \frac{(M+1) * (C+1)}{R+1} - 1$$

Suppose 100 Sikkim rats were captured, marked, and released on the 1st sample. In the 2nd sample, 90 rats were captured, of which 50 were previously marked. So, we have M = 100, C = 90, and R = 50, and

$$\widehat{N} = \frac{(M+1)*(C+1)}{R+1} - 1$$

$$\frac{(100+1)*(90+1)}{50+1} - 1 = 179.2\ Sikkim\ rats$$

The Lincoln-Peterson method has been extended for >2 sampling occasions (Schnabel and Schumacher methods) and are described in detail elsewhere (Krebs 1999, Mills 2013). We note only that these methods do not require animals to be individually identified, and that these models assume that the capture probability is the same for all animals and is constant over time.

Perhaps the most commonly used closed population CMR models are those implemented in program CAPTURE (Rextad and Burnham 1991, Williams et al. 2001). The program CAPTURE models are flexible and powerful because they can handle heterogeneity in capture probability due to individual heterogeneity, temporal variability, and behavioral response of animals to being captured. One notable difference in the study design between the closed population models described above and program CAPTURE is that animals must be marked in a way that allows one to identify each individual such that a capture history can be constructed for each animal in the sample. Let 0 = not captured, and 1 = captured on a sampling occasion. Then, capture history of each animal is a string of zeros and ones.

Here is an example capture history of 4 animals over 5 sample periods (occasions):

Capture period (t)					
Animal	1	2	3	4	5
A1	1	1	0	1	0
A2	1	0	0	0	0
A3	1	0	0	0	0
A4	0	1	1	1	1

This capture history tells us that Animal 1 was first captured in periods 1, recaptured in period 2, not captured in 3, and captured again in period 4. Animals 2 and 3 were first captured in period 1 and never captured again. Finally, Animal 4 was first captured in period 2, and recaptured in all subsequent samples. Given these data, program CAPTURE or MARK (http://www.mbr-pwrc.usgs.gov/software.html) can be used to fit models that allow heterogeneity in capture probability over time, among animals and between animals captured for the first time vs. those captured subsequently. Program CAPTURE models are by far the most popular closed population CMR models, and have been used to estimate abundance of many species of animals.

Open population CMR models relax the assumption of closure and allow population size to change over time. In fact, most open population CMR models allow estimation of important demographic parameters such as survival and recruitment rates, in addition to estimates of population size. The most commonly used open population models for estimating abundance are Jolly-Seber models and its variations, notably, POPAN or superpopulation models (Schwartz and Arnason 1996, Krebs 1999, Williams et al. 2001). General methodology and data structures

are the same as those described for program CAPTURE models, except that a closure assumption is no longer required. Freely available software packages such as MARK and POPAN (http://www.mbr-pwrc.usgs.gov/software.html) can be used to fit models and estimate relevant parameters. Additional details regarding these models can be found in Williams (2001).

Distance sampling methods

A class of abundance estimation methods relies on incomplete counts of study organisms, along with distances between organisms and the observer (or between two organisms). These methods are also called "plotless" methods, because there is no "fixed" sample area where data are collected (Krebs 1999, Buckland et al. 2001, Williams et al. 2001). Among the distance-based methods, the line transect method is most popular among wildlife ecologists, and has been used to estimate abundance of a large number of animal species (Buckland et al. 2001, Karanth and Nichols 2002). Buckland et al. (2001) provide a thorough treatment of the line transect methods. Here, we review basic concepts and methods as they relate to estimation of animal abundance.

The line transect method involves setting up line transects (straight lines) in the study area and searching for the species of interest along the transects (Figure 2.4). The line transects are laid out following a random or stratified random sampling protocol (or some other appropriate sampling methods). If an animal is detected, the observer records either the straight line distance from the line to the animal, or sighting angle and sighting distance (from which perpendicular distance can be calculated). When the sampling is completed with n animals being detected, the dataset will consist of n perpendicular distance measurements from the transect line to the animal and relevant covariates associated with the transect location, time of sampling or the locations of the animal at the time of detection. If n animals are detected along a transect of length L and width w (Figure 2.4), and assuming 100% detectability (i.e., all animals within distance w along the transect line were detected), the estimate of density is (Buckland et al. 2001, Williams et al. 2001):

$$\hat{D} = \frac{n}{\text{Area}} = \frac{n}{2Lw}.$$

The challenge, however, is that only a fraction of the animals present in the study area during the time of sampling are detected; that is to say, detectability is almost never 100%.

The fundamental principle behind abundance estimation from line transect method is that the probability of detecting an animal decreases as the distance between the observer (i.e., the line) and the animal being detected increases (Buckland et al. 2001, Williams et al. 2001). While it is generally true that chances of detecting an animal decreases as the distance between the observer and the animal increases, how do we estimate the probability of detecting animals at different distances? The line transect method focuses on modeling the probability of detecting animals as a function of perpendicular distance (x_i). Let g (x) be the detection function such that $g(x)$ = Pr(animal being observed | x). The challenge is to find an appropriate functional form for $g(x)$. A histogram of sighting distance provides useful information regarding the functional form of $g(x)$ (Figure 2.5), but in practice, a series of mathematical functions are used to describe this relationship using software packages such as DISTANCE (http://www.ruwpa.st-and.ac.uk/distance/), and the one that most parsimoniously describes the relationship is used for estimating abundance.

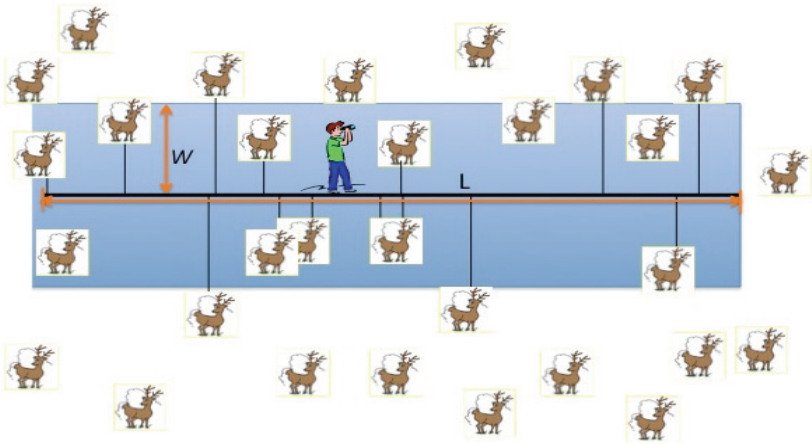


Figure 2.4. Schematic representation of the detection process in the line transect method (line transect with length L and width w). The observer walks a straight line transect detecting the species of interest. If an animal is detected, the observer records either the straight line distance from the animal to the transect line or the sighting angle and sighting distance from the observer to the animal. When the sampling is completed, the dataset will consist of perpendicular distance from the line to the animal (or sighting distance and sighting angle) for each animal detected and any covariates associated with the transect, time of sampling or the location of the animal when detected.

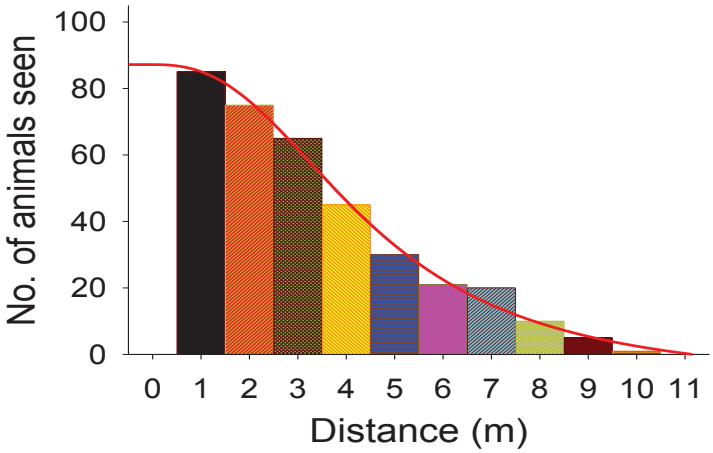


Figure 2.5. A hypothetical histogram of perpendicular distances. It is assumed that all animals directly on the line are detected with certainty, and the probability of detecting an animal decreases as the distance between the observer and the animal increases. The solid line is a hypothetical detection function relating the probability of detection as a function of perpendicular distance.

Once the functional form of $g(x)$ is found and relevant parameters are estimated, population density is estimated as:

$$\hat{D} = \frac{n\hat{f}(0)}{2L}, \text{ and}$$

$$f(0) = \frac{1}{\int_0^w g(x)\,dx}$$

where $f(0)$ is the probability that the observed animal is directly on the line

In practice, distance and covariate data are analyzed using software packages such as program DISTANCE. Program DISTANCE offers a powerful and flexible environment for estimating abundance using data collected from line transect studies (and other distance-based methods).

Count-based methods

If all animals present in the entire study site can be counted with certainty, one would not have to worry about statistical models to estimate abundance. Such counts may be occasionally possible for species that are easy to detect in a small area. If all animals within the entire study area are counted (β = 1, and α = 1), the resulting counts are referred to as *total counts*, or *censuses*. If the assumption of β

= 1 is violated, counts provide an estimate of the *minimum number alive* (MNA; a measure of relative abundance), rather than an estimate of absolute abundance.

A method that is closely related to total count is the method of *sample count*. The study area is divided into sampling units. A subset of sample plots is randomly selected and thoroughly searched to obtain a total count of the focal species within the selected sampling units. The estimate is then extrapolated for the entire study area. As an example, assume that 3 sampling units are randomly selected out of 9 equal-sized sampling units. A thorough search of the 3 sampling units resulted in a total count of 36 individuals. Then, the estimate of population size in all 9 sampling units is 36*3 = 108. Note that the sample count method assumes that detectability is 100% in surveyed sampling units.

A particularly useful count-based method is the double observer method (Nichols et al. 2000, Williams et al. 2001). Using this approach, two observers conduct surveys independently such that one observer does not know which animals the other observer counted. The observers obtain independent counts on the same sampling units using the same counting method. After the survey is completed, data are reviewed to determine which animals were detected by both observers, and which were missed by each of the two observers. Let:

x_{11} = number of animals (or any object) detected by both observers
x_{10} = number of animals detected by observer 1 but not by observer 2
x_{01} = number of animals detected by observer 2 but not by observer 1
p_i = detection probability for observer *i*
N = abundance (number of animals or burrows in sampled area)

Then, the estimate of detection probability is $\hat{p}_1 = x_{11} / (x_{11} + x_{01})$, and the estimate of population size is given by:

$$\hat{N} = \frac{x_{11} + x_{10}}{\hat{p}_1} = \frac{(x_{11} + x_{10}) * (x_{11} + x_{01})}{x_{11}}.$$

A variation (and a bit more complicated one at that) of this method is the so-called *dependent double observer method*, where there is a primary and a secondary observer. The primary observer communicates animals seen/heard to the secondary observer, so the secondary observer knows what the primary observer saw or heard. The secondary observer records animals detected by the primary observer, and additional animals he or she detects. Animals detected by the

secondary observer but not by the primary observer also are recorded. When the survey is completed, the data will consist of the number of animals detected by the primary observer, and the number of animals missed by the primary observer but detected by the secondary observer. Then, the observers switch roles; primary observer becomes secondary observer and vice versa. Let:

p = overall detection probability (assumed to be the same for both observers)
x_{11} = number of animals (or objects) detected by the first observer in the primary role
x_{21} = number of animals detected by the second observer in the primary role
x_{12} = number of animals detected by the first observer in the secondary role
x_{22} = number of animals detected by the second observer in the secondary role
$x.. = x_{11} + x_{12} + x_{21} + x_{22}$

Then, the overall detection probability is

$$\hat{p} = 1 - \frac{x_{12}x_{21}}{x_{22}x_{11}}, \text{ and}$$

$$\hat{N} = \frac{x..}{\hat{p}}$$

Program DOBOBSERV (http://www.mbr-pwrc.usgs.gov/software/dobserv.shtml) can be used to analyze data collected from double observer surveys (Nichols et al. 2000, Williams et al. 2001).

Index of Abundance or Relative Density

Estimating absolute abundance can be expensive and time consuming, and frequently impractical especially for species that are rare, elusive or those that occupy difficult terrain where capturing or observing animals can be challenging. For long-term population monitoring purposes, estimates of absolute abundance may in fact not be necessary if the goal is the long-time monitoring of population trends (rather than numbers per se). In some situations, population trends can be inferred using an index of abundance (or relative density) (e.g., Sauer et al. 2003, Sauer et al. 2005). An index of abundance is any quantity that is correlated with population size or density (Caughley 1977, Conroy 1996, Williams et al. 2001). One

hopes that the index of abundance contains information about the relative size or density of the population, and that changes in values of the index reflect changes in population abundance itself. An ideal situation would be when the index is linearly related to population size (Caughley 1977, Williams et al. 2001), but such an ideal situation rarely exists in real life, and frequently, the relationship between an index and abundance is unknown (Conroy 1996).

Count of animals is a commonly used abundance index. This can include the number of birds heard or seen along a North American Breeding Bird Survey route (Sauer et al. 2003, Sauer et al. 2005), the number of deer detected per kilometer along a road, or the number of pictures of tigers or snow leopards taken using a remote camera per week (Jackson et al. 2005). Another class of abundance index focuses on detecting animal signs (Jackson and Hunter 1996, Wemmer et al. 1996). Examples include counts of nests, dung piles, fecal pellets, tracks, scrapes, scent stations, or other measures of an animal's presence. Wemmer et al. (1996) thoroughly discuss various signs that can be used to monitor mammal populations. A third class of abundance indices that may be appropriate for harvested species are the measures of catch-per-unit effort (CPU); in Bhutan these may be most useful in aquatic sampling using standardized gear (e.g., cast netting, angling, electro-shocking).

In most studies using abundance index for population monitoring, changes in an abundance index is interpreted as reflective of changes in abundance itself. Furthermore, because the relationship between an index of abundance and population density is unknown, it is frequently assumed that they are linearly related to each other and that the slope defining those relationships is constant over time. If the abundance index is not related to population size or density, the relationship is non-linear, or if the relationship changes over time, inferences based on the abundance index can be misleading and can potentially lead to management blunders. Thus, despite their usefulness, one must be aware of the limitations inherent in using abundance indices for population monitoring purposes (Conroy 1996, Williams et al. 2001). For example, the number of pictures taken using camera traps is frequently used as an index of abundance for turkeys, a popular game bird in North America. A recent study found that this abundance index was not related to the population size of turkeys (Olson et al. 2012).

Estimating Population Trends and Persistence

Given a series of abundance estimates, or reliable indices, over time, what is the best way to characterize trend for the population? For a statistically reliable determination of whether the population is increasing, decreasing, or stationary,

we must estimate average trend, or growth rate, as well as its variance. A multitude of methods are available to estimate trend, incorporating all levels of biological complexity including density dependence, observer effects, age structure, and so on. However, the preponderance of trend analyses worldwide, especially in initial studies on species with no baseline data, tend to be based on simple exponential (density independent) evaluations of some sort of count data over time. Although we note in the previous section that we should strive to estimate abundance, instead of relying on indirect indices for count data, if the count index has a relatively constant relationship to actual abundance it may be appropriate to estimate trend. For simplicity, we will focus this section on estimating trend and its variance from count (abundance) data over time and assuming exponential models of population growth (see Mills 2013 and Humbert et al. 2009 for more details).

Terminology for understanding and estimating trends

Before describing how to estimate exponential trend, we must set the stage for some terminology to define both trend and its variance (Mills 2013). The abundance of a population (*N*) next year (at time *t*+1) is a function of both abundance now (N_t) and the population growth rate (λ) describing the proportional change in the population each time step:

$$N_{t+1} = N_t \lambda$$

Similarly, if we observe abundance changes over one time step we can rearrange the equation to determine population growth over one time step:

$$\lambda = \frac{N_{t+1}}{N_t}$$

Thus, λ (called **lambda**), otherwise known as the population multiplication rate or **geometric growth rate,** describes abundance next year as a proportion of the abundance this year. Note that even though we refer to this as the "population growth rate" it can describe a population that is growing, or declining, or remaining stationary; think of it as a "population change rate", where $\lambda = 1$ indicates stationary, $\lambda < 1$ indicates declining, and $\lambda > 1$ indicates increasing. λ is easy to work with because it easily converts to percentage change per year [% change = (λ-1)*100]. If $\lambda = 1.25$ the population will increase by 25% next year; if $\lambda = 0.75$ it will decrease by 25%.

Although λ is intuitive and easy to understand as a proportional change, the discrete growth represented by λ has some awkward mathematical properties, so

to really understand population growth requires the calculus-based continuous-time analog of λ, defined by r and interchangeably called the **exponential growth rate** or the **instantaneous per capita growth rate**. The two measures, λ and r, are interchangeable, after a simple conversion:

$$r = \ln \lambda \text{ or } \lambda = e^r$$

The *ln* is the **natural logarithm**, with the base e (which is about 2.718). A population with an $r > 0$ is increasing, and one with $r < 0$ is decreasing. As a simple rule, we typically use λ when we describe population growth to managers or the public because it is so easy to interpret, and we typically use r when we are doing mathematical calculations of population growth. Finally we note one more important symbol related to average trend: $\hat{\mu}$ (pronounced "mu") refers to an estimate of average trend, and is equivalent to average r ($= \bar{r}$) over time.

Of course, population growth (or change) is not constant; it changes over time. Sometimes growth rate changes from what we call **deterministic factors**. For example, we can think of fish population growth before versus after a dam, or macaques before versus after supplemental feeding has been ceased.

Often, however, the changes in growth rate are not deterministic but rather considered stochastic, arising from process variation, or process noise over space and time. Within a small population, process variance can arise from demographic stochasticity, the inevitable deviation in birth and death rates arising from the mean rates being probabilities across a population. For example, if the mean annual survival rate is 0.8, each animal can only live through a given year, or die. It cannot 0.8 live. For small populations, demographic stochasticity causes variation in population growth even when mean birth and death rates remain absolutely constant (demographic stochasticity disappears when the population is larger than about 100 individuals).

Process variance within a population can also arise from **environmental stochasticity** arising from extrinsic factors, often driven directly or indirectly by weather. For example, if a salamander requires a certain level of moisture to breed, then a particularly hot dry spring may lower the mean reproduction of adults and survival of juveniles across the population. For many wildlife populations, food supplies may affect average survival of young in a given year, as do random changes in predators or parasites. In all of these cases, mean vital rates for animals in the population—and therefore population growth rate—vary over time and

space in unpredictable ways. Unlike demographic stochasticity, environmental stochasticity does not decrease as population size increases.

And there is one final cause of variation in trends over time. Our perception of variation in a time series is also affected by **sample variance** in the abundance estimates. Because we almost never know abundance exactly, we must estimate it (as described previously this chapter), and the error in estimation (also called "**observation error**") causes variation in the estimate of $\hat{\mu}$. Although observation error is really just a "nuisance" form of variation—independent from real process variance that the animals are experiencing—it is critical to identify observation error because otherwise it might falsely lead us to overestimate actual variation in the trend.

Variation around the mean is used to construct a confidence interval for the estimated trend. Informally, the confidence interval provides a range in which we suspect the unknown true mean should be found. For example, a 95% confidence interval tells us that if we were to repeat our study many times, 95% of the time the true mean would fall within the confidence interval described. An estimate of trend should always be accompanied by a confidence interval because it lets us decide whether a population is really increasing or decreasing by more than we'd expect just by chance; if $\hat{\hat{\mu}}$ is positive (say, 0.01) or negative (say, -0.01) but the 95% confidence interval overlaps zero, we should be cautious about inferring that the population is, in fact, increasing or decreasing (because zero trend is a plausible possibility).

Estimating exponential trend and its variance

How should we actually estimate average trend for a wildlife population from a time series of count data? The most commonly used method is a simple linear regression of natural log *(ln)* of abundances against time (the natural log is used to account for the fact that birth and death processes cause wildlife populations to change geometrically, not arithmetically). The slope of the regression represents the average rate of change ($\hat{\hat{r}}$ or $\hat{\hat{\mu}}$). An increasing slope indicates an increasing population, while a decreasing slope indicates a decreasing population. Variance, standard error, and confidence intervals around $\hat{\hat{\mu}}$ are estimated using standard linear regression protocols. The simplicity of the method explains its popularity, but the method has a major limitation: it assumes that all variation in the trend arises only from the uncertainty in estimating *N* (i.e., pure observation error, or sample variance). That is, this method assumes that population growth is completely constant—not affected at all by weather, predators, or other environmental

conditions—and that all deviations in abundances from the trend line arise solely from the uncertainty of estimating abundances. For this reason, this model of exponential growth is referred to as "Exponential Growth with Observation Error" (EGOE), and the sample variance is symbolized by $\hat{\tau}^2$. Clearly, EGOE's assumption of zero process variation is problematic for nearly all wildlife populations, where environmental variation from year to year is a normal part of life.

A suite of other widely-used methods make the opposite assumption of EGOE, assuming instead that zero observation error exists and that all variation in the trend arises from process variance, or process noise. These are called Exponential Growth with Process Noise" (EGPN) estimators. For example, if the string of abundance values were collected each year (no missing years that create unequal time intervals between abundances), then you could simply calculate the mean and variance of the *q* consecutive values of *r*:

$$\hat{r} = \hat{\mu} = \frac{1}{q} \cdot \sum_{i=1}^{q} \ln\left(\frac{N_i}{N_{i-1}}\right)$$

$$\text{with variance } \hat{\sigma}^2 = \frac{1}{q-1} \sum_{i=1}^{q} (r_i - \bar{r})^2$$

Interestingly, this equation for $\hat{\mu}$ reduces to a really simple form, depending on only the first and last data points!

$$\hat{\mu} = \hat{r} = \frac{1}{\text{Total duration of survey}} * \ln\left(\frac{N_{last}}{N_{first}}\right)$$

This dependence of the $\bar{\mu}$ under EGPN on only the first two points has two important implications: First, it means you could really quickly estimate trend of even a long time series with this quick calculation. (However, you'd still need to use all the data to estimate the all-important variance needed to estimate the confidence interval; remember, confidence intervals are essential to help us know whether an apparent increase or decrease is actually likely to be different from zero). The second important implication is that a large sample size will not "fix" the estimate of trend if the first or last abundance estimates are poor. If you have concerns about the estimate for the first year (perhaps because the field crews

were just learning how to do the sampling) or the final year (perhaps because money was running out so effort was weak) then realize that the $\hat{\mu}$ will be affected.

The EGPN may also be estimated with what is known as the density-independent **diffusion approximation** method (we won't describe it here—see Dennis et al. 1991, Morris & Doak 2002 for details). This method may be used to estimate trend ($\hat{\mu}$), its process variance ($\hat{\sigma}^2$), and its confidence intervals even if abundance values are missing from the time series.

Although EGOE and EGPN methods are widely used in wildlife studies, recent developments allow us to go beyond their restrictive assumptions, and to allow for both process and observation error to occur simultaneously. These new methods are based on a "state space" statistical model, so the trend estimator is called "Exponential Growth State Space" (EGSS). The EGSS model extended to accommodate missing abundance estimates is not intuitive to explain, but in essence it contains a component to account for the stochastic fluctuations due to process noise and a component to accommodate the observation error in abundance estimates (Humbert et al. 2009)[1]. A program to run the EGSS in the R platform (adapted from Humbert et al. 2009) is available at UWICE in Bhutan or from L. S. Mills. The EGSS program will accept as input an excel file with abundances by year, and provide estimates for $\hat{\mu}$, $\hat{\sigma}^2$, and $\hat{\tau}^2$ under the EGSS (as well as EGOE and EGPN estimates).

For most situations, unless you are certain that your time series contains only observation error, or only process variance, you should use the EGSS to estimate exponential trend. The estimates from EGSS improve with time series length, and the method works well even with about half of the abundance estimates missing from the time series (Humbert et al. 2009). This suggests a somewhat radical notion for a monitoring program aiming for the best estimate of trend: consider skipping some years of sampling, if the money for those years can be spent in getting better estimates each year sampled, or in extending the length of the time series.

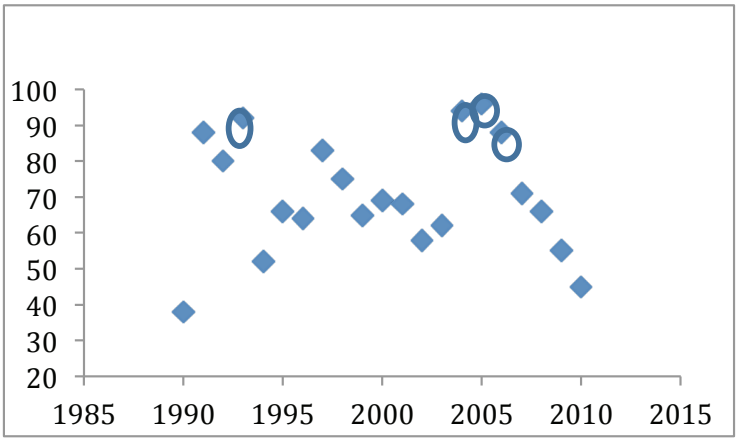
How long a time series is needed to estimate trends reliably? The answer depends on many factors, of course, but for the EGSS, a bare minimum is 10 years, with at least 5 samples of abundance during those 10 years (Humbert et al. 2009). Of course, unusual events that affect process variance (e.g., 20-year floods or 15-year fire events) will only be picked up with longer sampling.

[1] The EGSS can be estimated using either a "maximum-likelihood" (ML) or "restricted maximum-likelihood" (REML) approach. For technical reasons, you should always use the REML estimates.

Other approaches may also be used to estimate trends of wildlife populations over time. For example, Bayesian analyses (e.g., Taylor et al. 1996) are becoming more popular. Also, if mark-recapture data are available both λ and its variance can be calculated directly (see Nichols & Hines 2002 for a nice overview).

An Example of Estimating Exponential Trend

Let's go through an example of estimating trend from actual data (from Mills 2013). To represent the volatility typical of many time series as a result of both process and sampling error occurring simultaneously, we'll use a hypothetical salamander dataset (based loosely off a real time series for the salamander *Salamandra salamandra* in Schmidt et al. 2005). Figure 2.6 shows the complete dataset as a simple plot and table of the estimated abundance over time, and Table 2.1 shows the estimates of trend and variance for the dataset using the different exponential trend models. We analyzed both the complete dataset and also a more real-world situation where four of the counts were missing, as often happens due to funding breaks, logistics, weather, or other factors.



Year	N
1990	38
1991	88
1992	80
1993	92
1994	52
1995	66
1996	64
1997	83
1998	75
1999	65
2000	69
2001	68
2002	58
2003	62
2004	94
2005	96
2006	88
2007	71
2008	66
2009	55
2010	45

Figure 2.6. Time series data for a hypothetical salamander population, shown as a plot and as a Table so you can conduct the analysis yourself. All data were used in the "full dataset" analysis; for the "missing data" analysis the four years circled (in plot) and in gray (in table) were deleted as an example of estimating trend when real-world constraints lead to missed years of sampling (from Mills 2013).

Table 2.1. Analyses of the time series data in Figure 2.6. N/A means the parameter in the column can't be estimated with the method in the row.

Estimator	$\hat{\mu}$	$\hat{\sigma}^2$	$\hat{\tau}^2$	$Var(\hat{\mu})$	Lower 95%CI	Upper 95%CI
FULL DATASET						
EGOE	-0.00087	N/A	0.063	N/A	-0.020	0.018
EGPN – avg r	0.0085	0.082	N/A	N/A	-0.13	0.14
EGPN – DA	0.0085	0.082	N/A	N/A	-0.13	0.14
EGPN – 1st/last	0.0085	N/A	N/A	N/A	N/A	N/A
EGSS (REML)	0.0039	0.056	0.012	0.0029	-0.10	0.11
MISSING 4 YEARS						
EGOE	-0.0057	N/A	0.048	N/A	-0.024	0.012
EGPN – avg r	N/A	N/A	N/A	N/A	N/A	N/A
EGPN – DA	0.0085	0.072	N/A	N/A	-0.12	0.14
EGPN – 1st/last	0.0085	N/A	N/A	N/A	N/A	N/A
EGSS (REML)	-0.0053	0.0012	0.046	0.00014	-0.028	0.018

Here are some main points we'd like you to see in this example:

- Notice that the different estimators can give qualitatively different indications of trend, with at least one model indicating an increasing $\hat{\mu}$ and one a decreasing $\hat{\mu}$ for each of the sets of data. However, the 95% CIs overlap 0 for all estimates, which would lead us to infer in all cases that, regardless of whether $\hat{\mu}$ is positive or negative, we cannot exclude the possibility that the population is stationary, neither increasing nor decreasing.
- EGOE assumes the trend line is deterministic, with no process variance, so its variation (and confidence interval) is based entirely on observation (or sampling) error ($\hat{\tau}^2$). The confidence interval for this estimate says nothing about actual variation in the population size over time; rather, just variation in estimating the size of a population undergoing constant growth.
- EGPN can be estimated in three ways if the dataset is complete, but once missing values occur one method (averaging the string of r values) doesn't work because different r values encompass different time intervals.
- The fact that EGPN $\hat{\mu}$ can legitimately be estimated with just the first and last data point (see above) means, interestingly, that $\hat{\mu}$ never changes under EGPN no matter which points are removed—only the first and last matter! But as you see, the process variance and sample variance do depend on all the points— note that the $\hat{\sigma}^2$ and confidence intervals differ

for EGPN in the complete dataset and missing data scenarios. And remember, by definition the EGPN cannot accommodate any observation error in the abundance; all of the variation is assumed to arise from process variance ($\hat{\sigma}^2$).

- The EGSS gives both sample and process variance estimates. The relative amounts of sample versus process variance "perceived" by the EGSS model changes when we remove the four observations: for the complete dataset, process variance makes up 81% of the total variation (0.056/(0.056+0.012)*100). However, with four years of abundance removed, process variance appears to make up only about 2% of the total variation.

Conclusion

We have given a brief overview of the "what, how, where, and why" of wildlife monitoring. The state variables that are often monitored include occupancy (presence/absence), abundance, and trend over time. A key aspect of wildlife monitoring is the fact that animals present in an area can be hard to detect, especially in remote mountainous environments. This means that raw presence/absence data will underestimate presence (because sometimes the species is undetected) and raw count data will underestimate abundance (because some individuals in the population are not counted). Fortunately, the detection probability can be formally estimated using field data and some math. For species distribution studies, occupancy modeling can account for incomplete detection of species in an area to give a reliable estimate of the proportion of an area occupied, as well as turnover (extinctions and recolonizations). For individual detection probability and therefore abundance estimates, capture-mark-recapture and transect methods are commonly used. Once a series of abundance estimates over time are accumulated, trend can be estimated. The simplest trend estimators are based on exponential growth, and can account for various forms of variation, or uncertainty in the data.

With these approaches, we can conduct rigorous monitoring of wildlife in remote mountainous landscapes, guiding conservation and land management decisions.

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CHAPTER 3

Wildlife Genetics In Rugged Landscapes: Methods, Applications, and Examples

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Introduction

Over the last century, some of the greatest human achievements have been attained, unfortunately, often at the expense of Nature. We have tragically witnessed an ever-increasing number of extinctions, and many other species have rapidly declined (Vitousek 1997, Pimm et al. 2006). Despite these losses, there is also much to be hopeful for. In the past 50 years, dedicated conservationists have successfully recovered numerous species from the brink of extinction. Some of these, such as the American bison *(Bison bison)*, gray wolf *(Canis lupus*), and golden lion tamarin *(Leontopithecus rosalia)*, are now abundant in many areas (Kierulff and Rylands 2003, Freese et al. 2007, Wayne and Hedrick 2010). The paradigm of uninhibited progress has started to shift towards sustainable development. In addition, more tools and resources are available for conservation today than ever, making it possible to design realistic initiatives that enable preservation of our wild lands (Manel et al. 2003, Waits and Paetkau 2005, Mills 2007, Schwartz et al. 2007, Long et al. 2008, Frankham 2010).

It is important to have a balance between economic development and conservation, particularly in countries that are experiencing rapid growth, such as Bhutan, Nepal, and India (United Nations 2010). Knowledge is the foundation for such a balance. In order to guide development and effectively allocate conservation, we must first identify the species and populations most threatened by human activities, determine which factors are critical for their persistence, predict how our actions will influence them, design ways to mitigate any significant

negative effects, and monitor the success of our conservation efforts. That's a tall order. Only through carefully planned research initiatives can we hope to gain the knowledge we need.

Genetics has become a very important aspect of wildlife research and conservation. The genome is the blueprint for all life, and its interaction with the environment creates the remarkable biodiversity we see today. By exploring genomes, there are vast opportunities to understand the history of species, and in turn the evolution of life on Earth. Yet genetics is not only applicable to academic endeavors; there are also many practical applications. Because genetic variation is affected by population and ecological processes in a predictable manner, it is possible to understand life history and demographic parameters including population distribution and size, temporal fluctuations, connectivity, and social structure by interpreting patterns of genetic variation (see Table 3.1 for examples) (Kimura 1968, Schwartz et al. 2007, Hedrick 2011). Genetics has therefore become an important component of wildlife science.

Wildlife studies are particularly difficult because of the inherent limitations imposed on research. Much of the work must be done in the field by observing animals in natural environments. The elusive nature of many species, often combined with rugged topography or thick vegetation, can make direct observations next to impossible, particularly in places like the mountainous regions of Himalayas in Asia, Alps in Europe, Rockies in North America, and Andes in South America. Complementary to the other methods discussed in this book (e.g., sign surveys, telemetry, and camera-trapping), genetics provides an additional framework to get around these challenges (Waits and Paetkau 2005, Mills 2013, Long et al. 2008, Rodgers and Janecka 2012).

One of the most useful advantages of wildlife genetic studies is that individuals can be sampled noninvasively (i.e., without direct handling). As animals move through the landscape they often leave tracks and sign behind, along with biological material including hair, feathers, feces, and urine, that were traditionally incorporated into wildlife studies with a certain level of uncertainty. Fortunately, these biological materials include remnants of cells; therefore with genetics they can be unambiguously attributed to a species, and often to a specific individual (Waits and Paetkau 2005, Kelly et al. 2011). In this way, animals can be "directly" observed in their natural environment, without visual contact or capture. In contrast to visual detection by sight or via remote sensing cameras, a physical DNA sample is obtained to generate additional information on the population.

However, genetic information by itself is not very informative. The data generated on individuals and populations must be put into a biological context. The greatest benefit from genetics occurs when such studies are combined with more traditional methods including sign surveys, camera trapping, and telemetry. By using multiple complementary approaches we can better understand the status of species, how they relate to each other and their environment, and what factors may be influencing their persistence. Specifically, we can glean information on the biodiversity of an area, the distribution of species, the status of populations, landscape connectivity, and dispersal (Manel et al. 2003, Schwartz et al. 2007). More in-depth studies can even provide information on social structure and relatedness (Widdig et al. 2002, Jedrzejewksi et al. 2005, Janecka et al. 2006, Honer et al. 2007, Wagner et al. 2007). Every year, there are new developments in genetics along with a reduction in costs, facilitating such studies (Thomson et al. 2010).

As with all research approaches discussed in this book, the first and most critical step in a genetic study is to identify the specific questions to be addressed. The next step is to consider how genetics can be used effectively to answer these questions. Finally, the study design must be carefully considered so that the questions being asked can be realistically answered. Researchers have to decide how samples will be collected, if molecular markers are available for the species of interest, and how and where the genetic data will be generated. And finally, the entire project needs to be carefully reviewed in the context of the original objectives to confirm whether a genetic approach is indeed an effective allocation of resources.

This chapter is meant to provide a brief introduction to the world of wildlife genetics with a focus on noninvasive methods. We discuss where the information comes from, how it is generated, and what questions can be answered. Finally, we provide examples and additional information in appendices that can be used to develop a pilot population survey. We encourage the reader to explore other excellent books and review articles that focus on this topic (Waits and Paetkau 2005, Mills 2013, Long et al. 2008, Hedrick 2011). We hope that this chapter serves as a primer to generate additional interest for wildlife genetics in Asia, and other mountainous regions of the world.

Overview of Genetics: from DNA to Molecular Markers

DNA structure

All organisms, with the exception of some viruses, use DNA (deoxyribonucleic acid) as their genetic material. DNA has two essential roles: coding for and maintaining

the cell components via proteins and regulatory RNA molecules, and passing on the information to offspring.

A DNA strand is a relatively simple molecule composed of subunits called nucleotides. There are four types of nucleotides, each consisting of a phosphate molecule attached to one of 4 different bases: adenine (A), guanine (G), cytosine (C), and thymine (T). Within cells, these nucleotides are attached together in long chains, to form two strands that spiral round each other creating a double helix. The backbone of this helix consists of the phosphate molecules. The bases connect the opposing strands via hydrogen bonds (similar to the rungs of a ladder) in a specific pattern, with A only pairing to T, and C to G. Contiguous DNA strands are formed by millions of nucleotides that are usually arranged in structures called chromosomes.

Three fundamental DNA-related processes occur in cells: DNA replication, transcription of DNA into RNA, and translation of RNA into proteins. Replication is essential for passing on genetic material to other cells and to offspring, and transcription/translation for cellular development and processes. DNA replication occurs in the nucleus and mitochondria, and starts with the separation of the two strands (denaturation), which afterwards serve as templates that are also copied. With this process, the genetic information is maintained in each new cell.

The DNA in the nucleus is organized into chromosomes, which contain genes, noncoding regions, and structural elements. Genes were traditionally defined as segments of DNA that coded for proteins; however, this has been extended to include other regulatory molecules such as microRNAs. Genes ultimately determine how, when, and where proteins are made. Proteins are polymers of amino acids, the type and order of which define their form and function. These proteins are determined by the nucleotide sequence in the exons, which are composed of sets of three nucleotide bases (called a codon) that code for a specific amino acid. Proteins are synthesized during the process of transcription of DNA into RNA, followed by translation of the RNA into the respective amino acids that compose them.

Most genes consist of sequences of nucleotides that contain alternating sections of coding and non-coding regions (exons and introns, respectively). All cellular processes and structures are mediated, either directly or indirectly, through proteins and RNA molecules. However, the great majority of DNA within a genome does not code for proteins or regulatory molecules, and to date much of it has no known function. Only about 2% of genomes contain instructions for the synthesis of

proteins and other regulatory molecules. Genes are positioned in the chromosomes at specific physical locations called loci (locus in singular).

During DNA replication, transcription, and translation, occasional errors produce permanent changes in DNA or RNA sequences, these are known as mutations. If a mutation occurs during DNA replication in a gamete (a reproductive cell that fuses to form a zygote, i.e., egg and sperm) it is passed on to the offspring. There are several types of mutations including substitutions (called single nucleotide polymorphisms, SNPs) and insertion/deletion of nucleotides (called indels). Mutations accumulate over time leading to the variation (also called polymorphism) in genomes we see today (Kumar 2005, Schwartz et al. 2007). Mutations can also occur on larger scales, with entire sections of chromosomes being duplicated, deleted, or translocated. Wildlife genetics typically examines SNPs or indels. A genetic variant arising from a mutation in a specific position on a chromosome (referred to as a locus), is called an allele. Loci that have variation (i.e., more than one allele) can be used as molecular markers for assessing species diversity, evolution, population structure, individual identification, and relatedness (Avise 2004).

Mutations can occur in either genomic regions that influence gene function or in areas that do not, and therefore are considered neutral. Mutations in functional regions of proteins can change the amino acid sequence or expression patterns. If these alterations are dramatic and negatively affect biological processes, they can be lethal and/or cause diseases. In rare cases, they can be beneficial to an individual. The majority of mutations are neutral and do not affect fitness (Kimura 1968). Distribution and frequency of neutral alleles (the different variants) within and among individuals is largely determined by population parameters and processes such as the number of individuals, population size and fluctuations, selection on nearby loci, and migration (Avise 2004, Hedrick 2011). Therefore in wildlife genetics neutral variation often is used to understand populations.

Assaying genetic variation

Genetic information is inherited in four different ways depending on where in the genome it is located. For mammals, the genes on the Y-chromosome (~58 million nucleotides in humans) are passed only from the father to the male offspring. Those on the X chromosome (~160 million nucleotides in humans) are passed through both males and females, although males have only one copy. The majority of all other genetic material is located on autosomes (non-sex chromosomes, ~3 billion nucleotides in humans) and has bi-parental transmission (i.e., both maternally and paternally). Finally, mitochondria have their own genetic material

(mtDNA) organized in a small circular genome (~16,000 nucleotides in humans) only inherited through females. Because of differences in the size, content, structure, and modes of inheritance between the X, Y, autosomes, and mtDNA, their molecular markers have different applications.

Making sense of all the variation present in a ~3 billion nucleotide genome is very challenging. Until the latter part of the 20th century there were many technological limitations for applying genetics to wildlife. A revolution occurred after the invention of the Polymerase Chain Reaction, or PCR, and the use of a thermostable DNA polymerase enzyme enabling direct amplification of specific molecular markers (Saiki et al. 1988). The DNA polymerase enzyme used in the PCR was first discovered in a bacterium *Thermophilus aquaticus* in the hot springs of Yellowstone National Park (USA) (Antunes et al. 2008). All such enzymes are generically referred to as *Taq* (Chien et al. 1976). A PCR yields millions of copies of a targeted segment of DNA, achieving sufficient quantities for downstream analyses such as visualization on an agarose gel, sequencing, and genotyping (see Box 3.1).

BOX 3.1. Overview of the Polymerase Chain Reaction

In addition to the *Taq* polymerase, a PCR requires two short DNA sequences (called **primers**) with a nucleotide sequence complementary to the beginning and end of the targeted DNA segment (the molecular marker). To perform a PCR, the DNA from an organism (referred to as template) is added to a tube that contains primers, nucleotides (building blocks of DNA), *taq* polymerase, $MgCl_2$, and other salts and additives. The mixture is then placed in a thermocycling machine that can be programmed to increase and decrease the temperature of the samples.

First, the mixture is heated to separate the double-stranded DNA template into single strands (denaturation). Then it is cooled to allow the primers to bind to the DNA template (annealing). After annealing, the polymerase begins to synthesize new strands of DNA starting from the primers (extension). At the end of the first cycle, each double-stranded DNA molecule consists of one new and one old DNA strand. The new copies are used as templates in subsequent cycles. There is an exponential increase in the targeted DNA segment; after 20-25 cycles yielding millions of new copies of the particular molecular marker.

Molecular markers

One of the first steps in undertaking a genetic study is identifying the appropriate molecular markers for the population of interest. Molecular markers fall into two broad categories: short tandem repeats (STRs) and single copy gene segments. Short tandem repeats are also called microsatellites, and consist of repeats of a DNA sequence motif (typically 2–4 bases) that are surrounded by conserved flanking regions (Jarne and Lagoda 1996, Goldstein and Pollock 1997, Ellegren 2004). For instance, PUN100 microsatellite consists of 18–23 repeat units of "AC" in snow leopards (although additional alleles of different length may be found in unstudied populations) (Janecka et al. 2008b).

The number of repeats in a microsatellite is variable because DNA polymerase sometimes slips during replication of repetitive sequences, adding one more or one less repeat unit (Ellegren 2004). This type of mutation is more common than a substitution or indel in non-repetitive DNA segments (Jarne and Lagoda 1996). Microsatellites therefore tend to be more polymorphic than regions with unique sequence. Alleles in microsatellites are defined by the number of repeats, which can be inferred from the size of the PCR amplicons. It is important to note that the size differences for most microsatellites are too small to resolve on an agarose gel and must be differentiated with a sequencing instrument (e.g., Applied Biosystems 3730xl DNA Analyzer).

If enough variable microsatellites are genotyped (i.e., the alleles present at each locus are identified) each individual in a population will have a different combination of alleles (Waits et al. 2001). Therefore, the composite genotype (i.e., the combined genotypes of multiple microsatellites) can be used as a DNA "tag" to identify individuals (Figure 3.1) (Taberlet et al. 1997, Kohn et al. 1999, Waits et al. 2001). This approach has been used in many wildlife studies (Tallmon et al. 2002, Tallmon et al. 2004, Bhagavatula and Singh 2006, Schwartz 2009, Schwartz et al. 2009, Janecka et al. 2011a). We can also use the allele frequencies in the population to describe variation, structure, connectivity, hybridization, and to estimate parameters such as effective population size (Jarne and Lagoda 1996, Waits et al. 2001, Janecka et al. 2008c, Oliveira et al. 2008, Oliveira et al. 2010, Janecka et al. 2011b). Due to their high levels of variation and abundance in the genome microsatellites are currently among the most widely used class of molecular markers in wildlife research.

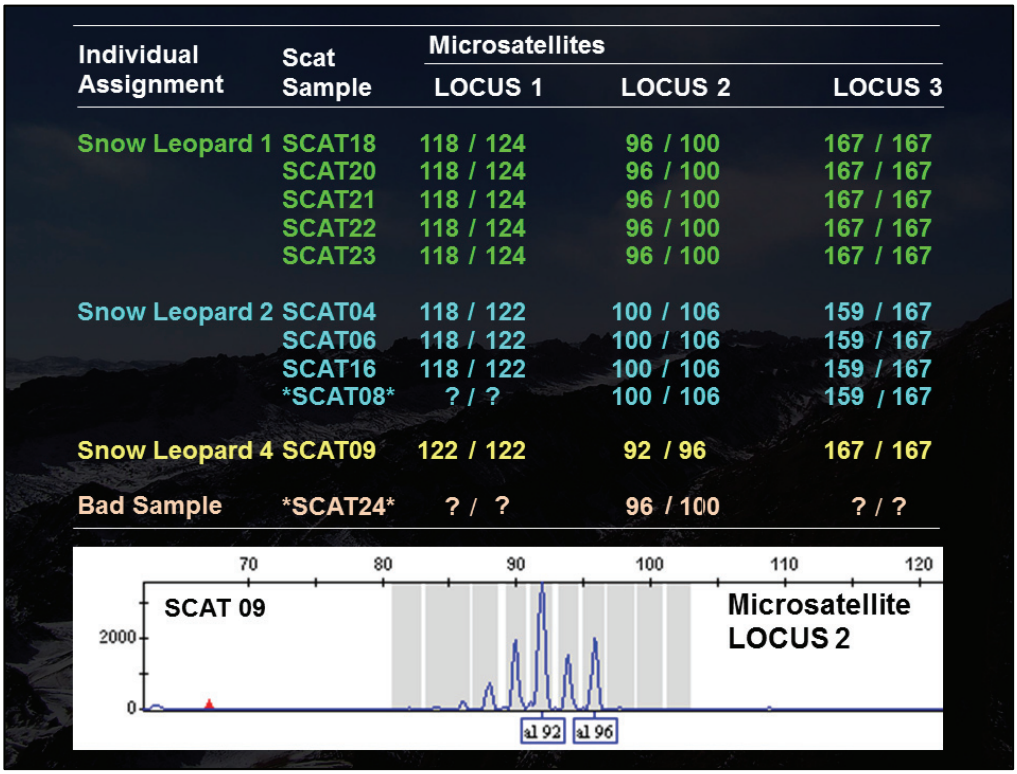

Figure 3.1. An example of the rationale behind individual identification of unknown scat samples using microsatellites. Allele sizes are obtained by analyzing electrophoretograms of PCR amplicons from a sequencer (e.g., ABI 3730). The inset shows an image of the genotype for microsatellite Locus 2 of Scat 09; note that the individual is heterozygous for 92 and 96 base pair alleles. Scats that have the same genotype at all loci (i.e., have the same genetic "tag") are grouped together and assigned to the same individual. For example, the "tag" 118/124-96/100-167/167 was assigned to Snow Leopard 1. Some scat samples will have missing data (e.g., Scat 08 and Scat 24) and these should be potentially removed from the dataset or re-analyzed.

The second broad class of molecular markers consists of single copy genes. Informative SNPs can be identified by DNA sequencing (Sambrook and Russell 2001, Avise 2004). For example, there are numerous SNPs in specific positions of the cytochrome b mtDNA that differentiate the snow leopard and common leopard, and other sympatric species (Figure 3.2) (Janecka et al. 2008b). If these differences are fixed between the species, they can be used for species identification. DNA sequencing of many gene segments across many animals can generate SNP information that can be analyzed to understand population structure and processes (Manel et al. 2003, Avise 2004, Kohn et al. 2006). This approach can be extended to understand the evolutionary history of species and their phylogenetic relationships (Kohn et al. 2006, Janecka et al. 2007, Janecka et al. 2008a, Davis et al. 2010, Eizirik et al. 2010).

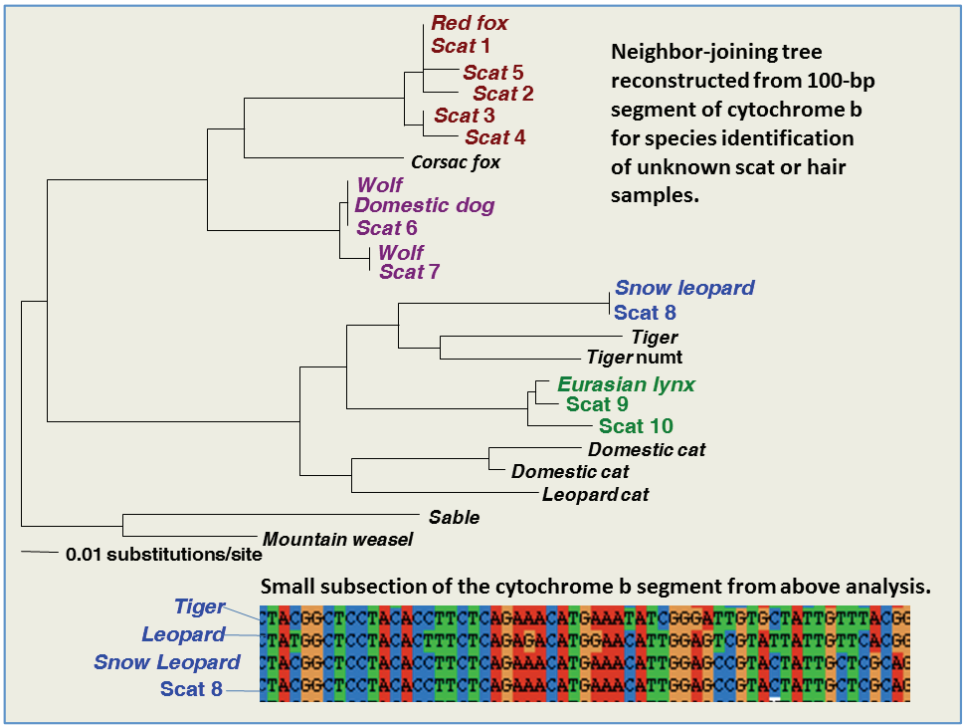

Figure 3.2. Phylogenetic approach to species identification. A small section of cytochrome b or another gene segment can be sequenced, aligned, and a tree reconstructed. Sequences from unknown scat that closely group with reference sequences and show >97% similarity can be assigned to a species. However, results must be interpreted with caution particularly in areas such as Bhutan, Nepal, and Tibet, where many species occur for which there is currently no reference sequence. Data from Janečka et al. 2008b.

Getting DNA – From the Field to the Laboratory

Sampling wildlife populations

Before embarking on a wildlife genetics study one must critically assess what kind of information is sought. The goals of the project need to be carefully identified and clearly outlined. One must also contemplate the predicted end point, and consider whether the outcomes will contribute to conservation and management. As with all endeavors “Begin with the end in mind” (Covey 1989). Never is this more applicable than in wildlife research. Think about the potential results from a successful outcome. If they would make substantial contributions towards your conservation or management goals, then proceed to develop a strategy for executing the project.

There are many kinds of concepts that can be addressed using genetics. These include intra-population (e.g., status, distribution, abundance, and trends), inter-populations (e.g., dispersal, gene flow), ecological (e.g., habitat use, predator-prey interactions), and evolutionary (e.g., describing new species, relationship between

species, adaptation to environments) (Table 3.1). Are you trying to map the distribution of a species in an area? Will you examine its taxonomic status? Do you want to get abundance estimates? Are you focusing on specific populations, or do you want regional information? What kind of sampling will be most effective for the species? Answering these questions prior to your study is critical because it will dictate not only the type and duration of sampling, but also the molecular markers and analyses that you will use (Figure 3.3). In some cases, you may decide that more traditional methods (e.g., mark-recapture, telemetry) may be more appropriate.

Table 3.1. Examples of studies that illustrate the application of molecular markers to understanding wildlife populations.

Purpose	**Species**	**Sampling**	**Molecular Marker**	**Reference**
Pilot study	Snow leopard	scat	cytochrome b, microsatellites, AMELY	Janečka et al. 2008b
Diet	Snow leopard	scat	cytochrome b, microsatellites	Anwar et al. 2011
Occupancy	Fisher	scat	16s, microsatellites	Zielinski et al. 2006
Abundance	Snow leopard	scat	cytochrome b, microsatellites	Janečka et al. 2011a
Population trends	Wolf	scat	microsatellites	Marrucco et al. 2009
Connectivity	Lynx	tissue	microsatellites	Schwartz et al. 2002
Effective population size	Ocelot	blood	microsatellites	Janečka et al. 2008a
Species delineation	Colugos	hide	multiple nuclear and mtDNA gene segments	Janečka et al. 2008c
Species identification	Carnivores	various	nuclear SNPs	Oliveira et al. 2010
Hybridization	Wildcat	tissue	microsatellites	Oliveira et al. 2008
Relationship among species	Felids	blood	multiple nuclear, Y, X, and mtDNA gene segments	Davis et al. 2010

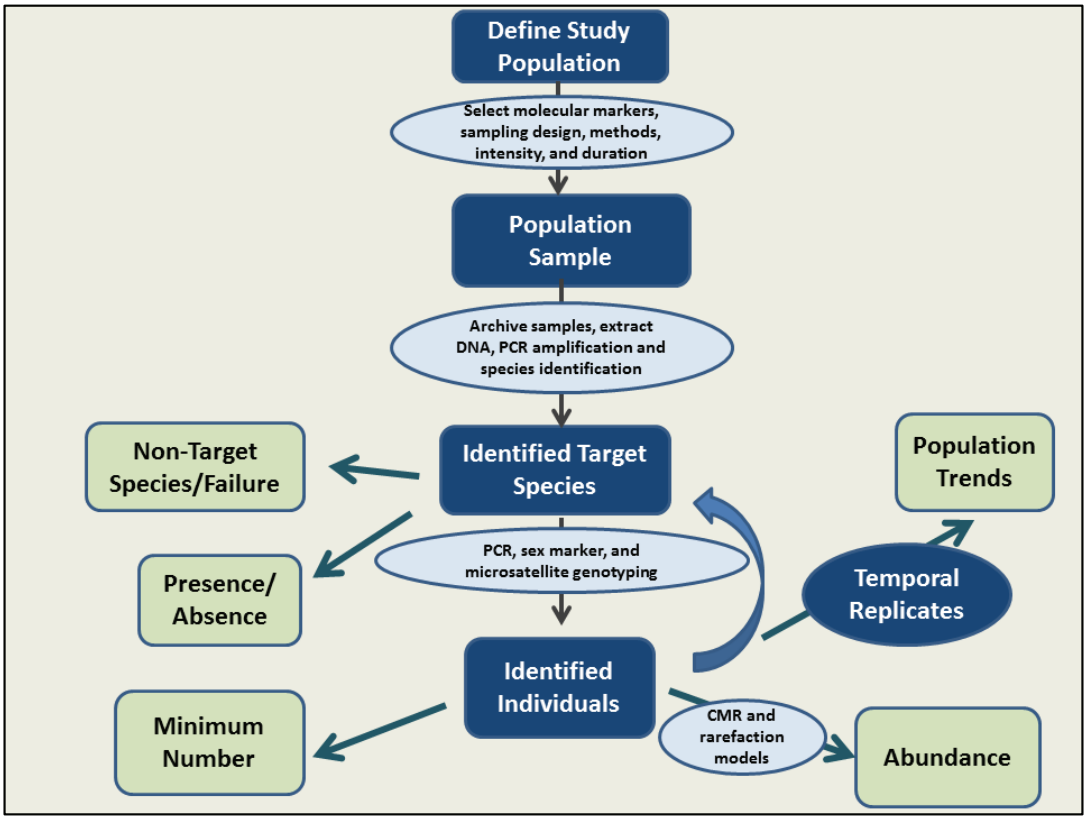

Figure 3.3. Flow chart depicting steps involved in wildlife genetic surveys that focus on estimating population parameters.

If genetic methods are indeed appropriate for achieving the goals of your project, the next important step is to assess whether the necessary molecular resources are available for your targeted species (Figure 3.3). The best way to do this is to search the literature for closely related species that have already been studied, ideally within the same genus, although many molecular markers will work across an entire family. For example, microsatellites developed in the domestic cat can be applied to other felids, such as snow leopards, tigers, and leopards (Menotti-Raymond et al. 2003, Bhagavatula and Singh 2006, Murphy et al. 2007, Janecka et al. 2008b). If markers are not available they can be designed. However, this requires additional time and expenses, along with close collaboration of a reputable genetic laboratory (Glenn and Schable 2005). At this stage, a laboratory where the genetic analysis will be completed should be identified.

The next critical aspect that will strongly influence the success of a project is the design of an appropriate survey scheme (see Appendix 3.1 for an example) (Long and Zielinski 2008, Schwartz and McKelvey 2009). In order to determine how many samples to collect from an area, and how they will be collected, one must again go

back to the goals of the project as they will influence the study design to be implemented. For example, different population state variables described in Chapter 2 (e.g., distribution, abundance, and trend) require different sampling protocols.

In most cases, the study area will be divided into a grid (Krebs 1998). This is then used to delineate sampling blocks. The blocks need to be of the appropriate size; it is common practice to make them roughly the same size as the mean home range of the target species (Long et al. 2008). Each block should be sampled if it is logistically feasible. However, if resources are limited, then the blocks can be selected based on a stratified sampling design (Quinn and Keough 2002). This means that they are randomly sampled in proportion to the available habitat in that area. Within each block, sites are chosen where the likelihood of detecting the target species is highest (Figure 3.4). The blocks sampled and the specific locations of transects will be largely influenced by physical access and the resources available for the survey, particularly in rugged, mountainous areas. The intensity of sampling is a very important variable to consider. It depends on capture probability, the objective (e.g., occupancy versus abundance), and the method used to estimate the parameters (MacKenzie et al. 2002, Wintle et al. 2004).

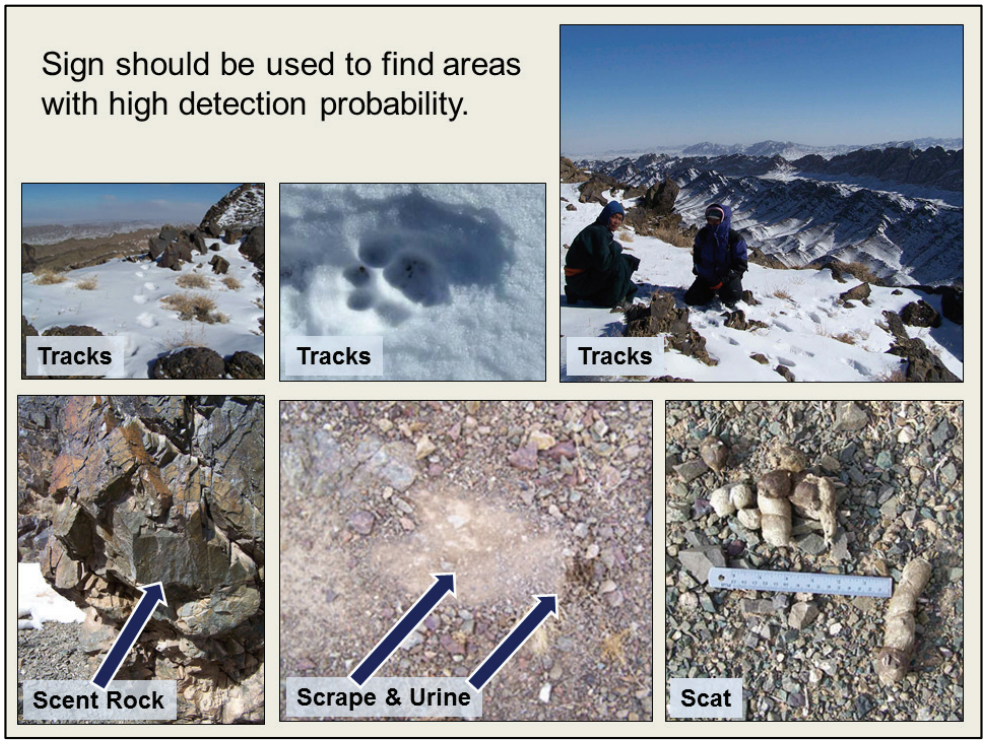

Figure 3.4. Examples of the type of sign used to identify sites that have high snow leopard activity within a sampling block. This information should be used to increase the detection probability during a noninvasive genetic survey. Photo credits J. Janečka.

In many regions of Asia, there is little information on the home range size and capture probability of species, making it difficult to design the most effective sampling strategies. In these cases, one must base the first survey on studies carried out in similar ecosystems and/or on closely related species, and also incorporate local knowledge. An initial population survey should be done, and subsequently the block sizes and sampling techniques adjusted based on the preliminary data. Even for well-studied species, survey strategies should be constantly reassessed and optimized to produce ever more reliable data (Boulanger et al. 2006).

The two primary methods for noninvasive genetic sampling of individuals include collection of scat and snagging of hairs (Waits and Paetkau 2005, Beja-Pereira et al. 2009, Kelly et al. 2011). Scat can be collected on wildlife trails and travel corridors for species including wolves, coyotes, bears, otters, elephants, bobcats, tigers, and snow leopards (Kohn et al. 1999, Eggert et al. 2003, Janecka et al. 2008b, Hajkova et al. 2009, Marucco et al. 2009, Mondol et al. 2009, Ruell et al. 2009). For some animals, such as snow leopards, which occur in dry, cold regions and leave scat in highly visible sites, it is an excellent method (Janecka et al. 2008b, Janecka et al. 2011a). Snow leopards and other carnivores often leave multiple scats at one site on distinct landscape features, such as saddles and outcrops, increasing the efficiency of sampling (Figure 3.5). Please refer to Appendices 3.1–3.5 for examples of scat survey methods, sampling techniques, and data collection sheets. Some species occupy habitats with dense vegetation, dung beetles, or hot, humid climates, and leave scat in sites with poor visibility, making it difficult to collect sufficient numbers of quality scat samples, therefore other techniques may be warranted.

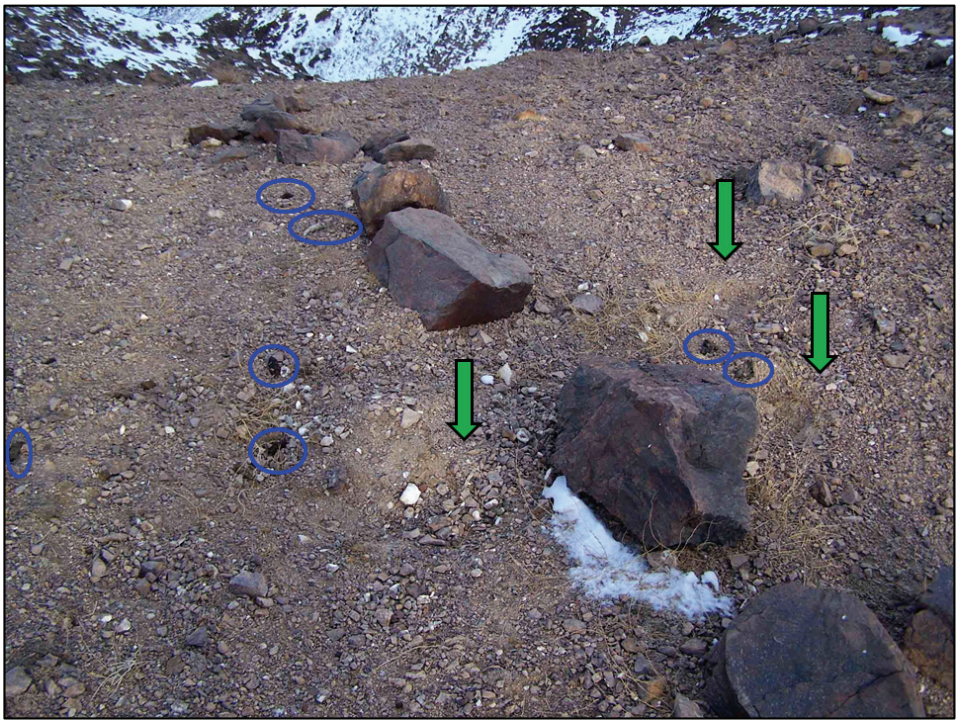

Figure 3.5. A saddle in the Gobi Desert of Mongolia that was on a sampled snow leopard scat transect. There were a total of 7 scats (blue circles) and 3 scrapes (green arrows) observed on the saddle. Sites like this are important to focus on during a survey in order to obtain sufficient numbers of noninvasive genetic samples. Photo credit J. Janečka.

In some cases hair collection may be the preferred means of sampling populations. One way that hair can be collected in areas with snow cover is to backtrack on fresh pugmarks, carefully searching for sites where the animal bedded down or rubbed against rocks or vegetation. Hair snares are an alternate option for some species. These "traps" are often either constructed out of barbed wire surrounding a container of bait, or carpet pads with roofing nails attached to a tree marked with scent lures that elicit rubbing behavior. Effective hair traps have been designed for lynx, bears, foxes, martens, fishers, and numerous other species (McDaniel et al. 2000, Poole et al. 2001, Bremner-Harrison et al. 2006, Schmidt and Kowalczyk 2006, Zielinski et al. 2006, Downey et al. 2007, Pauli et al. 2008).

When considering optimal sampling strategies one must bear in mind that there are species, population, behavioral, and site differences that will impact their effectiveness (Gompper et al. 2006, Long et al. 2007, Ruell and Crooks 2007, Ebert et al. 2010, Ralls et al. 2010). If no work has been done on your target species in habitat similar to where you are surveying, then sampling methods must be carefully tested before resources are allocated to a large-scale survey. Focus

transects on sites where there is a relatively high probability of detecting the target species. These include wildlife trails that are at the intersection of travel routes and landscape features, and have a high number of sign on them (i.e., tracks, scent rocks, claw rakes and scats, Figures 3.4 & 3.5).

Noninvasive samples (e.g., hair and scat) often have low quality and quantity DNA, making them very susceptible to contamination and genotyping errors (Taberlet et al. 1999, Waits et al. 2001, McKelvey and Schwartz 2004b, a, Pompano et al. 2005, Waits and Paetkau 2005, Rodgers and Janecka 2012). To minimize downstream problems it is essential that samples in the field are collected in a clean manner and properly stored. Various preservation methods (e.g., silica desiccant, ethanol, freezing) have been used with varying success; no technique has been found to be greatly superior (Frantzen et al. 1998, Murphy et al. 2000, Bubb et al. 2010). Often, the most practical method for the field is to store samples dry on silica desiccant (Appendices 3.2 & 3.3). Regardless of the technique used, the key is to minimize handling of samples and to maintain them in a stable environment. It is also critical to record information associated with each sample at the time of collection, including geographic location (GPS preferred), approximate age, size, nearby animal sign, vegetation types, landscape features, and any noteworthy observations (Appendices 3.4 & 3.5).

Population studies often produce large numbers of noninvasive samples (100s to 1,000s) and genetic data. Excellent organization is one of the most important means of preventing data errors, lost samples, and missing information. As soon as samples are collected, they need to be shipped to a central location. A specific person should be assigned to log samples into a collection archive and enter information into a database to prevent samples and information from being lost. Many field projects and laboratories have a frequent turnover in technicians; therefore it is critical that the principle investigator makes certain that all samples and data are being properly stored in a secure area.

Obtaining DNA from samples

Once the samples are archived in a laboratory the first step in genetic analysis is the extraction of DNA free of impurities and any contaminants (from other samples or even field personnel) (Figure 3.6). A good quality DNA sample ensures the analysis yields accurate data on species, gender, and individual identification (Taberlet et al. 1999, McKelvey and Schwartz 2004a, Pompanon et al. 2005). If the DNA is not properly extracted, downstream errors will jeopardize conservation initiatives and management plans, and will ruin the reputation of laboratories. The most common causes of error in the lab include poorly trained technicians, personnel that do not

have an invested interest in the project, rushing through protocols, and taking shortcuts to save money. It is critical that laboratory technicians care about the results, take their time, carefully follow the protocols, and practice excellent laboratory techniques.

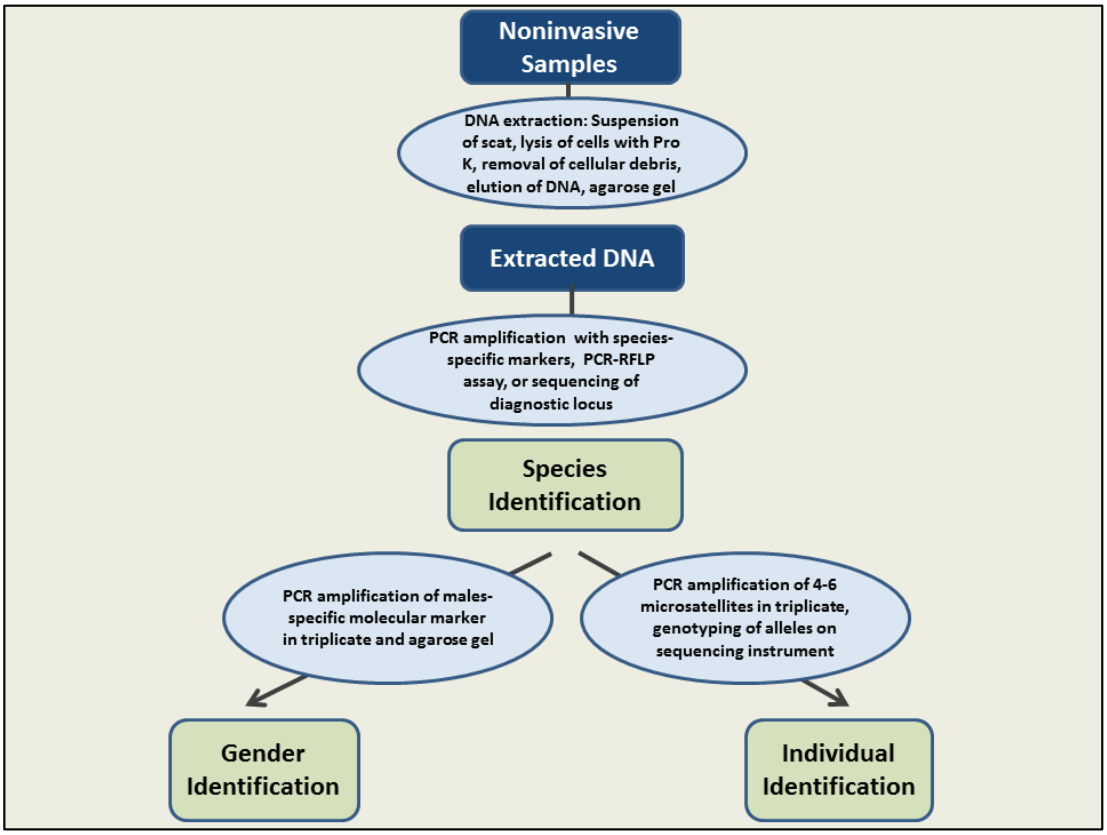

Figure 3.6. Flow chart of genetic analysis of noninvasive samples.

Cross-contamination of samples (i.e., DNA from one sample being present in the extraction of another sample) is a major threat with non-invasive samples. The reason these samples (e.g., hair and scat) are prone to contamination is that the DNA from the target animal is of low quantity and quality, and therefore even trace contamination from another source interferes with the analysis (Taberlet et al. 1999). In contrast, when working with DNA from tissues, there is enough DNA from the source individual that trace contaminants are usually not detected during the analysis. The elimination of cross-contamination starts in the field with careful sample collection and continues in the lab with meticulous handling during the extraction process. The use of barrier tips and manipulation of noninvasive samples

in an area physically separated from PCR products is essential (Waits et al. 2001, Waits and Paetkau 2005, Beja-Pereira et al. 2009).

The process behind different DNA extraction protocols is similar even though individual steps may differ (Sambrook and Russell 2001). In the beginning of an extraction, cells and their nuclei are lysed, releasing DNA into solution (Sambrook and Russell 2001). The cellular membranes are further broken down and proteins degraded (cut into small pieces with proteinase K). Subsequently, the DNA is separated from other cellular components and inhibitors. One efficient method (used by Qiagen) is to bind the DNA to a silica membrane, wash off the cellular debris, contaminants, and residual impurities, and then elute the DNA using a buffer. Extracted DNA is stable at 4°C for months, but it is best to maintain samples at -20°C for long-term storage. Several companies manufacture kits specifically for DNA extraction and tailor them to different types of samples (e.g., muscle, blood, buccal cells, etc.). To date, the QIAamp DNA Stool Mini Kit (Qiagen) is the most widely used product for scat. It is designed specifically for removing impurities in feces that could interfere with downstream analysis. Other techniques are available for hair samples (Walsh et al. 1991, Goossens et al. 1998, Vigilant 1999, Suenaga and Nakamura 2005, Bjornerfeldt and Vila 2007).

The quality and quantity of the extracted DNA needs to be determined after the extraction. The simplest method is to use an agarose gel (Sambrook and Russell 2001). Agarose can be envisioned as a matrix of molecules with spaces (or “holes”) between them (similar to a sponge). When an electrical current is applied, the DNA, which is negatively charged, moves through these “holes” towards the positive electrode. The DNA can be visualized by staining the gel in ethidium bromide (or a comparable stain such as SYBR green), which binds to DNA and fluoresces (i.e., glows) when exposed to ultraviolet light (λ = 312 nm). The DNA quantity is proportional to the intensity of fluorescence, and the DNA fragment length to the rate it migrates through the gel. Therefore an agarose gel provides information on the amount and integrity of the DNA obtained from a sample. (CAUTION: EtBr is a very strong carcinogen and UV is damaging to the eyes. Safer options such as SYBR green are available).

There are several excellent manuals that provide a step-by-step guide to DNA extraction, gel electrophoresis, and other important protocols (Sambrook and Russell 2001, Ausubel et al. 2002, Barker 2005). These should be closely studied to further understand these methods. Many details could not be covered in this chapter due to space limitations.

Application, Use, and Interpretation of Genetic Data

Measuring genetic variation

Genetic research explores the vast amounts of variation present in the genomes of individuals, populations, species, and higher-level taxonomic groups (Avise 2004). The interaction of genes and the environment leads to phenotypes (or physical traits) that characterize an organism. The genetic component is the heritable portion of these phenotypes. The influence of both natural selection and neutral processes upon genomes leads to the evolution of species.

The ultimate sources of genetic variation are mutations that occur during meiosis, as discussed above. Genetic variation of an individual is manifested in the number of alleles (i.e., variants) present across all loci. In population genetics we describe this variation by sampling a subset of loci, and measuring the number, distribution, and type of alleles or haplotypes (a series of linked alleles) (Hedrick 2011). At the population level, variation is described as percent of polymorphic loci, the mean number of alleles per locus, mean heterozygosity, and allele and haplotype frequencies (Hedrick 2011). Models have been developed that can be used to infer population parameters (e.g., effective population size and gene flow) based on the patterns observed in the genetic variation within and among populations (Avise 2004, Hartl and Clark 2007). In this section we will discuss how DNA polymorphisms are used to make species, sex, and individual identification, and to obtain other types of population information.

Species identification

Species identification is essential for noninvasive studies of wildlife as many samples collected in the field are of unknown origin. Previous studies have found high error rates in species identification of scat in the field, even by experienced biologists (Reed et al. 1997, Farrell et al. 2000, Davison 2002, Janecka et al. 2008b). It is also difficult to differentiate hairs and other tissues collected in the field or confiscated from wildlife markets (e.g., meat, claws, hides, bones, and horns) (Baker et al. 2007, Alacs et al. 2010).

Mitochondrial loci, most commonly cytochrome b and cytochrome c oxidase subunit 1, are favored for species identification because they are easier to PCR amplify in poor quality DNA samples. Primers are available for PCR amplification of portions of these genes across a wide range of taxa (Farrell et al. 2000, Hebert et al. 2003). The PCR products generated for unknown samples can be sequenced, aligned, and compared with sequences from known species (Figure 3.2) (Farrell et al. 2000, Janecka et al. 2008b, Janecka et al. 2011a). If the locus used for species identification has been characterized for all taxa present in a study area, and there

are known fixed differences between sympatric species, cheaper PCR or restriction enzyme assays can be developed for species identification (Mills et al. 2000b, Fernandes et al. 2008, Roques et al. 2011). In some populations or species, mtDNA can provide erroneous results due to introgression and nuclear mitochondrial translocations (numts). In these cases using nuclear markers may be necessary to differentiate closely related species (Alves et al. 2008).

Gender identification

Determining gender is important for inferring identity, examining social dynamics, and understanding the reproductive potential of a population. In mammals, gender identification is based on determining the presence of the Y chromosome. Primers are available for many species that amplify a small portion (100–400 bp) of male-specific genes on the Y chromosome (Pilgrim et al. 2005, Waits and Paetkau 2005, Janecka et al. 2008b). The results of the PCR amplification can be analyzed on an agarose gel, and presence or absence of the correct male-specific amplicon is used to determine the gender of the sample.

The results must be carefully interpreted because many noninvasive samples are degraded or have low amounts of DNA, which can lead to PCR failure. Therefore, it is important to determine whether lack of amplification of the Y-marker is due to the absence of the Y chromosome (i.e., female), or to PCR failure (i.e., degraded male sample). This can be done by repeating the PCR assay and testing the sample for PCR amplification of another locus on the X or on an autosome (e.g., any of the microsatellite loci used for individual identification). Gender assays based on amelogenin, zinc-finger Y, and sex-determining region Y loci have been developed for carnivores, ungulates, and other taxa (Palsboll et al. 1992, Griffiths and Tiwari 1993, Taberlet et al. 1993, Kurose et al. 2005, Statham et al. 2007, Janecka et al. 2008b, Kim et al. 2009, Rodgers and Janecka 2012).

Individual identification

The most common markers used for individual identification are microsatellites in the nuclear genome. Each locus is PCR amplified using one primer labeled with a fluorescent dye and the amplicons are then genotyped (i.e., exact size of alleles determined) with a sequencing instrument (e.g., Applied Biosystems 3730xl DNA Analyzer). There can be differences in allele sizes due to instrumental variation, the type of *taq* used, the dynamics of the PCR reaction, and interpretation of the raw data. For these reasons, careful steps must be taken to ensure the alleles are sized consistently across samples.

Allele sizes are not directly comparable across studies and laboratories; a subset of the same samples must be run in both labs to calibrate the alleles before genotype data can be combined. Sequencing instruments are very expensive (>US$100,000) and therefore there are few laboratories that can obtain such instruments. Fortunately, PCR amplicons are not restricted under CITES, so in most cases they can be shipped internationally to either collaborators or commercial service labs.

Microsatellite loci vary in the level of variation, PCR amplification failure, allele dropout, and false alleles. These factors affect both the precision and accuracy of the data, primarily when using noninvasive sampling (Selkoe and Toonen 2006). Genotyping errors in noninvasive studies stem from three primary sources: (i) Allele dropout that causes heterozygous individuals to be mistyped as homozygous, (ii) False alleles caused by primers annealing in areas of the genome outside of the target microsatellite locus, and (iii) False alleles resulting from small amounts of contaminating DNA (Paetkau 2003, Bonin et al. 2004, McKelvey and Schwartz 2004a, Pompanon et al. 2005, Miquel et al. 2006, Broquet et al. 2007, Zhan et al. 2010). It is critical that noninvasive studies quantify genotyping error and estimate levels of variation in the study population and then select loci and use protocols that minimize errors to a level that will not significantly affect population estimates (Taberlet et al. 1999, Mills et al. 2000a, Paetkau 2003, McKelvey and Schwartz 2004a, b, Pompanon et al. 2005, Rodgers and Janecka 2012).

The probability that two individuals will share the same alleles at a microsatellite locus and therefore have the same genotype (i.e., the probability of identity P_{ID}) is determined by the frequency of the alleles in their population (Paetkau and Strobeck 1994, Waits et al. 2001). The more loci that are compared between individuals, the smaller the P_{ID} for a composite genotype. If sufficient numbers of microsatellites are examined (usually 3 to 10, depending on the number of alleles) each individual in the population will have a unique genotype and therefore can be individually "tagged" (Mills et al. 2000a) (Figure 3.1). The tradeoff however is that increasing the number of loci will also increase cost, time, and the likelihood of error. The accepted criterion in most wildlife applications is to analyze enough loci to achieve a $P_{ID} < 0.01$ (Mills et al. 2000a, Waits et al. 2001).

Infectious diseases

Infectious diseases and parasites play an important role in wildlife populations, from influencing host diversity to altering species composition in ecological communities (Smith et al. 2009, Hedrick 2011). Pathogens can cause massive epidemics; small, endangered populations with reduced diversity are particularly sensitive to such outbreaks and should be closely monitored (Penn et al. 2002,

Acevedo-Whitehouse et al. 2003, Charpentier et al. 2007). Genetic methods can be used to detect the prevalence of pathogens. For example, elephant endotheliotropic herpesvirus (EEHV1) is a major threat in parts of Asia and Africa (Ossent et al. 1990). The presence of this virus can be monitored by testing trunk washes with an EEHV1-specific PCR assay (Stanton et al. 2010). Sequencing the genes from the virus or bacteria that is causing an outbreak can also provide information on the strain, virulence, morbidity, and dispersal through the population.

Population Inferences from Genetic Data

Abundance

Noninvasive genetic surveys provide detailed information on the distribution and abundance of individuals within an area (Figure 3.7). There are now numerous rigorous approaches that can use this data to estimate population size (*N*) (Luikart et al. 2010, Luo et al. 2010, Proctor et al. 2010, Stenglein et al. 2010, Stetz et al. 2010, Tallmon et al. 2010). Emphasis has been placed on complementary development of both field techniques and mark-recapture models. The fundamental approach to *N* estimation is to “capture”, “mark”, and “recapture” individuals over the course of multiple sampling occasions, and then to analyze the recorded histories of these events across sampling occasions (Chapter 2). Noninvasive genetics relies upon DNA from feces or hair so that individuals are “marked” and “recaptured” using a genetic tag as described above without physically handling them (Figure 3.1). This contrasts with traditional invasive approaches based on physically marking individuals with ear tags or unique collars.

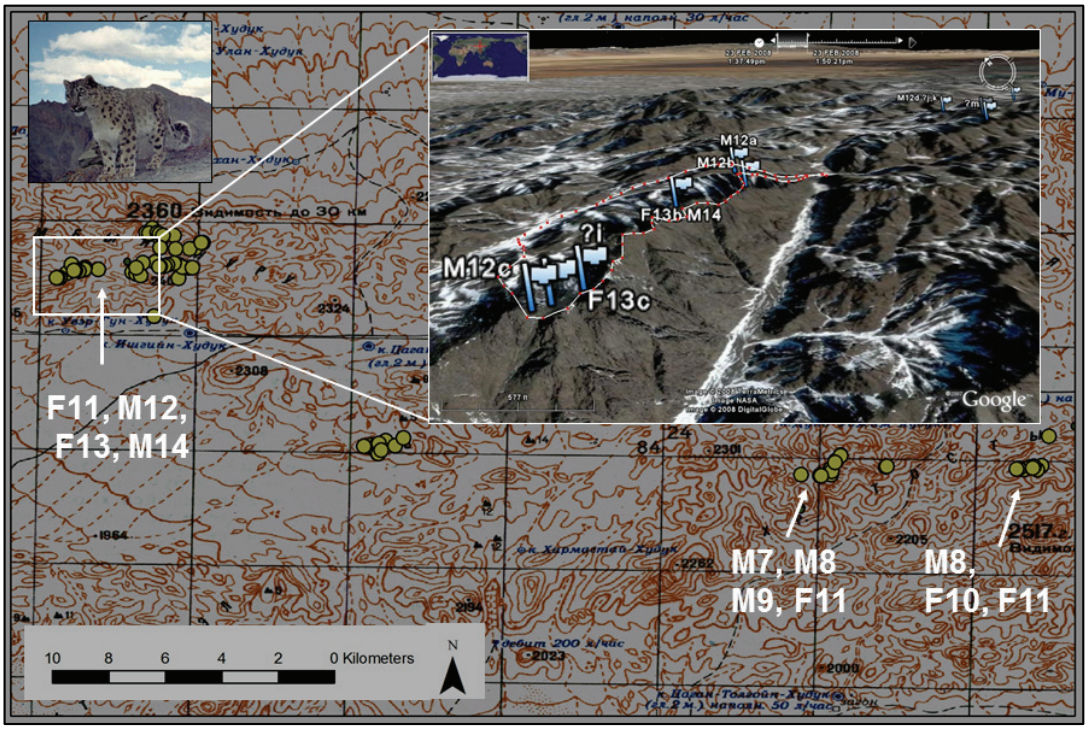

Figure 3.7. An example of the data that can be obtained from a noninvasive genetic survey. Yellow dots on topographic map represent scats collected along transects. Scat was analyzed using the snow leopard molecular panel for species, sex, and individual identification of Janečka et al. (2008a). The individuals observed at each transect are shown on the map. Note that F11 (female snow leopard 11) was detected on 3 transects. The inset shows the western scat transect. By focusing on ridgelines and saddles, Janečka et al. 2011b was able to detect 15 snow leopards in an 8-day sampling period covering 324 km^2. Data from joint survey between Texas A&M University (J. Janečka), Mongolian Institute of Biology (B. Munkhtsog), Irbis Mongolia (B. Munkhtsog), and Snow Leopard Conservancy (R. Jackson). Snow leopard inset photo-credit Snow Leopard Conservancy.

The encounter history of detections for each individual sampled can then be analyzed using an increasing number of methods and free software (Luikart et al. 2010). The most robust of these estimate *N* from data collected over multiple sampling occasions using mark-recapture models (White and Burnham 1999, Lukacs and Burnham 2005). However, because it is sometimes logistically or financially difficult to have multiple sampling occasions reliable methods are also available for single time periods (Kohn et al. 1999, Valiere 2002, Eggert et al. 2003, Miller et al. 2005, Petit and Valiere 2006).

Simulation models have shown that the minimum probability of detection should be at least 0.20 for precise *N* estimates (Boulanger et al. 2004). Pilot studies are needed to identify the sampling design and effort necessary to obtain this level of detection. The most obvious means of increasing the probability of detection is to increase the intensity and duration of sampling occasions (Boulanger et al. 2006).

There are many published examples to guide sampling designs for particular taxa (Boulanger et al. 2006). A recent review provides a nice summary of recommended steps to improve non-invasive studies (Marucco et al. 2010). In at least one case, improved study design led to substantially higher grizzly bear abundance estimates than were previously predicted for a remote, mountainous region in the US (Kendall et al. 2009).

The poor quality and quantity of DNA from non-invasive samples discussed above can lead to attributing a sample to the wrong individual, which can affect abundance estimates (Taberlet et al. 1999, Mills et al. 2000a, Waits and Paetkau 2005). In addition to minimizing errors in the lab there are now methods that incorporate genotype uncertainty into mark-recapture *N* estimation (Taberlet et al. 1999, Lukacs and Burnham 2005, Waits and Paetkau 2005, Schwartz et al. 2006, Schwartz and Monfort 2008). It is important to recognize that despite the best efforts to reduce contamination and degradation of samples, genotypes frequently can only be obtained from 15% to 90% of non-invasive samples taken from the field (Marucco et al. 2010). Consequently, many samples need to be collected to ensure enough data for abundance analyses.

A single *N* point estimate is an important goal for a study; however, the trajectory of *N* provides much more insight into the status of a population. By estimating *N* over time, we can understand how populations respond to specific stressors, such as habitat removal or road development. Repeated non-invasive sampling provides a way to monitor population trends with minimal disturbance, yielding useful information for conservation and management (Schwartz et al. 1998, Schwartz et al. 2007, Tallmon et al. 2010). In addition, it offers an opportunity to continually improve field and lab protocols.

Home range, parentage, mating structure

Non-invasive genetic studies can also provide insights on wildlife behavior and space use (Figure 7). For example, based on the location of sampled individuals, home range distribution can be estimated (Taberlet et al. 1997, Kohn et al. 1999, Taberlet et al. 1999). Similarly, questions on habitat use can be addressed by incorporating geographic, landscape, and habitat information into the analysis (Long et al. 2008). Combining genetic data with radio-telemetry can provide additional insights on relatedness and social structure (Ralls et al. 2001, Tallmon et al. 2002, Widdig et al. 2002, Di Fiore 2003, Janecka et al. 2006).

Landscape connectivity and population structure

The applications of molecular markers can be extended by sampling multiple populations. Movement between areas has important conservation ramifications because migration plays a large role in population dynamics and can greatly increase the likelihood of persistence (Sjogren 1991, Schwartz et al. 2002, Vila et al. 2003). The patterns of genetic variation can be used to infer population structure and history; the more isolated populations are the more genetic divergence there will be across loci (Avise 2004). Various methods including assignment tests and genetic-based clustering of samples can indicate contemporary dispersal, identify migrants, and estimate gene flow (movement followed by mating) between populations. Finally, there is an emerging field of landscape genetics that examines how genotypes are distributed across the landscape and to what extent landscape features hinder or foster connectivity (Manel et al. 2003).

From a practical perspective, the analysis of population structure and connectivity using genetic information requires random, representative samples from each population of interest. Typically, samples of 30 individuals are considered a minimum, although larger numbers (i.e., 50—100 individuals) provide greater statistical power. For rare species, such as snow leopards, this level of sampling may not be feasible. Based on some of the previous studies by the authors, samples of 10 individuals per area can be informative for population structure (Janecka et al. 2008c, Janecka et al. 2011b). Whenever possible, the sampling design used to generate single population estimates of abundance (described above) should be extended to multiple populations to address regional-level questions important for conservation and management of wildlife.

Summary

Genetics provides an important tool for both monitoring wildlife and understanding ecological processes. Noninvasive sampling has opened the horizon for the application of genetics to many wildlife species that previously could not be effectively studied. However, due to poor DNA quality and quantity, analysis must be conducted carefully to ensure errors do not lead to misinterpretation of the data. The data generated from noninvasive genetics can be combined with traditional studies to provide information on distribution and abundance, population trends, home range use, social structure, dispersal, and population connectivity. This knowledge is critical for making effective conservation and management decisions that ensure economic development occurs sustainably, without irrevocable damage to wildlife.

Glossary

Allele: Alternative form of a gene or locus that differs in size and/or composition.

Allele Dropout: The random non-amplification of one of the alleles in a heterozygous sample.

Gene Flow: Genetic exchange between populations as a result of migrants that successfully reproduce.

Genetic Drift: Random change in allele frequencies resulting from chance sampling of gametes. The process contributes to loss of genetic variation (e.g., reduction in the number of alleles) and increased divergence between populations. Its effect is greater when population size is small.

Genotype: The characterization of alleles present in a cell or organism. Diploid cells have 2 copies of each nuclear locus.

Homozygous: A locus is considered homozygous if an individual has two identical alleles at that locus.

Heterozygous: A locus is considered heterozygous if an individual has 2 different alleles at that locus.

Locus: A discrete location on a chromosome that is inherited as a unit. A locus may contain a gene; however, it may also contain no genes. Plural: loci.

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Appendix 3.1: Snow Leopard Scat Sampling Strategy

This sampling protocol was developed in Mongolia, where snow leopard habitat tends to be fragmented, rather than continuous like the situation in the Himalaya or parts of Tibet and China. The typical minimum home range size is assumed to be about 256 km^2, based on telemetry studies conducted in South Gobi (ISLT) and Baga Bogd (SLC). Please follow these guidelines in determining where to collect samples.

1) Obtain a 1:200,000 scale topographic map of the proposed survey region. These maps have latitude/longitude and grid lines that produce 4 x 4 km "cells". These are used as the basis for designated survey blocks.

2) Using the existing lat/long lines, mark 16 x 16 km sized "grids" covering the entire survey region. Each of these "grids" is a square with 4 contiguous 4 x 4 km "cells" on the map. The grids have now become "sampling blocks" (each about 256 km^2 in size). Number each one consecutively (e.g., 1 – 16 in attached diagram). Each sampling block area corresponds roughly to the size of home ranges of snow leopards in the Gobi. Sample the blocks according to rules described below. If it is possible, sample every block. If you cannot sample every block for logistical reasons, determine the total number that you can feasibly sample during the survey, and then randomly select which blocks you will sample. Remember to avoid sampling only the best sample blocks—this is achieved by first delineating areas of polygons each with a specific habitat suitability class, such as:

    - Good or prime habitat (see below)
    - Poor habitat (relatively large areas of flat or slightly undulating land, including desert plains, basins with lakes, wide river valleys, expansive forest)
    - Other—everything else
    - Later (using a GIS or mapping planimeter), you can estimate the proportion of each sampling block that falls within each of these categories

3) On the topographic map (or on tracing paper overlaid onto the map), outline the areas within the sampling blocks that contain suitable snow leopard habitat—broken, steep, rocky or rolling hills and mountains within the landscape. Delineate polygons that contain this type of habitat by following the contour lines.

4) Name (or number) each unique snow leopard habitat polygon (e.g., A—C in attached diagram). These polygons can also be used to guide a presence/absence survey. However, in that case non-snow leopard habitat polygons would also be sampled.

5) In each sampling block, identify specific sites within the snow leopard habitat polygons that can be physically accessed, and have the highest probability of snow leopards' presence and detection. The best sites to select are saddles, outcrops, distinct ridgelines, cliff bases, and distinct drainages that funnel animal movement and/or are used as marking sites. Please refer to the SLIMS manual for more details on sites with high snow leopard activity. Use the following rules to determine the transects that will be sampled:

   - In each 16 x 16 km survey block, locate 2 transects that have the greatest concentration of best sites within the snow leopard habitat polygons.
   - Transects should be 2–5 km length, depending on ruggedness, access, and number of scats found.
   - Transects are a minimum of 3–4 km, but no more than 10–12 km, apart, whether located within the same or in adjacent sampling blocks. Use the closest portions of two transect to estimate the minimum distance between them.
   - If possible within each sampling block, have different transects sample different drainages or watersheds, or on different ridgelines.
   - Sample both ridgeline and valley (drainage bottom) landforms within a sampling block.
   - Collect no more than 36 scats from a particular sampling block.

6) Collected scats should be:

   - Near snow leopard sign (i.e., scrape, rock spray, tracks). However, on some transects you may find very little sign. If this is the case continue to collect scats.
   - On distinct landscape features such as small outcrops, saddles, bottom of cliff faces, passes between two separate valleys (watersheds).
   - Fresh or very fresh. Older scats can be collected provided their surface is relatively intact. If you cannot find fresh scats, you can collect older scats.

Do not collect scats without an outer “shell” of fecal material. These scats look like the outer surface has been washed away, and they consist of only hair and small bone fragments.

7) Record information onto the datasheets. There are two datasheets to be filled out. The first is the TRANSECT DATASHEET. There is one for each transect. The second datasheet is the SCAT DATASHEET: Each individual scat collected will have a record on this sheet.

   Numbering Transects: Enter the name and number for each transect. These should be numbered consecutively with initials of primary field person (e.g., BM1 for Bariusha Munkhtsog transect 1). The numbering system should be run consecutively across sites, seasons and years, in order that each transect has a completely unique number. This serves to minimize potential errors arising from duplicate names and transect numbers.

   Numbering Scats: Please number all collected scats consecutively along each transect (i.e., BM1-1, BM1-2,…). Start at 1 again when you move to a new transect (ie, BM2-1, BM2-2,…). Be sure you record both the transect name/number and scat number on each collection tube.

   Take a photograph of scats before they are sampled (disturbed) with a labeled sampling tube next to it. Record GPS location and other scat / habitat parameters on existing form, then place and store scats in the provided collection tubes following the standard protocol distributed earlier.

   After running the transect (or back in camp) make sure to draw it on the map with the sampling blocks. It would be helpful if you were to supplement GPS scat locations with the location of major turning points along the transect, so that we can accurately map the route taken in a GIS for corridor and other spatial analyses.

Appendix 3.2: DNA Sampling Protocol – Scat

Notes

- Numerous scats believed to be snow leopard are often found together on active scrape sites, collect a sample from each intact scat found at each site.
- Do not touch, disturb, or kick, etc. the scat before you sample it.
- Handle samples with new gloves or rock/stick and then dispose of gloves immediately.
- Do not handle the scats with your bare hands.
- Once you collect a sample fill out "Sample Data Sheet".
- Keep samples cool and dry and in shade.
- Transfer samples to an appropriate lab as soon as possible.

Scat Collection

1) Prepare a new **Collection Tube with Silica Desiccant or 96% Ethanol** and label it with the Date, Sample ID, and Collector's Name / Transect number on the side of the tube and with the sample ID on top of the cap**.**
2) *Numbering Transects*: Number consecutively with initials of primary field person (e.g., BM1 for Bariusha Munkhtsog transect 1, or BM2 for transect 2). The numbering system should be run consecutively across all sites, seasons and years, in order that each transect has a completely unique number. This serves to minimize potential errors arising from duplicate names and transect numbers.
3) *Numbering Scats*: Number all collected scats consecutively along each transect (i.e., BM1-1, BM1-2,…). Start at 1 again when you move to a new transect (i.e., BM2-1, BM2-2,…). Be sure you record both the transect name/number and scat number on each collection tube.
4) Take the GPS location (decimal degrees, please) and record in *Sample Data Form*.
5) Fill out the rest of the *Sample Data Form*.
6) Put on a new pair of gloves. Or using a stone or stick to break up the scat, making sure the part touched by your fingers, never comes into contact with the part touching the scat. Use a new stone/stick for each different scat collected.

7) Break off bits and pieces from the outside part of the scat including pieces not directly in the sun (from the underside sitting on the ground). Collect scat material about the size of a pinkie nail in the tube. Do not fill the rest of the tube with scat. Do not compact scat—the scat should be loose. See Figure A and B on next page for correct amount of scat to store in tube. Close the tube and put it away. If you over fill the tube it will likely not dry and will lead to DNA degradation.

8) Dispose of gloves—keep used gloves away from sample tubes and new clean gloves, in a separate zip-lock bag. ALWAYS HANDLE EACH SCAT WITH NEW GLOVES OR WITH A PIECE OF ROCK OR STICK THAT HAS NOT BEEN USED ON A PREVIOUS SCAT.

9) Do not collect old scats lacking in a surface layer, and/or comprised only of hair, since these contain insufficient DNA for extraction.

10) Fill the form out carefully, ensuring the information you provide is complete, including:

- Species
- Scat diameter (cm), type of segmentation (see Data Code Sheet), sign age
- GPS location and site details, including any landmarks
- Relevant comments (e.g., presence of fresh scrape or rock-scent; human disturbance; scat from camera-trap site)

Figure 3.2.1. Only a small amount of material from the outside of the scat should be collected. The total should be about the size of a pinkie nail.

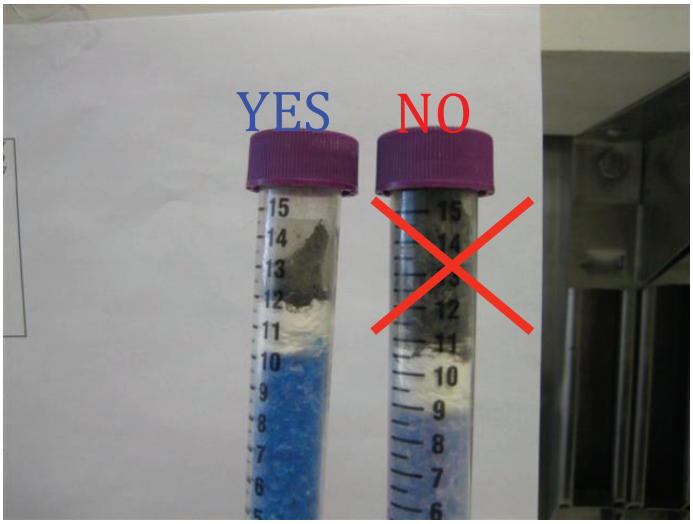

Figure 3.2.2. The scat should be loose in the tube and there should be additional space in the top of the tube as in the tube on the left. This is important in order to keep the scat dry. The tube on the right has too much scat, leading to poor DNA storage.

Appendix 3.3: Field Collection of Biological Samples

DNA Sample Collection

The most desirable samples are tissue:

- Muscle
- Tongue
- Skin (i.e., ear clip)
- Blood
- Hair
- Bone

Scat samples can yield DNA but they are of lower quantity and quality.

It is essential to avoid cross contamination (between samples):

- Wash your cutting instruments and hands (or wear fresh latex gloves) between the handling of samples from different individuals
- Sterilize cutting instrument with a flame

It is essential to properly label each sample and also write your name, date, and a unique ID with something that will not rub off.

Record information:

- Species
- Sex
- Date
- Geographic location (GPS if possible)
- Nearest landmark
- Approximate age (juvenile, subadult, adult)
- The way sample was obtained (road-kill, trapped, shot, etc.)
- Any relevant comments (e.g., parasites observed, morphological abnormalities)

Fill out sample sheet.

As soon as possible deposit the sample into a DNA collection. Many samples are misplaced, lost, or degrade because they are left somewhere (a shelf, in a truck, etc.)

How to sample:

- **Soft tissue (muscle, tongue, lip, ear clip, *etc.*)** – remove a portion of tissue (about ½" by ½") and cut up into smaller pieces. Place in vial with Longmire's storage buffer or 96% ethanol. Make sure the tissue is submerged and floats in the tube. The sample should take up no more than 1/3 the volume of buffer. If you put too much tissue in the tube it will not preserve properly. Most sampling errors occur because too much tissue is added to the tube. It take very little tissue (1 mm x 1 mm) to yield enough DNA for analysis.
- **Blood** – place volume of blood equal to the 1/3 volume of Longmire's storage buffer in vial (may need to remove some buffer from vial, preserve 2–3 ml of blood).
- **Hair** – remove a small tuft of hair (make sure hair bulbs will remain attached) or fragment of bone. Place hairs in coin envelope and tape shut. (store dry, in ziplock with silica desiccant)
- **Bone** – take a small fragment of bone and place in coin envelope and tape shut. (store dry, in ziplock with silica desiccant)

Emergency Method – If you find an animal and do not have sample vials remove some tissue using a flamed cutting instrument, place in a clean bag, and put on ice or freeze as soon as possible until you can preserve it properly (as described above).

Longmire's Lysis Buffer, 500ml

0.1M Tris-HCl, pH 8.0, 0.1M EDTA-Na2•2H2O, pH 8.0, 0.01M NaCl, 0.5% w/vol SDS

For Dry Reagents	For Reagents in Solution
Tris-HCl (or Trizma base), 6.06g	1M Tris-HCl, pH 8, 0.50ml
EDTA-Na2•2H2O, 18.6g	0.5M EDTA, 100ml
NaCl, 0.29g	5M NaCl, 1.0ml
SDS, 2.5g/300ml ddH2O	10% SDS, 5ml

Adjust pH to 8.0 and bring to 500ml (or 324ml ddH2O), store at room temp

Appendix 3.4: Transect Datasheet

Transect #: _____ **Date:** _____ **Sampling Block:** _____

Field Team: _____

GPS Location *Start*: _____ ***End*:** _____

Transect Aspect and Direction: (Aspect and lat-long where the transect changed directions). This is especially critical on transects where very little scat was found.

Compass Aspect (Direction) From Start Location: (e.g., 186°) _____

Intermediate GPS Points & Aspect: _____

_____

Elevation: _____ **Duration:** _____ hrs _____ km (approximate)

NOTE: BE SURE TO ALSO MAP TRANSECT ON THE TOPO MAP WITH SAMPLING BLOCKS

***Dominant Topographic Feature* (tick dominant one):**
___ Ridgeline ___ Hillside ___ Cliff Base ___ River/Drainage Bottom
Other: _____(specify)
General comments on topography:

***Landform Ruggedness* (tick dominant one):**
___ Very Broken/Steep ___ Moderately Broken ___ Rolling ___ Flat
General comments on ruggedness: (e.g., terrain changes from very broken to rolling at end of transect)

***Primary Vegetation Type* (tick dominant one):**
___ Barren ___ Grass ___ Shrub ___ Woodland
General comments on habitat: (e.g., grass only on southern slope)

***Grazing Status* (tick dominant one):**
___ Year-Round ___ Seasonal ___ Non-Grazing
Type of livestock:

General Comments on Grazing: (e.g., very little livestock sign, not much impact)

Other Wildlife Observed and Number:

Other Comments Relevant to Survey: (e.g., found an old ibex kill on transect)

Appendix 3.5: Scat Sample Data Sheet

SCAT SAMPLE DATA FORM		Contact:	Dr. Jan E. Janecka, jjanecka@cvm.tamu.edu	Texas A&M University
Collector's Name:			Dr. Rod Jackson, rodjackson@mountain.org	Snow Leopard Conservancy
Collector's Phone # or Address :			R. B. Munkhtsog, isltmon@magicnet.mn	Irbis Mongolia/Mongolian Academy of Sciences

Date	Sample ID	Transect	Species	Diamtr./ Segmnt.	Scat Age	Snow Leopard Sign and Age	Site	Nearest Landmark	GPS Location/ Elevation	COMMENTS

Data Codes And Definitions For "Scat Data Sheet":

1) Date = Date the sample was collected. *Example: 01 Oct 2010*
2) Transect and Sample ID = Provide a unique sample identification. Scats collected and transects conducted are consecutively labeled with the primary field persons initials.
    - Numbering Transects: Enter the name and number for each transect (see attached Transect Datasheet). These should be numbered consecutively with initials of primary field person (e.g., BM1 for Bariusha Munkhtsog transect 1). The numbering system should be run consecutively across sites, seasons and years, in order that each transect has a completely unique number. This serves to minimize potential errors arising from duplicate names and transect numbers.
    - Numbering Scats: Please number all collected scats consecutively along each transect (i.e., BM1-1, BM1-2,…). Start at 1 again when you move to a new transect (i.e., BM2-1, BM2-2,…). Be sure you record both the transect name/number and scat number on each collection tube.
3) Species = Record the name of the species that you believe deposited the scat based on the size, shape, smell, and associated sign. *Example: Snow leopard*
4) Diamtr./Segmnt. = Diameter/Segmentation = Record the maximum diameter of the scat and the type of segmentation pattern. *Example: 2.1 cm/Segmented*
5) Scat Age = Record estimate of the relative age of the scat (See the categories below). *Example: 1*
6) Snow Leopard Sign and Age = Record all sign (See the categories below) that the scat is close to and the age of each sign (See the categories below). Also record the number and age (in parentheses) of each sign. Example: 3 SC (2), 1 SC (0), 1 UR (2). Note if the collected scat was deposited on a scrape or if not, how far away from the nearest scrape which appears to be about the same age as the scat.
7) Site = Description of the geographic feature for the location where the scat is found. *Example: Ridge*
8) Nearest Landmark = A close well-known geographic location that is relatively close to where the scat was collected. This important so that the

GPS coordinates of the general area where this scat was collected is known. *Example: Dorj's gher*

9) GPS Location/Elevation = Record GPS location and save in GPS unit (in decimal degrees). *Example: 43 04 890, 101 59 758/2300m*

10) Comments = Record any additional information that may be useful. This includes parasites in scats, nearby snare-traps, etc. Also include which other scats were in the vicinity. *Example: Along river, had horse hair, there was a dead horse near by, next to LD31*

DEFINITIONS

Type of Sign:

Scrape (felid only)	SC	Scrape made by felid
Scratch (canid only)	SR	Scratch made by canid
Feces (scat)	FE	Scat or dropping
Urine	UR	Urination mark
Scent Spray	SS	Scent mark
Claw rake	CL	Claw mark on tree or rock left by felid
Pugmark	PU	Track impression

Scat Segmentation:

B = Block-like appearance, with one or more block-like segments with blunt ends and of uniform diameter (typical felid)

T = Tapered appearance, where scat has distinctly tapered tail or tails, often with irregular diameter (typical canid)

Note that a scat may exhibit both conditions, though one tends to be more dominant. Variations occur due to differences in diet or the condition of food.

Age Categories for Sign:

Scrape

Very Old	0	Extensive weathering and disintegration, scrape features poorly defined, often with vegetation growth in the depression and on the pile (age at least 3 to 6 months).
Old	1	Moderate weathering and disintegration, with the scrape showing a rounded form, occasionally with vegetation in the depression or on the pile (age several months or more).

Fresh	2	Slight weathering. Scrape has a well–defined form with “sharp” edges, is easily recognizable, and has no new vegetation growing in the scrape depression or pile (age 1 to 4 weeks).
Very Fresh	3	Little or no weathering has occurred, so that the scrape has a very sharp and “clean” form, is very easily recognizable, and has no vegetation in its depression or pile. Sand or gravelly material may cover some vegetation, causing it to “bend-down”. Other ephemeral sign such as tracks or urine may be observed, while scats deposited at the same time are obviously still fresh or very fresh (age less than 1 week).

Pugmark

Old	0	Pugmark is very poorly defined, with an obviously “weathered” appearance (more than 2 weeks old).
Fresh	1	Pugmark has sharply defined edges and shape (several days, but less than one week old).
Very Fresh	2	Pugmark is very fresh, showing fine surface details and having a very sharp edge (made less than 24 hours previously).

Feces

Old	0	Scat with a hard, dull surface and dry interior —some can be mottled and cracked (several weeks to several months of age).
Fresh	1	Scat is odoriferous and “fresh-looking”, with a glossy sheen inside (more than 2 days but less than 10 days of age).
Very Fresh	2	Scat is still wet outside and moist inside (no older than 2 days).

Scent-Sprayed Rocks

None	0	No detectable odor (more than 3 months old).
Slight	1	Odor is just detectable.
Moderate	2	Odor is readily detectable.
Strong	3	Odor is unmistakable.
Very Strong	4	Odor is very strong (can be detected from 25 cm or more away; less than several weeks old).

Chapter 4

Camera Trapping Protocols for Wildlife Studies (With Emphasis on Tiger Density Estimation)

Marcella J. Kelly[1], Tshering Tempa[2], and Yeshi Wangdi[3]

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Introduction

Camera trapping, or photographing wildlife through the use of automatic trip cameras, has a long history in wildlife biology, first employed in 1877 (Guggisberg 1977) to photograph animals for aesthetic reasons. Recently, there has been a dramatic increase in commercially available, lightweight, relatively inexpensive, digital cameras and this has led to widespread use of remote camera traps for a variety of purposes in wildlife science. Camera traps can be used to document presence of a target species or to conduct a species inventory for a target area. In the 1990s a major advance came with the linking of capture-mark-recapture (CMR) statistical analyses to large-scale camera-trap grids for abundance and density estimation (Karanth 1995, Karanth and Nichols 1998). This technique is well-suited for animals such as most felids that are already marked with bold coat patterns that make them individually recognizable in photographs.

Tigers once roamed in the variety of habitats in Asia from the Caspian sea to the Russian Far East (Global Tiger Recovery Program 2010). Since then, the human world population has increased dramatically, causing large portions of natural habitats to vanish, squeezing tigers into only ~7% of their historic range (Sanderson et al. 2006) and reducing their population to only ~3,000 individuals (Global Tiger Recovery Program 2010). Recognized as endangered since 1975 (Morell 2007), the global tiger population and its habitat have steadily declined (Chundawat et al. 2010). Therefore, this chapter will use tigers as a focal species for conducting density estimation. In addition, we provide protocols for camera survey design, camera field set up, data entry and organization, and data summary and analysis for all photographs returned from field studies.

Warning: Due to the ability of digital cameras to take multiple shots with each triggering and their high sensitivity, there is often an enormous amount of data (i.e., photographs) to sort through to gain meaningful information. It is important to plan for ample time in photographic data entry and to enter all data on *all* animals including humans as these data can often be used as predictor variables for target species in future analyses. Plus they provide much-needed species inventory data on the animals of Bhutan.

Camera Placement and Maintenance in the Field

Camera set up

Camera traps are particularly well-suited to surveying terrestrial mammals, especially those known to use roads or trails as travel paths. Placing cameras on such paths is efficient and increases trapping rates. In forested environments, cameras can easily be attached to trees with bungee cords or nylon webbing straps. In areas with few trees, stakes can be used effectively. It is important not to place cameras too close to trails because digital cameras tend to have slow trigger speeds and many animals may be missed resulting in numerous blank photos and/or tail tips only. We suggest placing cameras at 2–4m from the center of the trail (Figure 4.1). Conversely, cameras should also not be placed so far off the trail that the night flash cannot illuminate the field of view—often ~6–8m for white flash and longer for infrared. Each camera brand should have its specifications for flash illumination. However, past 5m, it may be hard to distinguish animals—especially for smaller species and/or individual ID. Finally, we have seen the best placement is on level, flat ground and fairly low to the ground (20–40cm — or knee height).

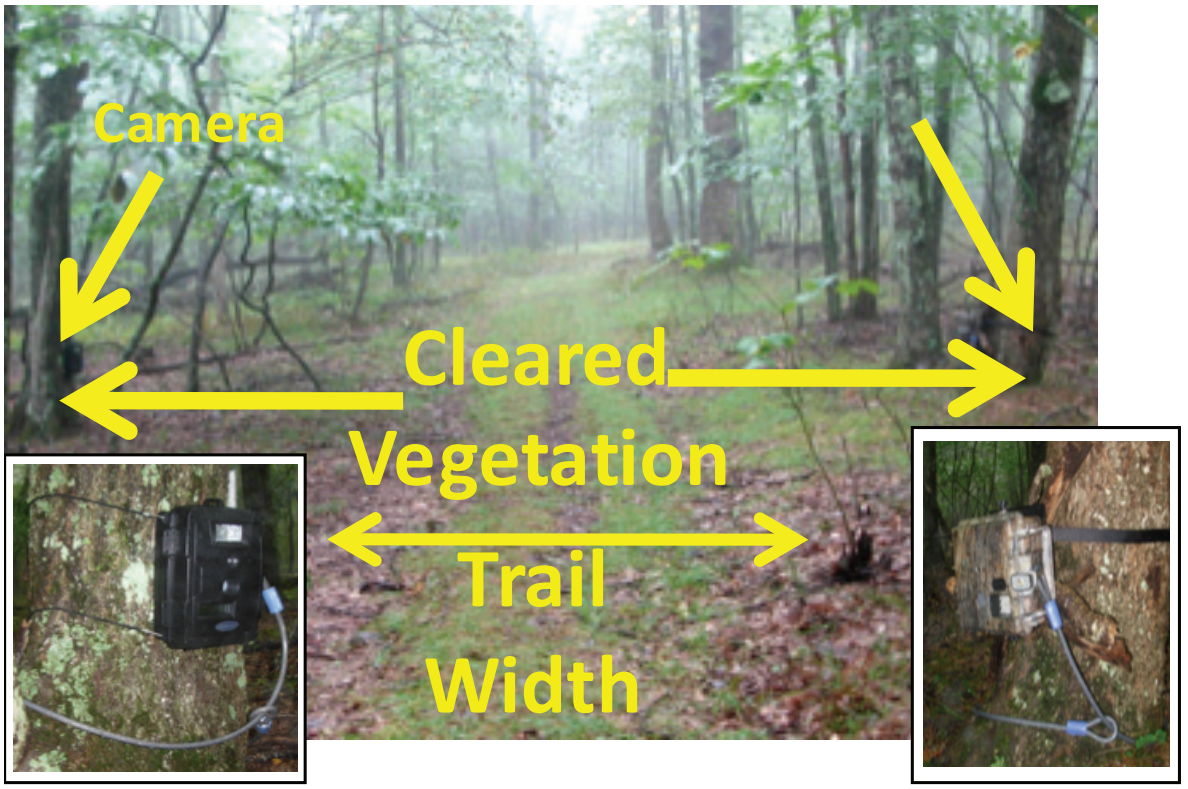


Figure 4.1. Example of field camera placement with 2 cameras per station. Cameras are attached to trees with bungee cords (left) or nylon webbing straps (right) and are backed off from the center of the trail (2–4m) so that they can capture the whole image of the animal (rather than a tail tip). Trail width in this instance is ~2m. It is important to clear vegetation surrounding the cameras' view finders and sensors as this will prevent false triggering and will provide clear, unobstructed, images. Repeated clearing of vegetation is often necessary. Trail width measurements are useful in predicting trap rates for some species.

However, on steep and rugged terrain, it is difficult to find such ideal location for camera placement that would accommodate two cameras as in Figure 4.1. In such cases, we recommend finding a location that can accommodate one camera on one side of the trail and another within 50 meters along the other side of same trail. If cameras are placed over ruts in a road/trail, or high on a tree, animals can escape "capture" by being under the camera's sensor. We have seen this many times when obtaining only ear tip photographs, as an animal travels in ruts or investigates a camera trap at close range. Lowering cameras to knee height and parallel to the ground does not hinder photographs of larger species such as tigers, but be prepared to obtain only knees and bellies of elephants or other large ungulates!

When individual ID is needed, it is necessary to use 2 cameras per station to obtain both sides of the tiger because the stripes are different on the right and left sides. Some researchers argue that one should not place cameras directly facing each other because white flashes can create washout in the opposing camera. This is not

an issue with infrared flash, and it is a relatively minor issue even with white flash cameras. A very slight angle is usually sufficient to prevent wash-out. Having the second camera within the view field of the first allows for photographs that can reveal interesting behavior as animals investigate or vandalize cameras (Fig. 4.2).

When setting up cameras for the first time, we advise using a "set up" data sheet that has some basic information such as: GPS location (UTM coordinates usually preferred), unique station number, unique camera number(s), physical description of location, and some basic habitat features such as: type of habitat, land use code (e.g., protected, unprotected, private, etc.), canopy cover, trail type (Appendix 4.1).

In particular trail width and canopy cover have been shown to be a good predictors of species trapping rates for some felids (Davis et al. 2011).

Figure 4.2. Camera traps placed in opposing pairs can capture interesting animal behaviors such as this bear and cub in Virginia USA.

Camera checks for maintenance and proper functioning

Camera equipment placed at a field site is usually subject to extreme weather conditions and malfunctions are commonplace. Therefore, frequent camera checks are necessary to ensure proper functioning and researchers should always bring extra cameras to replace malfunctioning ones. We advise doing a first camera check at about 10 days into a study to make sure everything is operational and to

determine photographic rates and battery drain (most modern camera traps have a battery meter). After this initial camera check, digital cameras can be checked every 14–21 days. However, cold climates may require more frequent check at ~every 10 days. Going beyond 21 days is risky—especially if animal damage is an issue—because you can lose weeks of data if an early malfunction occurred or a camera was damaged by an animal. We recommend not going > 14 days between camera checks.

Appendix 4.2 gives an example of a camera checking data sheet (different from a set up data sheet) useful for keeping track of battery drain, photographs taken, and general malfunctions. At each camera check, it is useful to bring the previous camera checking sheets, or copies thereof, into the field to evaluate the performance of the camera at the last camera check. Alternatively, you can create a list of potential malfunctions noted from examining the previous data downloaded from the memory cards. Past experience has revealed that there is a temptation to rush camera checks and assume everything is in order when, in fact, some cameras have minor or major malfunctions. Checking sheets of possible malfunctions help prevent mistakes.

It can be very easy to lose track of what data came from which camera when downloading camera memory cards to a computer. An easy solution is to trigger each camera with a placard that, at minimum has: station code, camera number, and date (Figure 4.3).

Figure 4.3. Camera traps need frequent field checks (left above) and general maintenance (e.g., replace malfunctioning camera, check battery life, and change memory cards). Additionally, at each check, all information should be recorded on a data sheet (see Appendix 4.2) and placards should be used to check that cameras trigger properly and to double-document the date and station (right

Alternatively, a stake can be placed into the ground within the camera's viewfinder that documents the station code and camera number. However, we prefer the placard method, because the date (even time) written on the placard, can later be used to recalibrate a camera whose data/time stamp has become corrupted. All cameras should be set to display both the time and the date on the photographic image as this information is essential in future analyses. All cards should be downloaded at the end of each camera check and images examined to determine if possible malfunctions are occurring.

There is often a lot of field gear and equipment to bring when setting and checking remote cameras in the field. It is easy to forget critical items such as keys to padlocks (when cameras are locked). Appendix 4.3 provides gives a list of useful items to bring when camera trapping to prevent forgetting something important.

Should cameras be baited?

Given the extensive use of remote cameras in the field today, it is surprising that there have been relatively few studies systematically addressing the impact of baited versus non-baited camera traps. While using bait (olfactory lures or meat) to draw in carnivores is commonly done in presence/absence studies, most studies estimating abundance do not bait cameras for fear of changing animal behavior and luring animals in that would otherwise not already be present in the camera grid. But there are studies that have used bait in order to increase trapping rates for the purpose of mark-recapture analysis (sardines for ocelots: Trolle and Kery 2003; chicken pieces for Malagasy carnivores: Gerber et al. 2010). Additionally, Gerber et al. (2011) found that bait did not change abundance estimates for Malagasy civets. Still other studies do not mention if they used bait or not. In some instances, trap rates may be so low for very elusive species that baiting is necessary. Baiting is probably not a concern for inventory studies but should be further explored for abundance/density estimation. In general, baiting takes more time and can be very messy (especially for meat), and logistically problematic. Tigers, and many other felids, have been successfully surveyed without baiting camera traps.

Species Inventory or Distribution Studies from Camera Traps

Survey design

The design of any camera-trap survey depends on the purpose of the study and can change for different target species. In areas where not much on species compositions and distributions are known, use of camera traps would be highly valuable and provide great insight and baseline data on species occurrence in these

areas. It can even be done as part of a tiger density estimation survey. For documenting species presence or conducting species inventories, there is currently no standard for number of camera stations, spacing between cameras, or duration of surveys (Kelly 2008). However, Carbone et al. (2001) suggested through simulation modeling that at least 1000 trap nights would be needed to document tiger presence if tigers occurred at densities of 0.4 to 0.7 tiger per 100km^2. Wegge et al. (2004) provide some insight into how increasing camera saturation can decrease the total number of trap nights needed to detect individual tigers. In their species inventory, Tobler et al. (2008) captured 86% of species assumed to be in the area in 2340 trap nights. Most studies use a minimum of 1000 trap nights but more may be needed for rare species and many current studies strive for 2000 trap nights per survey.

Camera placement and spacing are flexible for inventory studies and often include targeting likely areas with more cameras while not surveying unlikely areas. However, studies addressing habitat use should stratify by habitat type to make meaningful comparisons. Use of 1 camera per station is sufficient for this type of study since individual identification in not necessary, but note that all data is lost if the camera malfunctions or is vandalized at a particular station.

Data entry, summary, and analysis: trap nights and trap success

Number of trap nights (or trap days) is calculated as the number of camera *stations* times the number of nights each station is operational. When there are 2 opposing cameras per station, this is still only considered one camera *station* since cameras are at the same location. Therefore, only distinct camera stations, and not distinct cameras, should be used in calculating trap nights. It is important to subtract any days where a camera station was non-operational due to malfunction, battery drain, or human/animal vandalism. If using 2 opposing cameras, as long as one camera is operational (i.e., if only 1 of the 2 cameras malfunctions), the station is usually still considered operational. If an event occurs that knocks cameras askew (e.g., pointing directly up into the air or at the ground) these should not be counted as operational even if photographs are obtained of tree tops and dirt.

Useful summary data to present include the total number of trap nights for an entire survey, the total number of photograph “events” for each species and the trap rates for each species for an entire survey. Trap rates require determining the number of trap nights and dividing the photo events by trap nights. In addition to calculating total number of trap nights across an entire survey, it is important to determine trap nights for each camera station independently to determine if stations have high malfunction rates and need replacement cameras or need to be

excluded in future analyses due to low samples sizes. Additionally trap rates for each camera station are useful in determining hotspots (or coldspots) of animal activity. Finally trap rates per camera station should be presented in addition to the total number of photographs of each species at each camera station because it is unlikely that all camera stations will be operational for the same number of days, due to unpredictable malfunctions and some stations being in the field longer than others. Obviously, a camera station that is up for a longer time is more likely to obtain more photos, therefore dividing the number of capture events by number of trap nights (i.e., trap success) is more appropriate than the number of raw photographs of each species.

Trap success (a.k.a. trap rate, photographic capture rate, photographic capture index, etc.), is usually calculated as the number of independent photographic capture "events" per 100 trap nights (See Appendix 4.4).

$$Trap\ Success = \frac{\#\ of\ capture\ events\ of\ target\ species}{\#\ of\ trap\ nights} \text{x } 100$$

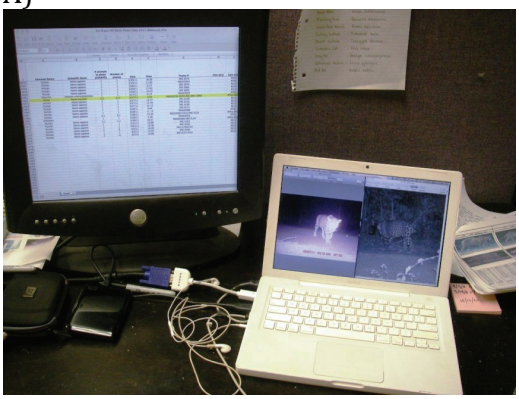
Some studies do not multiply by 100, but this can lead to very small numbers that are difficult to graph or interpret for very rare species. Using the 100 multiplier also allows relatively easy interpretation. For example, if trap success was 6.0, this would be interpreted as obtaining 6 photographs of the target species in 100 trap nights (i.e., 6 photos with 1 station running for 100 nights or with 10 stations running for 10 nights). It should be noted however, that this is not a direct percentage because it is possible to photograph more than one target species per day per station, and this can lead to a value of over 100 for trap success of very common species.

In past studies a capture "event" has been defined as an independent photograph of a species that occurs within either a ½ hr or a 1 hr time frame from the date and time stamp of the first photo of the species (Kelly 2003). The choice of time frame is somewhat arbitrary and is up to the researcher but either ½ or 1 hr should be sufficient and probably will not make much too much difference. If you use ½ hr data, however, that can be combined later to 1 hr if need be, whereas if you use 1 hr, you cannot go back to using ½ hr unless you go back to the raw data. So ½ hr is perhaps more flexible.

If there are numerous photographs of an individual within the specified time period, care should be taken to determine if the event is 1 individual, or several. If

two animals can be distinguished in the photographs, or even in a single photograph, it should be recorded as 2 capture events. If it is not possible to tell if there are 2 or more animals, then err on the side of caution and add the animal as a single event. If the study is using 2 cameras per station, it is important not to double enter the same animal photographed by both opposing cameras. Even if both opposing cameras record the animal, there is still only 1 capture event. This can make data entry extremely tedious because it requires examining photographs from both sides of the trail simultaneously to prevent double entries. Setting up a data entry system with 2 laptops or a computer with extra monitors can greatly ease data entry from multiple cameras simultaneously (Figure 4.4).

A)



B)

Figure 4.4. Example for how to enter data when you have more than one camera operational per camera station. Attaching a laptop to an extra monitor A) or using 3 monitors attached to one computer B), can ease data entry and aid in avoided double-entry of the same animal photographed in separate cameras at the same station.

Appendix 4.5 gives an example spreadsheet for organizing data entry on all species, including humans (and their vehicles), for inventory or distributional studies, but we advise that this be part of all studies (even abundance/density studies). In this spreadsheet, each row or record represents a trap event within a ½ hr time period, and notes the species, # of photos, # animals in photos, etc. (Appendix 4.5). Once the data is entered into such a spreadsheet, it is fairly straight forward and

relatively simple to use “pivot tables” in Microsoft Excel to summarize data by species or by camera station (or both) and to convert into trap rates. This data provides a very useful summary of total species occurrence over a whole survey (Figure 4.5a) and the trap rates across the study site of a target species (Figure 4.5b). The data also can be useful to indicate what influences target species presence or trap rate (Figure 4.6).

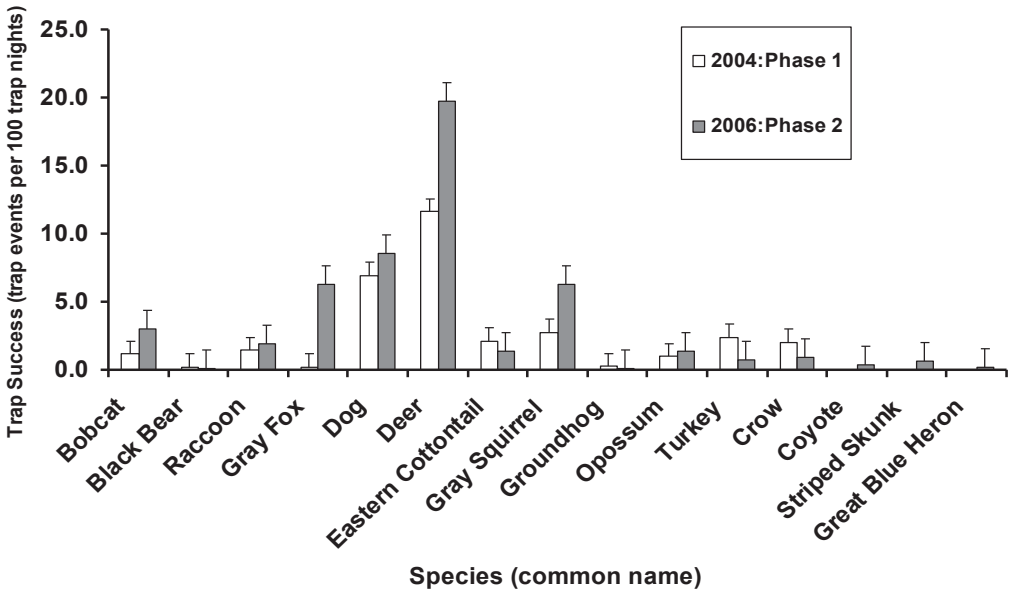


Figure 4.5a. Average trap success (and SEs) for each species across 15 camera stations in 2 different years of the study. This gives an indication that deer and domestic dogs may be easy to trap with camera traps, but that the carnivores have low trapping rates and the effort may need to be large to gain information on these species.

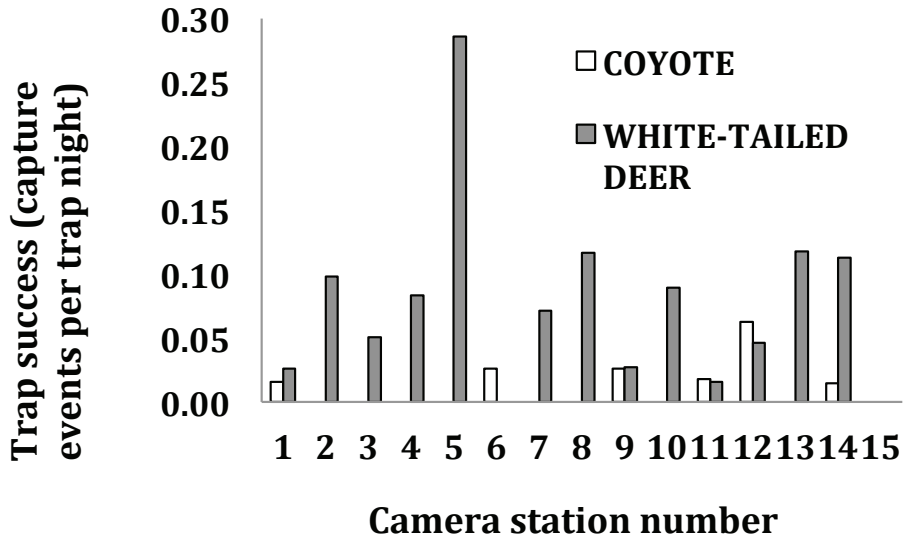


Figure 4.5b. Trap success for coyotes and white-tailed deer across 15 camera stations during 1 year of study. Deer are ubiquitous being found at all stations except station 6 and 15; whereas coyotes are much more rare across the study site.

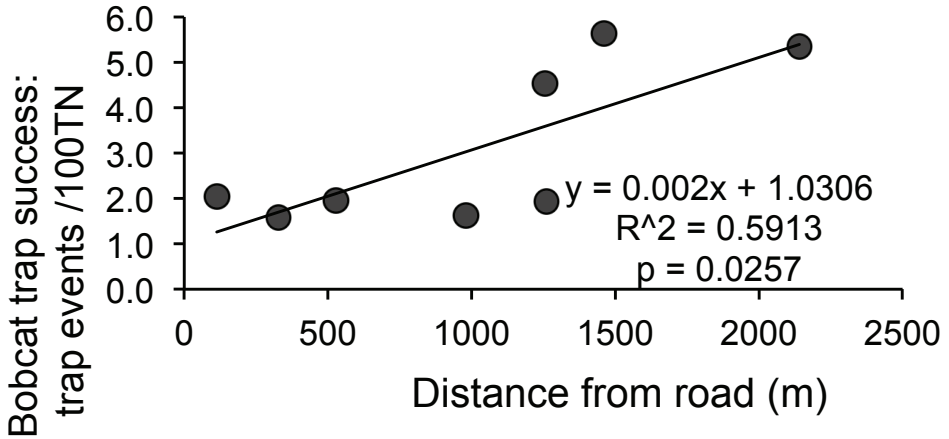


Figure 4.6. Trap success for bobcats across 8 camera stations where bobcats were captured, increases as the distance to the main road increases. This implies that bobcats avoid using areas closer to roads. (Adapted from Kelly and Holub 2008).

Species Abundance / Density from Camera Traps

Survey design

Unlike species inventories, which have highly variable survey design, there are well-established protocols and active research regarding camera station survey design for abundance and density estimation. There are 2 main requirements for abundance/density surveys: 1) individual identification is necessary and 2) two cameras per station are usually needed to photograph both sides of the animal for positive ID. Because most wild felid species such as tigers, leopards, marbled cats, clouded leopards, leopard cats, etc., have unique coat pattern that enable individual ID, and they readily use trails, camera trapping techniques are particularly well-suited for felids.

Most studies within a survey use a fixed grid with a minimum of 20 stations (with 2 cameras per station), spread across the landscape with systematic spacing. For example, camera spacing for jaguars is often cited at 3 km based (conservatively) on the smallest home range recorded for 1 female radio-collared jaguar of 10 km^2 (Rabinowitz and Nottingham 1986). This ensures every 9 km^2 will contain a camera trap; hence no individual jaguar should be missed due to holes in the trapping grid. In fact, most animals will have 3–4 camera stations within their home range. This also ensures that every animal has a probability of being captured, a necessary assumption of mark-recapture models commonly used in data analysis (Otis et al. 1978). The spacing is often larger for tigers at 3–5 km between stations due to their much larger home range. An approach for Sumatran tigers used a 2X2 km^2 grid, overlaid upon the study site and camera stations were placed in every other grid cell for a spacing of roughly every 4 km^2 (Sunarto 2011). In Royal Manas National Park, Bhutan we used a grid size of 2.5x2.5 for a tiger survey in 2010 (Tempa et al. 2013). In another approach at a site with high road density, researchers in Belize used hand-held GPS units to determine distances to nearest camera stations and placed cameras at 3 km intervals from at least 2 other camera stations across the study site (Davis et al 2011).

In Bhutan, an initial site was chosen for tiger surveys based on accessibility by trails, roads, and rivers (Figure 4.7). The original placement of camera traps at 2.5 km spacing (Figure 4.8) is sufficient for pilot study work, but it may be too small at ~25 km^2, to encompass enough tiger home ranges to obtain a rigorous density estimate. The pilot study data, however, is highly useful and will give much needed information on how and where to expand the camera trapping grid into the future. In any case, grid trapping is essential for density estimation and bigger is usually better for wide-ranging species. Thus, for a tiger survey in Jigme Singye Wangchuck National Park in 2013, we used a 5x5 km^2 grid.

Data entry, summary, and analysis: abundance and density

We advise entering data on all species in addition to the target species following the protocol laid out above. However, data entry and formatting for abundance/density estimation is unique and does differ depending on the software used to analyze the data (see Chapter 2). In general however, capture histories must be created for each individual tiger identified. A good way to keep track of tiger IDs and to provide a quick way to check IDs of incoming photos, is to create a spreadsheet displaying both sides of the animal and all the dates and locations recorded (Appendix 4.6). This can also form the basis for creating capture histories and calculating distances between camera stations and for keeping track of animals from year to year.

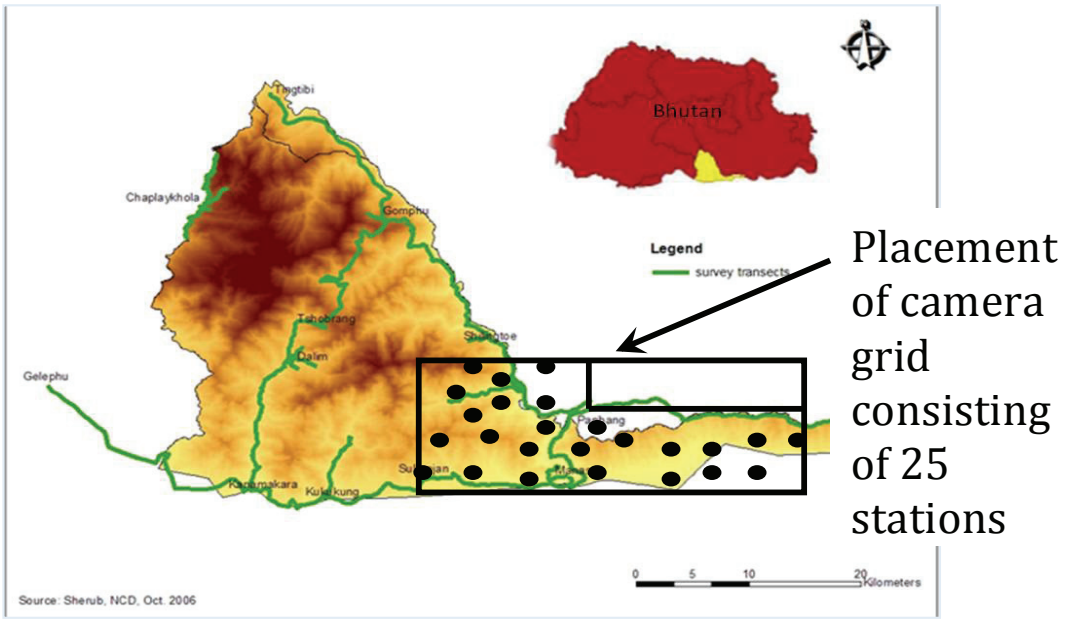


Figure 4.7. Royal Manas National Park. The black box denotes the suggested location of an initial tiger camera trap grid centered on a network of trails, rivers, and roads. A distance between traps of ~2km, with a minimum of 25 camera stations (2 cams per station) will result in a survey area ~25 km^2.

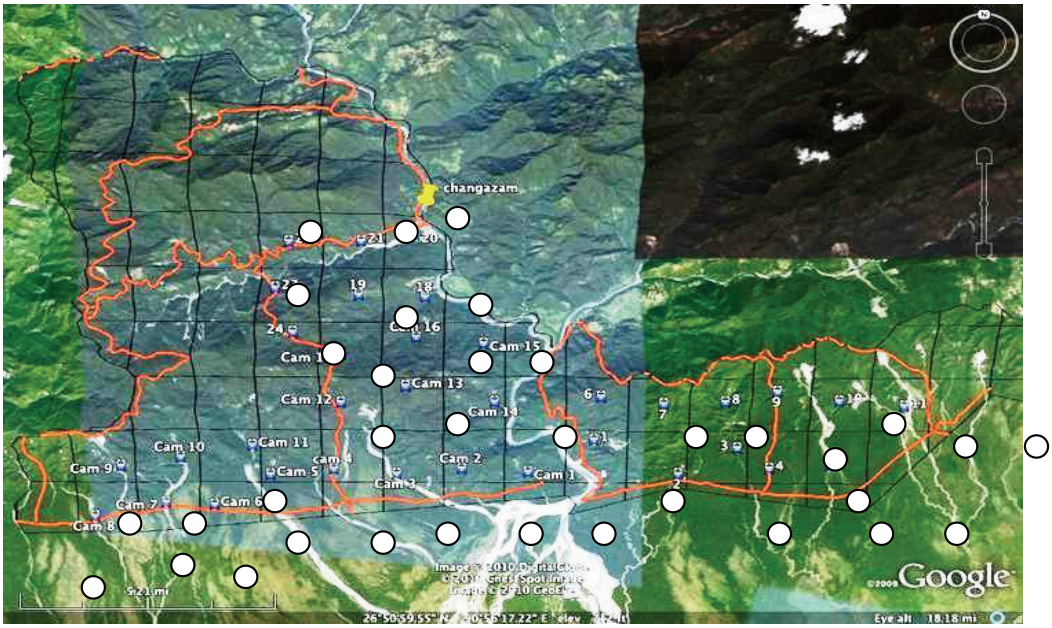


Figure 4.8. Actual placement of initial camera trapping grid for tigers in Royal Manas National Park placing cameras in each 2x2 km grid cell placed across the landscape to systematically survey for tigers. Blue symbols represent camera stations.

A capture history for an individual tiger consists of a set of 0s (non-capture) and 1s (captures). Some researchers use each day that a camera station is operational as a trapping occasion such that a 60-day survey would have a set of 60, 0s and 1s. However, due to the low capture rates (i.e., high number of 0s) for many target species, this can present problems in data analysis. Therefore we suggest collapsing data such that a few days or even a week represents a single capture occasion. This is particularly true if running the data through Program CAPTURE (Rexstad & Burnham 1991; White et al. 1982) or MARK (White and Burnham 1999), but it is actually not necessary if using spatially explicit capture recapture (SECR) programs such as DENSITY (Efford 2004; 2007) or SPACECAP (Royle et al. 2007; Singh et al. 2010).

Program CAPTURE utilizes numerous models including heterogeneity, M(h); behavior M(b); time M(t); and combinations of these effects, to determine which model fits the CMR data best (Otis et al. 1978). Included in the analysis is a test for population closure whereby a high p-value indicates that you cannot reject the null of “closed” population (i.e., high p-value is good in this case!). CAPTURE can be run as a stand-alone program (freely available) or from within Program MARK (however the closure test is not automatically available in MARK). The stand-alone CAPTURE and the one embedded within Mark use discriminant function analysis to rank models. Data can also be run in Program MARK to estimate abundance using maximum likelihood estimation (MLE) and Akaike information Criterion (AIC; Akaike

1973) for model selection. While the statistical implementation in MARK is thought to be superior, we have found that sometimes datasets with very low numbers of animals and low capture rates run better or more consistently in Program CAPTURE.

Because camera grids are often different sizes and can change size and shape over time in longitudinal studies, it is necessary to convert abundance estimates from CAPTURE or MARK into densities for comparative purposes. Therefore, researchers must divide the resulting abundance estimate by an effective trap area. Determining the effective trap area is the sticky, problematic part. Because animals roam far and wide, and not all animals detected live within the camera trap grid but can be photographed at the edges (edge effects), there is uncertainty about the area sampled. The most common methods to estimate survey area are 1) to create a minimum convex polygon (MCP) connecting camera stations and add a buffer surrounding that MCP (commonly done in tiger studies), and 2) to create circular buffers surrounding each camera station and dissolve the buffers (commonly done in jaguar and ocelot studies). Buffering points is more consistent because camera "grids" are often oddly shaped across the landscape and can lead investigators to create MCPs somewhat subjectively. Buffering points eliminates creating an MCP around trapping grids.

To date, most studies determine the buffer size using the mean maximum distance moved (MMDM) between camera locations among all individuals recaptured at least once (Dice 1938; Wilson & Anderson 1985). Traditionally ½ MMDM is added as the buffer, and is meant to represent a surrogate for the radius of the animal's home range. Determining the distance moved between cameras can be done using ARC GIS or some other mapping software. Alternatively, using the Pythagorean Theorem is do-able over relatively short distances. Determining the variance in the density estimate is tricky because it requires incorporating variance in the abundance estimate and variance in the area surveyed, which is based on the variance in the distances moved across individuals. The delta-method is commonly used and well-documented in Nichols and Karanth (2002).

The ad-hoc techniques for estimating effective survey area are problematic because they are influenced by trap spacing and size of trapping grid (Dillon & Kelly 2007; Maffei et al. 2008). Additionally, ½ MMDM has been shown to be a poor proxy for home range radius for some populations (Soisalo and Cavalcanti 2006—jaguars; Dillon and Kelly 2008—ocelots) but not others (Maffei et al. 2008—ocelots). Because of the problem noted above, new camera trapping analysis techniques for abundance/density estimation are rapidly developing and will likely

replace the common method of using program CAPTURE/MARK and MMDM methods. We therefore recommend also using Program DENSITY, which is a free download and is fairly user friendly. DENSITY is a simulation-based method of fitting models to the trap array data. Resulting estimates do not depend on trap layout. Probability of detection declines radially with increasing distance from the fixed home-range centers, and the density of the centers is the parameter of interest (Efford et al. 2009). We supply input files and descriptions for entering data into Programs CAPTURE (Appendix 4.7), MARK (Appendix 4.8), and DENSITY (Appendix 4.9).

At this time, Program SPACECAP is not particularly user friendly, sometimes requires a working knowledge of R programming, and requires many hours of computing time to run analyses. While we foresee the use of SPACECAP increasing in the future, we do not provide more information here but refer the reader to Singh et al. (2010).

Presentation of results for abundance/density estimation should, at a minimum, include the number of camera stations, number of trap nights, number of individual animals captured and recaptured, MMDM (if used), effective survey areas size, CMR technique used, and best model selected, closure test results (if using CAPTURE), abundance estimates, and density estimates with standard errors.

Some Challenges and Limitations to Consider in Camera Trapping

Start-up costs for camera trapping surveys can be high, particularly for abundance estimation which requires a minimum of 20 stations (40 cameras) and we advise starting with at least 60 cameras because malfunctions always occur. In addition, camera lifespan is only ~3 years especially if used for extensive time periods. Additionally, camera models vary widely in price (currently $65–$1500 USD per unit), quality (image, durability, trigger speed) and features (event delay, sensitivity, video capability). Some are very easy to use and others require programming or are less intuitive. Many websites are available that rank camera models and supply user input. New users should seek this type of input. In addition, several papers evaluate camera types (Swann et al. 2004, Kelly and Holub 2008)

The trade-off between image quality and quick trigger speed for digital cameras has not yet been resolved. Studies of carnivores that require individual ID need both clear images and a quick trigger, and many users are currently frustrated with most digital cameras.

Probably everyone using camera traps has experienced some theft or animal damage, even with cameras that are locked down and secured. This can be

devastating to a study, especially if theft is large. Certain animals, like elephants and black bears, find cameras offensive or just plain fun toys. Bears in Virginia, for example, bit, chewed and otherwise rolled around with, ~20 out of 40 remote cameras destroying a large number (Kelly pers. exp.). In south Asia, elephants are a major cause of camera loss. Researchers should be prepared and perhaps refer to other studies that have found creative solutions to deter theft and animal damage (Grassman et al. 2005, Karanth and Nichols 2002, Fiehler et al. 2007).

Camera trapping is greatly enhanced by an established trail system. Carnivores especially, readily use trails and if a study site lacks trails, time should be spent creating a trail system, both for the ease of research and to increase capture success. Animals will come to use such trails over time (Maffei et al 2004). Use of old roads (e.g., old logging roads) is highly desirable for larger carnivores.

Open habitats may be at a disadvantage relative to closed forest habitats in camera trap studies since they lack natural “funnels” to channel animals in front of remote cameras. Animals will use game trails in these open habitats, but high trail density, which often occurs in savannahs, can also lead to decreased trapping rates for carnivores (Henschel and Ray 2003) likely due to inability to saturate all trails with cameras.

Finally, data organization and input is intensive for camera trap studies. But it is essential to spend the time to complete it. The photographs are “proof of life” for species occurrence. Camera trapping can provide an excellent means to attain this inventory data and to obtain the density estimates for tigers and other species. This is highly relevant for tiger conservation as we strive to prevent tiger extinction in the wild.

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Appendix 4.1: Example of a "Set-up" Camera Data Sheet for the Initial Deployment of Camera Traps in the Field

Data Sheet for initial camera set up only

SITE: Royal Manas National Park (RMNP): March- May 2009

Survey #1: camera station SET UP

Camera Station #	Camera #s	Physical location	date (D/M/Y)	GPS location Easting (UTM X)	GPS location Northing (UTM Y)	Road (R), Trail (T), New Trail (NT), Game Trail (G)	width of road or trail in meters	Canopy cover (%) at station *	Land use **	Habitat type ***	Notes
RMNP01	MT04 MT15	Baisuki	11/03/09	90°42.739	26°47.189	T	1.6	80	PA	F	tiger tracks nearby
RMNP02	MT01 MT08	Kawapani	11/03/09	90°38.811	26°47.532	G	1.0	90	PA	F	small animal game trail
RMNP03	MT12 MT14	Zomrong	12/0						PA	F	Old logging road - still occassionaly used by vehicles, cameras locked

* Canopy cover: 0 = 0-10%, 10 = 10 - 20%, 20 = 20 -30%, 30 = 30 -40%, 40 = 40-50%, 50 = 50-60%, 60 = 60 - 70%, 70 = 70 - 80%, 80 = 80 - 90%, 90 = 90 - 100%. ** Land use: P pastural, C crops, PL plantation, PA protected area, R roads, BA built up area. *** Habitat: F forest, W woodland, G grassland, B bushland, T thicket, WG wooded grassland, AA Afro-Alpine, M mangrove, FS fresh water swamp, SS saline swamp (/ = transition between two types), R riverine

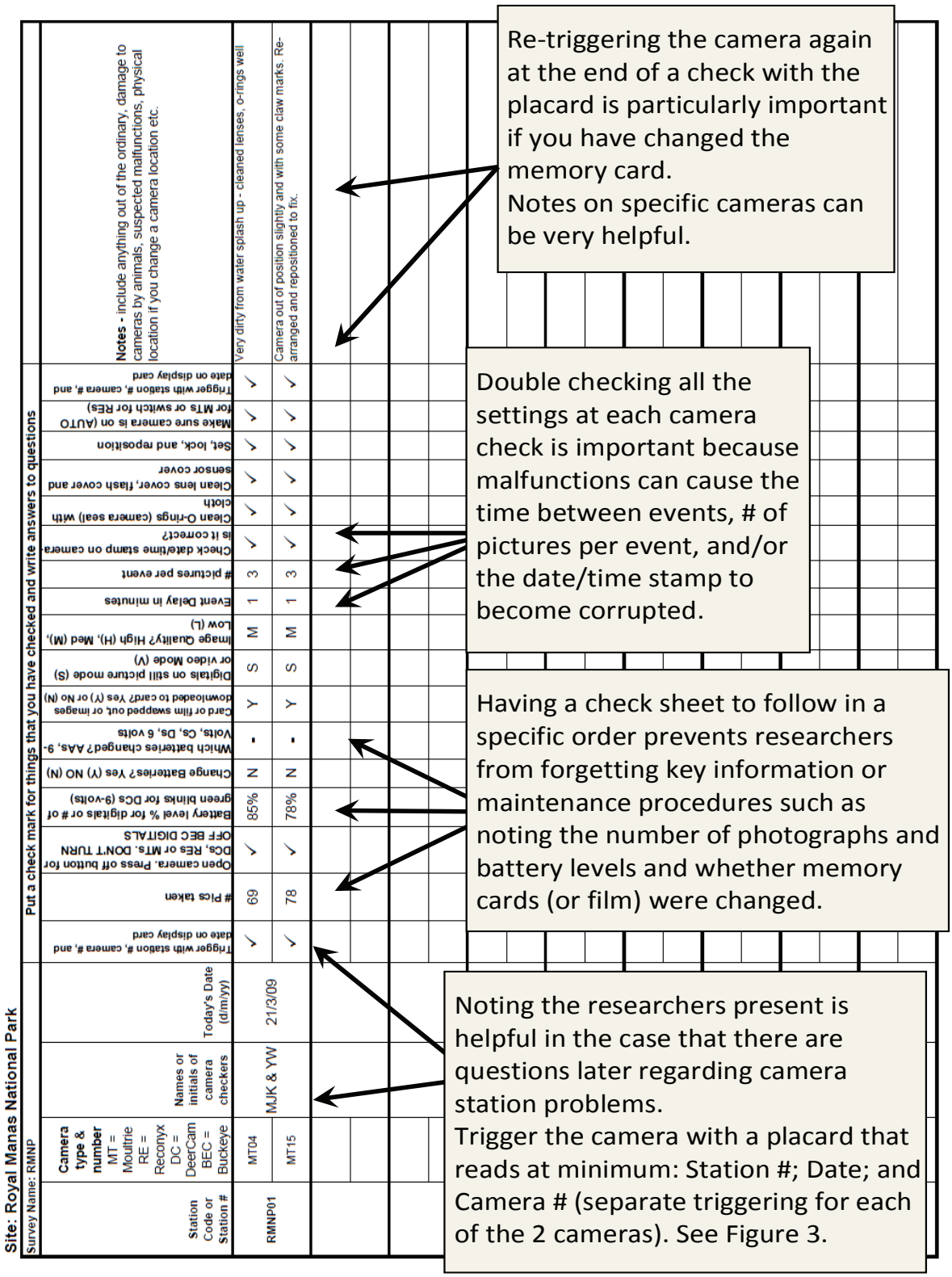
This example uses 2 cameras per station. In this case MT stands for Moultrie brand cameras.

Ultimately, it may be better to record these as UTM coordinates rather than lat/long or decimal degrees since UTM is becoming standard for most studies. In addition, since UTM is in meters, it is very easy to determine distances away from cameras or between cameras even when GPS fails.

Trail width and canopy cover have been shown in other studies to be predictors of detectability and / or habitat use.

Can make codes very specific to the particular study

Appendix 4.2: Example of a Camera Checking Datasheet (Different From a Set-up Datasheet) Used When Monitoring Cameras for Proper Functioning in the Field



Appendix 4.3: Example of List of Equipment to Bring When Camera Trapping

EQUIPMENT FOR CAMERA TRAP CHECKING

Equipment to Bring for All Camera Types

- Map
- Compass
- GPS unit
- GPS coordinates of cameras
- Extra AA batteries for GPS unit
- Radio and/or cell phone
- Data sheet
- Keys for padlocks (if cameras are locked)
- Laminated sheet (or dry erase board) or placard, for writing date, camera, and station number
- Sharpie
- Ball point pen or pencil
- Dry erase pen
- Rag to wipe off dry erase pen
- Extra Bungee Cords or nylon webbing
- Extra ziplock baggies to put film or cards in from cameras
- Extra sign (camera trapping "project sign" if needed)
- Alcohol prep pads for cleaning debris from camera O-rings.
- Umbrella –if raining
- Tape measure (for taking trail measurements)
- Machete, panga, or other vegetation cutting device (for clearing vegetation around camera)
- Swiss army knife, leatherman or some kind of multi-tool
- Weapons to protect yourself from dangerous animals (mace, shotgun, etc.).

Equipment for DEERCAM Cameras

- Two 9-volt batteries per unit
- 2 AA batteries per unit
- Film for each camera unit
- Extra DEERCAM UNIT for malfunctions

Equipment for MOULTRIE Cameras

- D batteries (6 per unit)
- Extra Moultrie camera for malfunctions
- Extra SD memory cards to swap out

Equipment for BUCKEYE cameras

- Charged 6-volt battery (1 per unit)
- Extra Buckeye camera for malfunctions
- Extra SD memory cards to swap out

Equipment for RECONYX cameras

- C batteries (6 per unit) or 8 AAs for some models
- Extra Reconyx camera for malfunctions
- Extra compact flash (CF) memory cards or SD cards depending on the model number to swap out

Note: If it is raining use EXTREME CAUTION because cameras are very susceptible to moisture which causes malfunctions. Use an umbrella or wait until rain ceases.

Appendix 4.4: Example Spreadsheet for Keeping Track of the Number of Traps Nights per Station and Total

Mountain Pine Ridge Forest Reserve 3rd survey (3P) Camera trapping: July - Sept. 2005							
Station # or Code	**Physical Location**	**UTM X**	**UTM Y**	**Date Begin**	**Date End**	**Minus Days of Cam. Malfunc.**	**# of Trap Nights**
3P1	1967 Line off of Oak Burn	0291788	1881313	06/20/05	09/19/05	0	91
3P 2	Pinol Line, 0.5km from Granite Cairn	0292955	1879718	06/20/05	09/19/05	0	91
3P 3	Log trail of Granite Cairn near 1961 line	0295416	1879718	06/20/05	09/19/05	21	70
3P 4	Little track off of North Line	0295115	1881838	06/20/05	09/19/05	11	80
3P 5	Block 8 East Line	0297997	1880908	06/20/05	09/19/05	0	91
3P 6	Butler Line; 1km from Orchard Hill Line	0300005	1882818	06/20/05	09/19/05	0	91
3P 7	Butler Line; 0.5 km from end of line	0297661	1883578	06/20/05	09/19/05	0	91
3P 8	Codd Line	0297180	1878163	06/20/05	09/19/05	0	91
3P 9	Track off Granite	0301235	1877953	06/21/05	09/19/05	19	71
3P 10	Baki Line	0298996	1876168	06/21/05	09/19/05	6	84

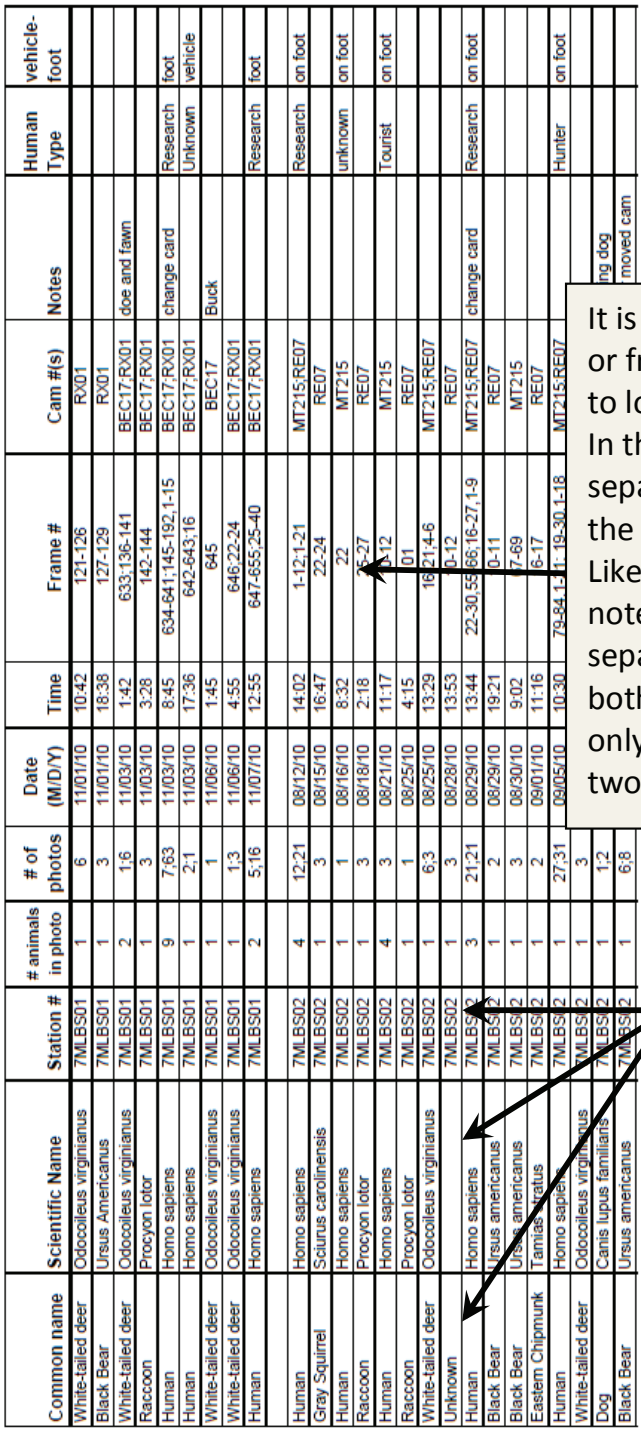
3P 11	Brunton South of Baki Line	0300607	1874659	06/21/05	09/19/05	0	90
3P 12	Devil's Drive	0300635	1872131	06/21/05	09/19/05	0	90
3P 13	Kin Lock	0297143	1870786	06/21/05	09/19/05	0	90
3P 14	Granite Basin Road	0297608	1873835	06/21/05	09/19/05	0	90
3P 15	Morris Road off Winward	0294867	1873067	06/20/05	09/19/05	0	91
3P 16	Rainbow Creek of of Raspa Road	0292615	1872103	06/21/05	09/19/05	26	64
3P 17	Inner Circle near #2 Line	0289947	1873822	06/21/05	09/19/05	0	90
3P 18	Mountain Cow Road by Creek	0295070	1877388	06/22/05	09/19/05	0	89
3P 19	1960 Line near Anderson	0292936	1875862	06/22/05	09/19/05	0	89
3P 20	1960 Line near Windward	0295824	1874995	06/22/05	09/19/05	0	89
3P 21	Espat Road	0289528	1867797	06/22/05	09/19/05	0	89
3P 22	Brunton near Espat Junction	0292463	1869231	06/22/05	09/19/05	1	88
3P 23	Tower Road #2	0289345	1870852	06/22/05	09/19/05	10	79
Average days operational per camera station							**86.04**
Total number of trap nights							**1979**

In the spreadsheet, format the columns 'Date Begin' and 'Date End' as date then use the functions to subtract 'Date End' from 'Date Begin'. Finally, subtract days of malfunction from that to automatically calculate trap nights for each station.

The 'minus days of cam malfunc' column is not automatic and is somewhat tedious to determine as researchers must painstakingly go through the photographs from each station to determine how many days a station may have malfunctioned. In this case, most stations had zero malfunctions but a few had days to 2-3 weeks of malfunction problems. Only if both cameras malfunction at the same time, is the whole station considering non-functioning.

Appendix 4.5: Example of Data Entry Spreadsheet for Raw Photo Data with Two Cameras per Station

We suggest entering all data on all species including non-target species and humans as this information can be important in predicting target species presence or abundance.



Common name	Scientific Name	Station #	# animals in photo	# of photos	Date (M/D/Y)	Time	Frame #	Cam #(s)	Notes	Human Type	vehicle-foot
White-tailed deer	Odocoileus virginianus	7MLBS01	1	6	11/01/10	10:42	121-126	RX01			
Black Bear	Ursus Americanus	7MLBS01	1	3	11/01/10	18:38	127-129	RX01			
White-tailed deer	Odocoileus virginianus	7MLBS01	2	1;6	11/03/10	1:42	633;136-141	BEC17;RX01	doe and fawn		
Raccoon	Procyon lotor	7MLBS01	1	3	11/03/10	3:28	142-144	BEC17;RX01			
Human	Homo sapiens	7MLBS01	9	7;63	11/03/10	8:45	634-641;145-192;1-15	BEC17;RX01	change card	Research	foot
Human	Homo sapiens	7MLBS01	1	2;1	11/03/10	17:36	642-643;16	BEC17;RX01		Unknown	vehicle
White-tailed deer	Odocoileus virginianus	7MLBS01	1	1	11/06/10	1:45	645	BEC17	Buck		
White-tailed deer	Odocoileus virginianus	7MLBS01	1	1;3	11/06/10	4:55	646;22-24	BEC17;RX01			
Human	Homo sapiens	7MLBS01	2	5;16	11/07/10	12:55	647-655;25-40	BEC17;RX01		Research	foot
Human	Homo sapiens	7MLBS02	4	12;21	08/12/10	14:02	1-12;1-21	MT215;RE07		Research	on foot
Gray Squirrel	Sciurus carolinensis	7MLBS02	1	3	08/15/10	16:47	22-24	RE07			
Human	Homo sapiens	7MLBS02	1	1	08/16/10	8:32	22	MT215		unknown	on foot
Raccoon	Procyon lotor	7MLBS02	1	3	08/18/10	2:18	25-27	RE07			
Human	Homo sapiens	7MLBS02	4	3	08/21/10	11:17	10-12	MT215		Tourist	on foot
Raccoon	Procyon lotor	7MLBS02	1	1	08/25/10	4:15	01	RE07			
White-tailed deer	Odocoileus virginianus	7MLBS02	1	6;3	08/25/10	13:29	16;21-6	MT215;RE07			
Unknown		7MLBS02	1	3	08/28/10	13:53	0-12	RE07			
Human	Homo sapiens	7MLBS02	3	21;21	08/29/10	13:44	22-30,55;66;16-27,1-9	MT215;RE07	change card	Research	on foot
Black Bear	Ursus americanus	7MLBS02	1	2	08/29/10	19:21	0-11	RE07			
Black Bear	Ursus americanus	7MLBS02	1	3	08/30/10	9:02	7-69	MT215			
Eastern Chipmunk	Tamias striatus	7MLBS02	1	2	09/01/10	11:16	6-17	RE07			
Human	Homo sapiens	7MLBS02	1	27;31	09/05/10	10:30	79-84,1-11;19-30,1-18	MT215;RE07		Hunter	on foot
White-tailed deer	Odocoileus virginianus	7MLBS02	1	3					ng dog		
Dog	Canis lupus familiaris	7MLBS02	1	1;2					moved cam		
Black Bear	Ursus americanus	7MLBS02	1	6;8							

It is a good idea to note the image or frame number in case you need to locate a particular image later. In this case the semi-colon separates the image numbers of the first camera from the second. Likewise, the camera numbers are noted in the next column separated by a semicolon when both cameras fired. When there is only 1 camera noted, only 1 of the two cameras captured the image.

We suggest starting with common and scientific names followed by the camera station code. In this example 7MLBS01, and the following Station 7MLBS02, are separated by a blank row. The 7 refers to the fact that this is the 7th survey at Mountain Lake Biological Station (MLBS) and the 01 and 02 are different stations.

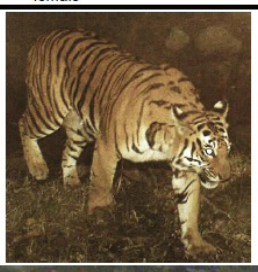
Perhaps the trickiest part is determining the number of independent “events” or “captures”. Most camera studies use either 30 min or 1 hr as the cut-off for when to consider a new capture as another record or a new event. In this spreadsheet each row is an event and new events are not counted until 30 min has passed. However, there can be more than 1 event in a photo such as in the third record where 2 deer were photographed in one picture. The numbers of photos taken are noted with the first number being the one taken by the first camera noted in the Cam #(s) column, and the second number, after the semicolon being the number of photos taken by the second camera.

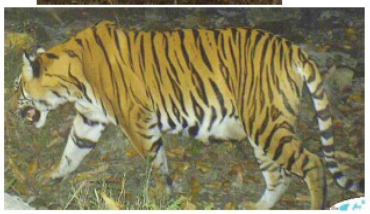
Appendix 4.6: Example of Data Organization for Tigers "Captured" at Remote Camera Stations

Using 2 cameras per stations allows photographs to be obtained from both the right and left sides of the animal. Printing out such reference sheets as this makes for easier identification as new photographs come in from the field. Additionally it is easy to build capture histories for each individual from this reference sheet.

Tigers from Royal Manas National Park (RMNP)

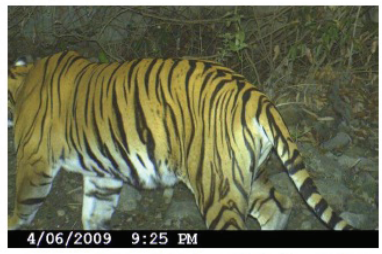
ID	sex
T01	female





dates	time	x-location	y-location	place
02/08/09	19:45	26°79.103	90°97.991	Zomrong
01/11/09	3:43	26°79.103	90°97.991	Zomrong
03/11/09	12:15	26°48.134	90°00.893	Reebalingmin
26/02/10	8:51	26°47.394	90°55.344	Batasi
13/03/10	18:14	26°47.394	90°95.049	Balukhola
15/04/10	11:12	26°52.302	90°42.312	Dangkhapong
12/04/10	20:55	26°79.103	90°97.991	Zomrong
01/05/10	13:18	26°48.134	90°00.893	Reebalingmin

ID	sex
T05	female



dates	time	x-location	y-location	place
04/04/09	20:34	26°47.532	90°38.811	Kawapani
14/04/09	11:35	26°52.532	90°41.910	Serthang
05/05/09	22:31	26°46.320	90°43.214	Rekhajuli
04/06/09	21:26	26°46.320	90°43.214	Rekhajuli
07/06/09	23:25	26°46.320	90°43.214	Rekhajuli
15/06/09	0:38	26°52.532	90°41.910	Serthang
20/6/09	20:34	26°47.532	90°38.811	Kawapani

ID	sex
T06	unknown

dates	time	x-location	y-location	place
21/03/09	5:02	26°46.289	90°42.639	Baisuki
15/04/09	0:44	26°52.302	90°42.312	Dangkhapong
26/04/09	15:19	26°46.289	90°42.639	Baisuki
07/05/09	14:14	26°48.134	90°00.893	Reebalingmin

We suggest using a space between years or surveys for ease in building capture histories. Time should be recorded in military time and we suggest converting to UTM locations rather than lat/long.

While names of places can be used, we suggest using a code that incorporates the survey number or date. For example these stations could be labeled as RMNP01 for Royal Manas National Park camera station 01. The following year could be labeled 02RMNP01 – signifying the 2nd survey as Royal Manas National Park but same location 01.

Appendix 4.7: Example Input Files for Program CAPTURE

Can easily be run from the USGS website by creating input files in notepad and then copying and pasting input files such as these notepad files below in the text boxes directly into the site and click perform analysis.

http://www.mbr-pwrc.usgs.gov/software/capture.html

```
title='jaguar abundance 4Pine 6/17/06 - 9/7/06'
task read captures occasions=42x matrix
format='(T1,A3,1X,42F1.0)'
read input data
J25 000000000010000000001000000000000000000000
J27 011101101111111100101110011111111011111110
J36 100000000000001000000000000000000000001100
J39 000000001001001000001000000000100000000000
J40 000000000000000000000001000000000000000000
J41 101100001010100101001001001110100011100100
J42 000000000000000000001000000000000000000000
J43 000000000101001000000000001000000000000000
J44 000100000001000000000000000000000000000000
J47 000000000001000000000000010000001000000000
J49 000000000001000000000000101001000000000000
task closure test
task model selection
task population estimate all
```

First line – title in quotations, the program does not read things in quotations.

Second line – tells the program there are 42 capture occasion or "days" of the survey.

Third line – what is the data format? T1 is tells the program that the data starts in column one. A3 says that the individual animal ID is 3 characters long (if it said A4 that would mean 4 characters long). 1X means that there is 1 space before the capture history starts. 42F1.0 means that there are 42 capture occasions and that 1 is a capture and 0 is a non-capture

Fourth line – read the input data that follows the above format.

Following the 4th line is the capture history for each individual animal. In this case, 42 capture days.

The final lines are telling the program to conduct the closure test, conduct model selection, and give the estimates for all the models: M(o), M(h), M(b), M(t) and various combinations.

```
title=jaguar abundance 4Pine 6/17/06 - 9/7/06'
task read captures occasions=21x matrix
format='(T1,A3,1X,21F1.0)'
read input data
J25 000001000010000000000
J27 111111110111111111111
J36 100000010000000000010
J39 000011010010000100000
J40 000000000000100000000
J41 110011111011011101110
J42 000000000010000000000
J43 000011010000010000000
J44 010001000000000000000
J47 000001000000010010000
J49 000001000000111000000
task closure test
task model selection
task population estimate all
```

Same data as above, but this time "collapsed: such that there are only 21 instead of 42 "days" of the survey. Each 2 days in collapsed into 1 "day" or capture occasions. This often works better in program CAPTURE due to the large number of zeros in the original data which can cause difficulty in analysis. Too many zeros can cause the program to crash or have difficulty in finding any structure in the data ,leading to the M(o) model being selected as the best model.
We highly recommend collapsing the data in this way – but note the changes in the 4 input lines to reflect the different number of 'days' in the capture history.

Appendix 4.8: Example Input Files for Program MARK

Input files (file_name.inp) created in notepad for a single group or divided by sex. Do not include titles below.

Input- One group – capture history for each individual for 34 days. Capture (1) and no capture (0). Last column indicates 1 groups: one abundance estimates for whole population

```
0110001000000110010000010000000000  1;
0000000100000101001000010000000000  1;
1010010100101110011110001100001010  1;
1111101111100100111111100111110000  1;
0001000000010101010100000110111010  1;
1101001110110101101111101101110001  1;
1001100011010100000010100011100101  1;
0100100000011110001100110010010011  1;
0000000000000100110000000010010100  1;
0000100100001011000011010000000000  1;
1000100000001000000000110000000000  1;
0000100000000000001110000000010000  1;
0000000000000000 0 00000000000001  1;
0000000110101010000000000001001000  1;
0000000000000000000001000001000000  1;
Etc…
```

Input- Two groups – capture history for each individual for 34 days. Capture (1) and no capture (0). Last 2 columns indicate 2 groups: male (1 0) and female (0 1). Two abundance estimates – 1 for each sex.

```
0110001000000110010000010000000000  0 1;
0000000100000101001000010000000000  0 1;
1010010100101110011110001100001010  0 1;
1111101111100100111111100111110000  0 1;
0001000000010101010100000110111010  1 0;
1101001110110101101111101101110001  1 0;
1001100011010100000010100011100101  1 0;
0100100000011110001100110010010011  1 0;
0000000000000100110000000010010100  1 0;
0000100100001011000011010000000000  1 0;
1000100000001000000000110000000000  0 1;
0000100000000000001110000000010000  1 0;
0000000000000000 0 00000000000001  0 1;
0000000110101010000000000001001000  0 1;
0000000000000000000001000001000000  0 1;
Etc…
```

Input- One group sex as a covariate– capture history for each individual for 34 days. Capture (1) and no capture (0). Last 2 columns indicate 2 groups: male (1 0) and female (0 1). Two abundance estimates – 1 for each sex.

```
0110001000000110010000010000000000  1  1;
0000000100000101001000010000000000  1  1;
1010010100101110011110001100001010  1  1;
1111101111100100111111100111110000  1  1;
0001000000010101010100000110111010  1  0;
1101001110110101101111101101110001  1  0;
1001100011010100000010100011100101  1  0;
0100100000011110001100110010010011  1  0;
0000000000000100110000000010010100  1  0;
0000100100001011000011010000000000  1  0;
1000100000001000000000110000000000  1  1;
0000100000000000001110000000010000  1  0;
0000000000000000 0 00000000000001  1  1;
0000000110101010000000000001001000  1  1;
0000000000000000000001000001000000  1  1;
Etc…
```

Appendix 4.9: Example Input Files for Program DENSITY

Input files (File_name.inp) created in notepad for a single group or divided by sex. Do not include column headings or titles. Below, there is only one "session" or survey.

Camera station codes and locations – required for all data entry formats

Station	UTM_X	UTM_Y
VOH01	749271.1006	7650068.358
VOH02	749333.5212	7650599.186
VOH03	749545.745	7651120.356
VOH04	749317.8578	7651757.134
VOH05	749806.2043	7651663.242
VOH06	750056.9424	7651180.293
VOH07	750395.6051	7650723.296
VOH08	749810.8982	7650780.468
VOH09	749308.4597	7649553.647
VOH10	749752.6581	7649832.653
VOH11	750082.4613	7650326.717
VOH12	750570.7086	7650158.59
VOH13	750596.9657	7649677.087
VOH14	750130.7628	7649408.716
VOH15	750367.3902	7648739.212
VOH16	750851.2446	7648376.684

1 Group = 1 density estimate for all animals

Session	animal ID	day of capture	place of capture
1	F02	1	VOH07
1	F010	2	VOH10
1	F011	2	VOH13
1	F011	2	VOH12
1	F015	2	VOH16
1	F09	2	VOH09
1	F010	3	VOH11
1	F015	3	VOH16
1	F05	3	VOH07
1	F09	3	VOH09
1	F010	4	VOH11
1	F010	4	VOH10
1	F013	4	VOH22
1	F016	4	VOH17
1	F05	4	VOH07
1	F01	5	VOH01
1	F01	5	VOH20
1	F015	5	VOH16

Etc….

2 Groups = males (1 0) and females (0 1) – two density estimates one for each sex

Session	animal ID	day of capture	place of capture	sex code
1	F02	1	VOH07	1 0
1	F010	2	VOH10	1 0
1	F011	2	VOH13	1 0
1	F011	2	VOH12	1 0
1	F015	2	VOH16	0 1
1	F09	2	VOH09	1 0
1	F010	3	VOH11	1 0
1	F015	3	VOH16	0 1
1	F05	3	VOH07	0 1
1	F09	3	VOH09	1 0
1	F010	4	VOH11	1 0
1	F010	4	VOH10	1 0
1	F013	4	VOH22	1 0
1	F016	4	VOH17	1 0
1	F05	4	VOH07	0 1
1	F01	5	VOH01	1 0
1	F01	6	VOH20	1 0
1	F011	7	VOH26	1 0
1	F011	7	VOH12	1 0

Etc…..

1 Group = 1 density estimate - sex as a variable that influences the single density estimate

Session ID	animal capture	day of capture	place of covariate	sex
1	F015	25	VOH16	0
1	F015	29	VOH16	0
1	F016	4	VOH17	1
1	F016	9	VOH17	1
1	F016	24	VOH17	1
1	F019	26	VOH24	0
1	F02	1	VOH07	1
1	F02	18	VOH07	1
1	F02	19	VOH06	1
1	F02	21	VOH05	1
1	F020	15	VOH25	0
1	F020	21	VOH25	0
1	F020	23	VOH25	0
1	F020	33	VOH25	0
1	F021	13	VOH26	0
1	F021	26	VOH26	0
1	F022	12	VOH25	1
1	F022	13	VOH25	1

Etc….

CHAPTER 5

Application of Radiotelemetry to Wildlife Conservation in Mountainous Asian Landscapes

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Background

Understanding animal behavior can be fundamental to effective conservation. Knowing where an animal spends its time as it goes about its daily business can tell us a lot about its habitat needs, resources that are key to its survival and reproduction, what it considers "home," and ultimately the portions of a landscape critical to the population to which the animal belongs. Radiotelemetry has become the primary tool for understanding these important aspects of animal behavior.

Placing transmitters on animals allows us to collect geographic locations for those animals over time, providing a wealth of information. Analysis of these locations allows us to answer questions that would be difficult to address any other way. For example, locations can tell us about an animal's home range, i.e., that part of the landscape where an animal confines its movements, presumably finding all the resources (food, water, shelter, mates) that it needs to survive and reproduce. If we can relate locations to environmental characteristics, we can learn more about what those resources are and thus begin to understand what constitutes habitat for our study animals. Looking at sequences of locations allow us to understand how an animal moves through its habitat, e.g., where it moves quickly and where it lingers, providing insights into how it makes use of the resources available within its habitat. Answering such questions for a sufficient number of animals gives us insights into how a population of animals uses a landscape (e.g., areas of high densities, seasonal home ranges, and movement corridors), providing key information for conservation and land use planning.

As with any other scientific technique, though, the full potential of radiotelemetry for research and conservation cannot be achieved without a clear understanding of the questions it is used to address, and the technology, sampling design, and analyses appropriate to answering those questions. In other words, simply

deploying telemetry collars on animals and hoping to learn much from them without careful prior planning is very unlikely. This chapter is designed to familiarize the reader with the questions that can be answered using radiotelemetry, the equipment currently available for producing location data, and a general introduction to analyses that use those data to answer the questions.

Questions Appropriate to Telemetry Studies

The fundamental motivation for research designed to inform effective conservation is providing information that is needed but presently unavailable. The best way to identify such information gaps is to ask questions that make clear how research should be designed to address them. For example, it is scientifically ineffective to begin with the notion that animals need to be radiocollared so we can follow them. *Why* do we need to follow them? *What* is it we do not presently know that locations, or survival status, will help us to understand? These are critical questions because they strongly affect how a telemetry study should be designed—no one approach to collecting radiotelemetry will be appropriate to answering all questions equally well. Questions that are good for motivating and designing a telemetry study include (but are not limited to):

- What are the annual home ranges for animals?
- Do animals have seasonal home ranges?
- How big are animal's home ranges?
- Where do animals spend most of their time?
- What features of the landscape are most important to animals?
- What migratory routes do animals use?
- How important are mature forests (or grasslands, or other vegetation types) to animals?
- What is the distribution of habitat quality for animals on a landscape?
- How do planned human activities affect habitat quality?
- What are the effects of human activity on animal movements and habitat use?
- What are the survival rates of animals, and what are the primary causes of mortality?

Knowing which questions are best to ask in order to address a given conservation need can be challenging. The following definitions of concepts common to radiotelemetry can be useful for developing good questions.

Home range

Animals that exhibit site fidelity, where they confine their movements to a distinct area for extended periods of time, are said to have home ranges, in contrast to animals that are nomadic or dispersing that do not exhibit confined movements. An animal's home range is the place it chooses to live, given what is available. Burt (1943) outlined the basic concept of an animal's home range:

> *That area traversed by an individual in its normal activities of food gathering, mating, and caring for young. Occasional sallies outside the area, perhaps exploratory in nature, should not be considered part of the home range.*

This definition is conceptually complete, but can prove challenging to quantify using radiotelemetry because it can be hard to know what is a "normal activity" or an "occasional sally." Nonetheless, locations collected with radiotelemetry can provide unprecedented insights into an animal's home range if we assume that time spent by an animal in different areas represents the importance of those areas. Thus, where telemetry reveals clusters of locations, we know an animal spent a portion of its time in a specific area, suggesting these areas contain something important to the animal. Similarly, dispersed and infrequent locations suggest places of little importance that animals rarely visit.

This thinking underlies nearly all analyses of home range behavior, though it can sometimes be misleading. Consider an animal with access to a single water source regularly attended by predators. That water source is obviously critically important to the animal, but spending a great deal of time at it increases the likelihood of being killed by a predator. Thus, one might not see a lot of locations at that water source, although it is critically important. In this case, only a solid biological understanding of the animal being studied, as well as some knowledge of how the resources it needs are distributed on the landscape, can prevent drawing the erroneous conclusion (with potentially dire conservation consequences) that little time spent at the water source indicates the water source is not important.

A home range estimated from telemetry locations can provide a number of insights into animal behavior. Home range size reveals how much of the landscape an animal needs. Also, a strong relationship exists between size of a home range and abundance and productivity of resources that contribute to survival and

reproduction; where home ranges within a species are relatively small, one can infer rich resources, where they are relatively large one can infer poor resources. Where animals are territorial (i.e., they exclude conspecifics from their home ranges), the size of home ranges within a population can help determine the maximum density of those animals on a landscape. Perhaps more interesting than home range area, though, are the behavioral dynamics telemetry locations reveal within a home range—where animals concentrate their time, and how such concentrations vary spatially (revealing areas of high and low importance) and temporally (revealing areas whose importance changes seasonally) within a home range. Many animals will exhibit one or more "cores" to their home ranges, a smaller area than the entire home range where they spend most of their time, and analyses of such cores often reveal resources critical for animals.

Habitat use

Few words are more carelessly used in wildlife ecology than "habitat." Considering all the ways habitat is presented in the literature, it can mean almost anything we want it to—land cover classes (e.g., blue pine forest habitat, mixed conifer forest habitat, warm broad-leaved forest habitat), species-specific classifications (e.g., tiger habitat), and ecological classifications (e.g., riparian habitat). Before embarking on habitat analyses using telemetry data, careful thought is needed to understand just what habitat means for a particular study—no single definition of habitat will be universally appropriate.

Most wildlife ecologists recognize that habitat is defined by the resources an animal needs to survive and to reproduce. This is the essential beginning point for defining habitat in any radiotelemetry study. Note that resources in this case can be defined very broadly, including the actual food an animal consumes, conditions needed for mating, places for escaping predators, travel routes, etc. The important point for a telemetry study is to identify those resources believed to structure how an animal spends its time within its home range. In some cases, perhaps where an animal is highly specialized, a single recognizable type of environment might provide all such resources. Defining habitat can be relatively simple for such species, provided sufficient information is available to map where their needed environment occurs and where it does not. Most animals, however, must obtain their needed resources from a variety of environments, particularly species that occupy large portions of a landscape. In this case, habitat definitions convenient to researchers (e.g., land cover classes) may provide little or no information on how critical resources for an animal are distributed on a landscape, and are thus likely to be poor predictors of where animals spend their time.

Under most circumstances, therefore, defining habitat in a way likely to reveal insights useful to effective conservation will require much thought and effort in advance, along with creative uses of available data. This latter point is a critical one for a radiotelemetry study that seeks to understand an animal's habitat. Few environmental data are available that directly depict the resources contributing to survival and reproduction of animals, therefore surrogates that depict such resources indirectly (e.g., cover type, stand age, soil type, etc.) are commonly used. Valuable information about resources is generally lost to some degree whenever a surrogate is used, but this simply cannot be avoided. When surrogate habitat variables are selected, therefore, it is worth thinking about how much information is lost; in other words how tightly linked is the surrogate to the actual resource? Those surrogates where linkage is tightest will be the most useful to understanding an animal's habitat. Where linkage is loose, at best, surrogates can actually be quite misleading. The overwhelmingly important consideration here is to ensure that the biological justification for selection of environmental characteristics, be they GIS layers or field data on plant or prey distributions, is as strong as possible.

Movement

Sequences of location data obtained using radiotelemetry allow us to infer how animals move, including the distances they cover and the speed at which they were traveling. Obviously, animals move for a variety of reasons: to reach new food sources, to find mates, to find a new home, to protect territorial boundaries, to make use of seasonally available resources. Before embarking on a telemetry study of movement, it is important to distinguish between 3 types of animal movement: 1) dispersal, 2) migration, and 3) day-to-day movements within a home range. Dispersal is the act of abandoning a natal home range and setting out to find a new home range on the landscape. Dispersal is thus unidirectional (from an old home range to a new one, with no return), generally occurring once in an animal's life. Dispersal movements can be very long compared to those observed within a home range, prior to or after dispersal. Migration can also consist of very long movements, but unlike dispersal it is generally bi-directional, with animals regularly returning to places within their annual home ranges. Most commonly, migratory movements are periodic over the course of a year, where animals move back and forth between different seasonal home ranges to take advantage of resources available during only a portion of the year. Relative to dispersal or migration, day-to-day movements of an animal within its home range tend to be relatively short, reflecting the daily activities of an animal as it seeks and consumes food, makes use of available shelter, and interacts with other animals (e.g., conspecifics, predators, prey).

Survival rates and cause of mortality estimation

Sometimes the questions of interest center on estimating survival (1 - mortality) or identifying the primary factors affecting survival. Although survival can be estimated from capture-mark-recapture data, the required number of captures is prohibitively large except for all but the most common species. With radiotelemetry, you can not only know if the animal died, but with regular monitoring you can determine when the animal died, and even how (provided the carcass is located quickly following death). Thus, radiotelemetry is the method of choice for estimating survival, often in concert with some of the other objectives described in this chapter.

If you are planning to estimate survival with radiotelemetry, be sure to consult with the manufacturer before ordering radiocollars. Often, additional features can be added to the transmitter to help you identify mortality. For example, with VHF collars, a "mortality sensor" can be added that changes the pulse rate after a pre-determined set time of very limited activity. For example, the pulse rate might double after the collar has been still for 4 hours (live wild animals rarely stay completely still for very long).

As with all the other issues we've discussed, it is important to clearly define the objectives of a survival study before beginning. Here are just 3 reasons why clearly defining objectives is important. First, it determines your sampling schedule. Generally weekly determinations of alive vs. dead are adequate for survival estimates, but depending on the study species and questions asked, you may need to sample more often (it would be rare to sample less often than weekly for good survival estimates). Second, although telemetry can be quite powerful for facilitating estimates of how particular environmental variables (e.g., weather, disease) or individual variables (e.g., sex or age, dispersal status) affect survival, if you are going to include these so-called "covariates", you must be sure to record them at the animal's location as you collect data on whether the animal is alive. Third, your question will determine sample sizes, which are not trivial for accurate survival estimation, especially with covariates. We'll describe these issues more in the section on analysis methods below.

Radiotelemetry Basics

Radiotelemetry involves placing a radio transmitter, often in the form of a collar, on an animal that then carries that transmitter as it goes about its daily behaviors. The transmitter broadcasts a radio signal that can be detected by a receiver, which allows the estimation of where the animal was at the time a radio signal was received. This approach was first employed by John and Frank Craighead in 1965

during their studies of grizzly bears *(Ursus arctos)*. Technology has improved considerably since then and radiotelemetry has become a highly advanced and diverse methodology for studying and monitoring animals, revolving around 2 distinct technologies: 1) radiotracking using very high frequency (VHF) transmitters and receivers, and 2) transmitters that communicate with global positioning satellites to obtain locations. This section will cover general concepts for each. Technical considerations and field methods for addressing each of these will be covered in the methodologies section, below.

VHF telemetry

Radiotelemetry using VHF is essentially the same technology first developed by the Craigheads; though technology and techniques have advanced, the approach remains essentially the same. The transmitter attached to the animal broadcasts a repeating, omni-directional radio signal in the VHF band of the radio spectrum (30 to 300 megahertz, MHz). An observer equipped with a radio receiver tuned to the same frequency of the transmitter will hear the radio signal as a series of continuous beeps. When a directional antenna is attached to a receiver, moving the antenna in an arc from side to side will result in variation in the loudness of the beeps, with the loudest beeps generally occurring when the directional antenna is pointing directly at the transmitter. Thus, an observer can discern the direction to the animal.

Knowing the direction to an animal allows the researcher to determine location in one of two ways: 1) the observer can walk toward the animal in the direction indicated by the loudest beeps until making visual contact with it (i.e., homing), or 2) the observer can collect multiple bearings toward the same animal from different, known locations in quick succession; plotting these bearings allows estimation of the animal's location through triangulation. Which of these approaches is best to use will depend on the frequency of desired locations (homing can take longer than collecting multiple bearings for triangulation), the extent to which observer effects on animals are a concern (homing may disturb an animal if it sees the observer, causing it to change its behavior), and study specifics (homing may be impractical for a large number of collared animals or those that inhabit mountainous country; on the other hand, if study objectives require sighting the animal, homing will be required).

Transmitters that broadcast in the VHF spectrum are ideal for homing and for triangulation because, unlike transmitters using different portions of the radio spectrum (e.g., ultra high frequency; UHF) they do not require that the space between the transmitter and receiver be free of obstacles (i.e., clear "line of sight").

Thus, a VHF radio signal can be received even if the transmitter is behind a modest hill or ridge line (although extreme terrain features such as deep canyons or large mountains can block a VHF signal). The reason this is possible is because VHF signals can "flow" across terrain, not unlike air or water currents. This provides the obvious advantage of allowing location of animals using VHF telemetry in a variety of topographical conditions. The disadvantage of this characteristic of VHF radio waves, however, is that topography can "bend" radio signals to an extent, much in the same way it changes the direction of wind. Thus, it is imperative that observers be aware of the terrain inhabited by animals wearing transmitters and how it can affect VHF radio signals.

Effective sampling using VHF radiotelemetry can be challenging, requiring the observer to quickly and accurately identify the direction to an animal using radio signals, maintain a constant awareness of how terrain might be affecting those signals, and covering a lot of ground while homing or moving between sampling points. VHF telemetry is thus a learned skill, and for many practitioners an art that is continually improved through experience. Further, for most radiotelemetry studies, multiple observers are required to collect locations on multiple animals over long periods of time. Therefore, one of the most challenging (and expensive) aspects of VHF telemetry is maintaining a cadre of well-trained observers. Effort and funding needed to ensure high-quality observers is well-spent for a VHF study; without trained observers that can consistently and reliably produce accurate locations under even the most demanding circumstances, even a large number of collared animals are unlikely to produce robust insights useful for conservation.

The benefit of using VHF telemetry is that it is a well-established and generally cost-effective methodology. Until the advent of satellite telemetry, VHF telemetry was the only means of obtaining animal locations and therefore the research backbone of home range, habitat, and movement studies over the past few decades. Accordingly, much of the software readily available for analyzing radiotelemetry locations is well-suited to VHF data. VHF collars are also relatively inexpensive, such that approximately 15 VHF collars can be purchased for the price of a single satellite collar. The most obvious downside of VHF telemetry is that the number of locations obtainable is limited to the sampling schedule which trained observers can maintain; even the most ambitious and well-manned VHF telemetry crew will collect at least an order of magnitude fewer locations than satellite collars, and with larger associated location error. Further, the relatively low expense of VHF collars can be more than offset by the relatively high expense of using human observers, once training, salaries, transportation, and logistics to support field work are considered.

Satellite telemetry

The most widely used satellite telemetry system is the Global Positioning System (GPS). It is maintained by the U.S. government and is freely available to anyone with a GPS receiver. The system consists of receivers on the ground and over 24 satellites orbiting the earth. When a GPS receiver on the ground has an unobstructed line of sight to ≥ 3 GPS satellites it can calculate its latitude (i.e., distance north or south of the equator) and longitude (i.e., distance east or west of the prime meridian) which is known as a 2-dimensional (2D) position fix. When a GPS receiver has an unobstructed line of sight to ≥ 4 GPS satellites it can calculate its latitude, longitude, and altitude which is known as a 3-dimensional (3D) position fix. To calculate positions, GPS receivers compute the distance to each satellite. These distances and the satellites' locations are used in an algorithm to compute the position of the receiver to an accuracy of within 15 meters.

Another satellite telemetry system is the Advanced Research and Global Observation Satellite system (Argos). It is a cooperative program between the United States' National Oceanic and Atmospheric Administration and France's Centre National d'Etudes Spatiales. The system consists of polar-orbiting satellites, platform transmitter terminals (PTTs) which, for example, are attached to wildlife telemetry collars, and global data processing centers. The PTTs are located using the Doppler Effect and send signals with their location information to satellites. Data from satellites are then sent to data processing centers where the data are made available to users in an internet-accessible database. Location data collected via Argos are only accurate up to 150 meters and locations are collected irregularly throughout the day. If greater accuracy and more regular locations are needed, a GPS receiver is added to an Argos PTT. When GPS is included, GPS locations collected by the receiver are coded in Argos messages and relayed to the data processing centers.

Obviously, satellite telemetry does not require observers because the collars are self-locating. Both GPS and Argos collars must be programmed prior to placement on animals to specify the frequency with which the collars will attempt to locate themselves. The frequency of locations desired for a particular application is determined based on study objectives (i.e., there may be no need for locations recorded every 10 minutes if research questions revolve around seasonal movements) and the trade-off between frequency of locations and battery life (more frequent locations means shorter battery life).

The most obvious benefit of satellite telemetry is that it allows the collection of a large number of locations 24 hours a day and in all weather conditions with

minimal personnel time and logistical requirements. As a result, satellite collars are better suited than VHF collars for studies evaluating highly detailed questions about wildlife movement, space use, and resource selection where large sample sizes of locations with a high degree of precision and accuracy are needed. Satellite telemetry, however, still has several limitations, the most obvious being cost. The high initial costs of satellite collars often limits the number of animals that can be monitored compared to VHF collars, which can substantially reduce the ability to generalize the findings of a satellite collar study. The cost of satellite collars increase from GPS store-on-boards, to store-on-boards with remote download capabilities, to store-on-boards with an Argos uplink. When using Argos, in addition to the cost of the collar there are also nominal monthly fees that depend on the number of collars with an Argos uplink and the frequency and duration of Argos transmissions. Finally, the size and weight of satellite collars can limit their use on small animals, though ongoing refinement of collar and battery technologies is constantly reducing satellite transmitter size and weight and thus increasing the number of species that can be studied using this technology.

Telemetry Equipment

VHF collars, receivers, and antennas

VHF transmitters can be attached to animals in a variety of ways; they can be mounted on backpacks for birds and bats, glued to the shells of turtles, or implanted internally in small animals (e.g., fish, rodents, and snakes). Attachment of transmitters to animals using collars is perhaps the most common approach, and this chapter will thus focus on radiocollars, though the principles of VHF telemetry apply equally to other forms of transmitter attachment. VHF collars are relatively simple, generally containing a combined radio transmitter and battery pack molded permanently into a portion of the collar (Figure 5.1A).

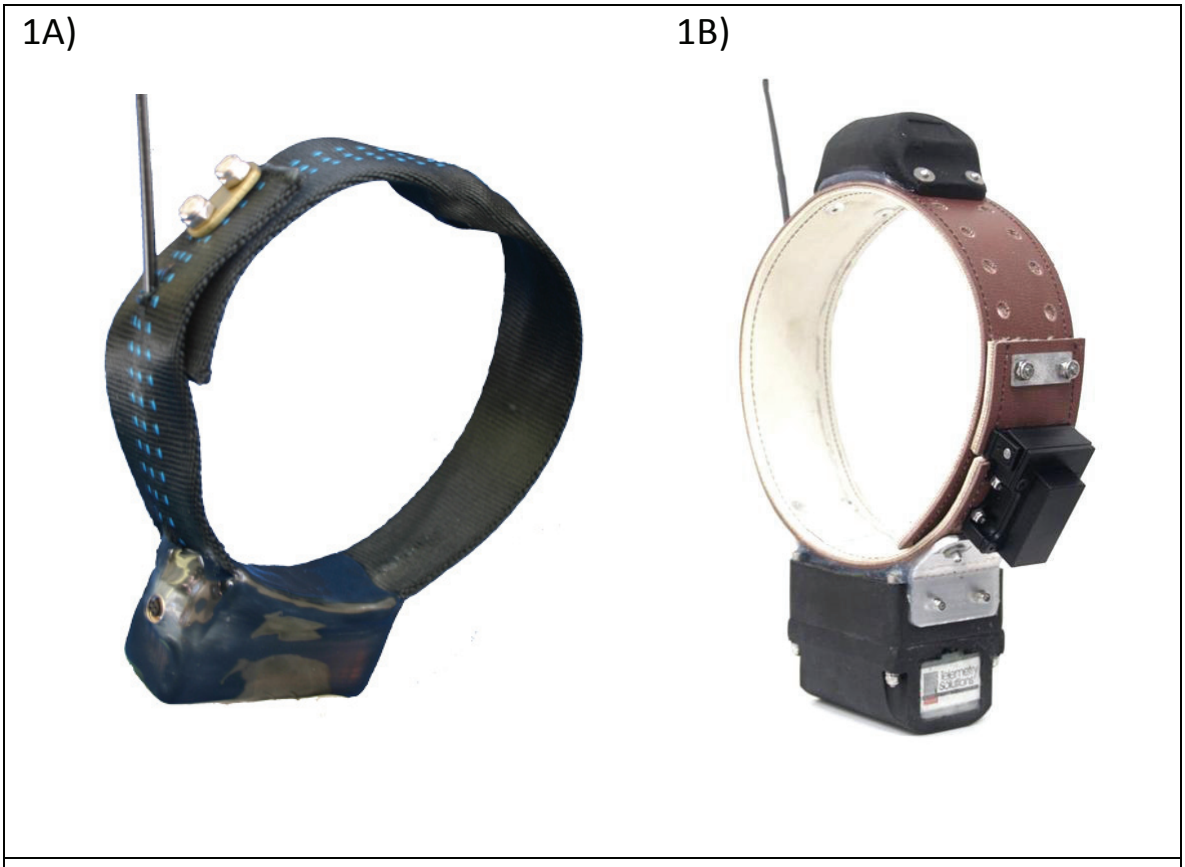

Figure 5.1. Collars commonly used for radiotelemetry: 1A) Very high frequency (VHF) collar, 1B) satellite collar.

Depending on the researcher's preferences, the antenna for the transmitter can be external to the collar or embedded within it (reducing transmission range slightly). Collars are generally fixed about an animal's neck by bolting the 2 loose ends of a collar together. Some collars come with spacers included in the collar that are made of a material that will deteriorate over time so that the collar will drop off once the material has rotted through. Each collar broadcasts on a unique VHF frequency; upon ordering a set of collars, the user specifies the preferred portion of the VHF band to the manufacturer of the collar (i.e., 164 MHz), and the manufacturer sets individual frequencies of collars within this band by varying them from 0.1 to 0.001 MHz (i.e., 164.995, 164.985,..., etc.). Whereas manufacturers will take great care to ensure that collars delivered in the same order will all have unique frequencies, they will have no way of knowing that collars from different orders, perhaps from different projects entirely, being used in the same study area will have different frequencies. This can be highly problematic if a project studying takin (*Budorcas taxicolor*) in Jigme Dorji National Park orders collars that have the same frequencies as those produced by a different company for a study on snow

leopard in the Park. Thus, coordination among researchers using VHF collars to study animals in the same area is essential.

Telemetry receivers for hearing radio transmissions from collars come in a variety of styles, capabilities, and prices (Figure 5.2a). Telemetry receivers tend to be one of the most expensive investments made for a VHF telemetry research project; this is because they are highly sensitive in order to pick up the very weak transmissions produced by VHF transmitters in collars. Each receiver is generally programmed to receive only a portion of the VHF band, therefore the programming of the receiver must match the frequencies at which collars are broadcasting. Every receiver comes with the capacity to change frequencies (within its pre-programmed portion of the VHF band), and to change the volume and gain of incoming radio signals. Additional features found on some receivers include the ability to scan across a band of frequencies and the ability to program desired frequencies that can be called up as needed.

To pick up radio transmissions from transmitting collars, a telemetry receiver requires an antenna. Antennas come in multiple forms. The simplest is an omni-directional, "whip" antenna (Figure 5.2b) that has the ability to detect a radio signal but cannot be used for direction finding. An omni-directional antenna is generally mounted on a vehicle to search for a collared animal over large areas. When the VHF beacon from a collar is heard on an omni-directional antenna and the user wishes to determine the direction to the collared animal, switching to a Yagi antenna is required (Figure 5.2c). There are multiple forms of Yagis, but all of them consist of parallel "elements" placed along a "beam", with width of elements increasing from the front of the beam to the rear. Antennas with longer beams and more elements are more sensitive (i.e., can pick up weaker signals, or signals from longer distances) than those with shorter beams and fewer elements. The width of the elements, as well as the distance separating them on the beam, is unique to a particular frequency range. Thus, the frequency range capabilities of the antenna, the receiver, and the collars all have to match.

Satellite collars

Because satellite telemetry uses power-hungry GPS technology, the components and large batteries needed to collect GPS locations has historically limited satellite telemetry to large animals capable of carrying the bulky, heavy collars. This is changing rapidly as components become smaller and efficiency of batteries continues to improve, such that satellite telemetry is now being used on large birds and medium sized carnivores. Currently, collars remain the predominate means of tracking animals using satellite telemetry. Satellite collars superficially resemble

2a)

2b)

2c)

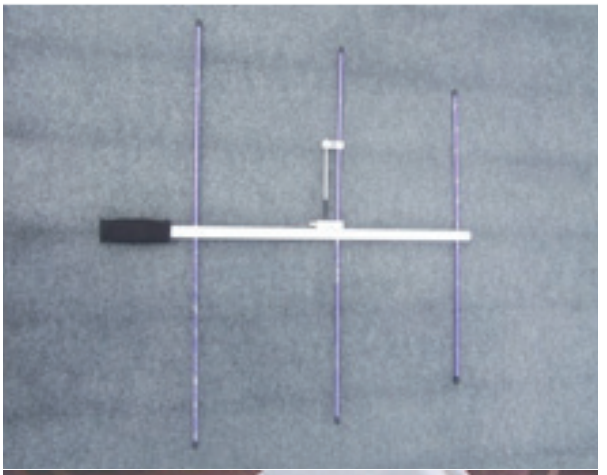

Figure 5.2. Equipment used for conducting radiotelemetry using very high frequency (VHF) equipment: 2a) telemetry receivers, 2b) omni-directional "whip" antenna, 2c) yagi antennas.

VHF collars, except they are larger. They will typically include a VHF transmitter (which transmits a beacon for locating the collar via homing), transmitters, processors and antennas for communicating with satellites, and a large battery pack (Figure 5.1b). Satellite collars currently are available in 4 types that vary in how data are collected and processed: 1) GPS store-on-board, 2) GPS remote download, 3) GPS with Argos uplink, and 4) Argos only. Global Positioning System store-on-board collars acquire GPS locations and store them in the collar's memory but do not transmit them to the user remotely. The user must physically recover the collar to download the GPS data. Collars are recovered when an animal dies or is killed, if an animal is recaptured, or with the help of an optional drop-off mechanism. Drop-off mechanisms are devices built into collars that are either pre-programmed to cause the collar to detach after a specified time interval or are remotely activated by the user causing the collar to detach. Once the collar has detached, the user can use the VHF beacon included on the collar to home in to its location. Store-on-board collars are limited in that data can only be downloaded when the collar is recovered. If an animal migrates or disperses and their VHF beacon cannot be found or if the collar's battery dies before it is recovered then the collar is lost and no data can be recovered.

Global Positioning System store-on-board collars may be accessorized to have remote download capabilities. With this feature, users can periodically download GPS locations stored in the collar's memory using a handheld command unit (HCU). The HCU is attached to 1 of 2 antennas; an omni-directional antenna for a range of < 200 m or a Yagi antenna for a longer range. The maximum range from which a user can successfully retrieve GPS locations depends on the terrain and the position of the animal. If a VHF beacon is clearly audible then the user should be able to remotely download that collar's GPS locations. By periodically downloading location data the user is not completely reliant on recovering the collar to get data. However, collars with remote download capabilities are limited by transmission range and constraints on personnel time, logistics, weather, and daylight requirements for data recovery by foot, vehicle or aircraft.

GPS store-on-board collars may also be accessorized to have an Argos uplink. An Argos uplink allows GPS locations stored on the collar's memory to be transmitted to an internet-accessible database via Argos satellites. The amount of location data transmitted depends on the user-programmed duration and frequency of Argos transmissions. Location data not transmitted via Argos are saved on the collar and can be downloaded when the collar is recovered. The Argos uplink allows users to retrieve GPS data from the internet instead of having to go into the field which minimizes personnel time requirements and eliminates any logistic, weather, or

daylight requirements. However, the Argos uplink is very expensive and Argos transmissions cause the collar's battery to be depleted faster. It is also possible to have a collar with an Argos uplink only (i.e., no GPS receiver). Location data collected via Argos are only accurate up to 150 meters and locations are collected irregularly throughout the day.

Satellite telemetry is a developing technology with new, more advanced satellite collars being developed annually. For example, collars have been developed that couple Globalstar transmitters, a satellite voice and data service, with GPS receivers. This allows location data to be sent from the field to the internet in real time. Collars have also been developed that transmit GPS location data to the user over the GSM mobile phone network. In the following sections we will only focus on GPS and/or Argos collars.

Methodologies

Collaring animals

It is beyond the scope of this chapter to address handling capture animals in any kind of detail. Obviously, in order to fit an animal with a radio transmitter it must be compliant, requiring either restraint or drug-induced immobilization. Methods for restraint and immobilization that ensure safety of both the animal and the researcher are species-specific and require extensive training. Even a safe handling of a captured animal, however, is traumatic and so can come at a cost to that animal, either in the form of stress, injury, the energetic cost of lugging around the telemetry device the researcher attaches to it, or in the worst case, death. Therefore, before attempting to fit an animal with a radiotelemetry device it is paramount to address the ethical justification for doing so. Simply put, are the costs you as a researcher are asking the animal to bear worth what you will gain in terms of knowledge or conservation? For many species under many circumstances, the answer can be "yes" because knowledge and effective conservation are so badly needed that costs (minimized as much as possible) to study animals can be deemed justifiable. But this is not always the case. Non-invasive methods (several are also covered in this book) are making great advances in the information they provide on animals. Though they may never offer the exact same information as radiotelemetry, it may be more than sufficient to address the questions that need to be answered for effective conservation.

Once the decision has been made to collar an animal, great care needs to be taken to minimize the chance the collar will cause harm. This is based not only on ethical obligations, but also on sound research sense—an animal that is suffering will not behave in a way that will provide the scientific insights that are being sought. The

most obvious way to minimize chance of harm is to ensure the collar will fall off once it is no longer useful (i.e., once the battery has died). Inserting spacers made of a material that deteriorates over time (e.g., cotton, surgical tubing) into collars can be a very effective way of accomplishing this.

Another way to minimize harm is to fit the collar to the animal properly. Again, this is generally very species-specific, but some rules of thumb can be useful for most animals. First, do not fit a collar of fixed circumference to an animal that is still growing, or whose weight significantly changes seasonally, to avoid strangling or chaffing it as it matures or goes through cycles of weight loss and gain. If collaring a juvenile animal is deemed necessary, some collar manufacturers make collars that are expandable, allowing the collar to adjust to the animal's growth. The appropriate tightness of collar fit will vary among species, depending on what risks to the animal are imposed by the collar. Animals with relatively long limbs and small feet (e.g., lagomorphs, ungulates) have the potential to slip their forelimbs through loosely-fitted collars, which can be lethal if their legs become stuck; collars fitted to such animals should be snug enough to preclude this. Animals with relatively short limbs or large feet (e.g., carnivores) are unlikely to tangle their forelimbs in a loose collar, but much more likely to snag the collar on something like a tree branch, which could lead to strangulation if a collar is fit too tightly. In this case, the collar should be fit to the animal just tightly enough so that if it really wants to remove the collar, it can do so with a lot of hard work, allowing the animal to wriggle free of the collar if it becomes hung up. Third, when in doubt, do not collar the animal. It is very common in telemetry studies to occasionally be presented with a situation that is completely unanticipated or at least very unusual, and it is not clear whether collaring a particular animal will result in harm or not. When such doubt exists, choosing to not collar an animal is almost always the best option, except perhaps under the most demanding of research needs.

Locating collared animals: VHF

Sampling design

When planning how collared animals will be sampled during a telemetry study, it is important to bear 3 things in mind: 1) sampling should result in as many locations as possible, 2) locations should be representative of all behaviors of an animal, and 3) locations should be as independent from each other as possible. It almost goes without saying that large sample sizes are needed to derive reliable inferences in any study, but this is one of the major challenges of any study based on VHF telemetry. Because locations must be obtained by observers, and the number and availability of observers is limited, obtaining enough locations for statistical validity

is often challenging. A good rule of thumb for the minimum number of locations per collared animal is 30/year, but even this will be insufficient for some analyses; researchers should determine a reasonable sample size of bearings/animal for the analyses they wish to conduct prior to designing a sampling schedule.

A common pattern in some telemetry studies is animals are located regularly during times convenient to human observers, i.e., the middle of the day, on days when weather is good, or during seasons when accessibility is relatively easy. This can be highly problematic. Consider a scenario where an observer collects locations at noon each day and uses these data to estimate habitat use, the home range, and movements for an animal. Now, what if the animal likes to rest in the same spot each day? Imagine what the estimates of habitat use would look like—they would only show where the animal likes to rest! Imagine what the home range estimates would look like—very small! And movement—very little! The important thing here is that locations should sample all the behaviors of an animal—where it rests, where it forages, where it travels, where it finds mates, etc. Only then can analyses of habitat use, home ranges, and movement reveal useful information. Therefore, it is critically important for a sampling schedule to suit the behavioral schedule of the animals being sampled and the questions being asked. Commonly asked, general questions about habitat needs, habitat use, or entire home ranges of animals will usually require sampling at all hours of the day (e.g., sampling periods 8 hours in length, each beginning 24 hours after the last one ended, would result in sampling around the clock over time), and during all seasons of the year that the animal is active. Alternatively, samples may target specific space use patterns for questions related to behaviors at particular times of day (e.g., roosting or nesting during the day or night) or specific seasons (e.g., summer or winter home ranges).

Finally, locations of collared animals are a sample of their behavior and thus are subject to the same statistical considerations as other forms of sample data, one of which is independence of observations. There is considerable debate among statisticians and biologists about the need for locations of animals to be statistically independent. From a statistical point of view, autocorrelation of sequential locations has the potential to bias some estimates of habitat use and home ranges. Collecting locations at a rate (i.e., hourly, daily, weekly) such that there is no chance of statistical autocorrelation between locations is the obvious solution to this problem. Analytical approaches for estimating statistically independent sampling intervals are available (Kernohan et al. 2001). From a biological point of view, however, no sequence of locations along an animal's behavioral path is truly independent because the locations sample a continuous behavior by the same animal. In practice, it makes little sense to discard precious locations that are hard

to obtain, just because they may not be statistically independent from others. Further, many current analytical approaches (e.g., kernel home range estimators; see *Estimating Home Ranges* section, below) are relatively unaffected by autocorrelation, thus many ecologists are less worried about autocorrelation in their data than obtaining enough locations to characterize the behavior of the study animals accurately. A practical approach to balancing concerns about autocorrelations among locations and adequate number of locations for solid inferences is to collect as many locations as possible while attempting to standardize autocorrelation between them, rather than eliminating it. This can be done by collecting locations at a fixed frequency (e.g., once every X minutes, hours, days, etc.) so that whatever statistical correlation between fixes might exist among locations is consistent across all of them (i.e., the potential for bias due to autocorrelation is not greater for some locations than others).

Finding bearings

Animals wearing radio transmitters can be located from the ground and from the air. Locations from the ground can be obtained using homing and triangulation. When homing, an observer uses a telemetry receiver and directional antenna to "home in" on an animal by moving in the direction of the peak signal; once the animal is sighted, the observer can then record its observed location on a map or using a hand-held GPS device. Alternatively, an observer can use the same telemetry equipment to collect several bearings to an animal from known locations within a limited time period; once these bearings are collected, they can be triangulated to estimate the animal's location. Both techniques require extensive training of observers (and often a fair amount of field experience) in order to produce reliable results.

Observer training begins with becoming comfortable with how to use telemetry equipment and the information it can provide. To determine the direction to a collared animal using telemetry equipment, the observer must find the peak signal, but simply pursuing the loudest beeps produced by a telemetry receiver often results in misleading directions. It is far more productive to use all the information produced by the correct use of telemetry equipment, which begins with sweeping the directional antenna ≥180°, slowly and constantly back and forth in the suspected direction of the animal. If the space between the animal and observer is unobstructed by terrain, over the course of several sweeps, the observer will hear the beeps quietly appear, gradually get louder, then softer, then disappear. If the observer imagines the distribution of these beeps along the sweep of the antenna as bars that indicate the strength and the direction of the radio signal, it might look something like Figure 5.3. Clearly, the peak signal is indicated by the longest

imaginary bar, but 2 other factors also assure us it is the peak. First, it is nicely centered between the places where the beeps disappear on either side of the sweep (points A and B in Figure 5.3). Second, the increase in signal strength going from both A and B toward the center is nicely symmetrical. All 3 indicators are important to consider when finding a peak signal; relying on any of them alone can result in poor bearings.

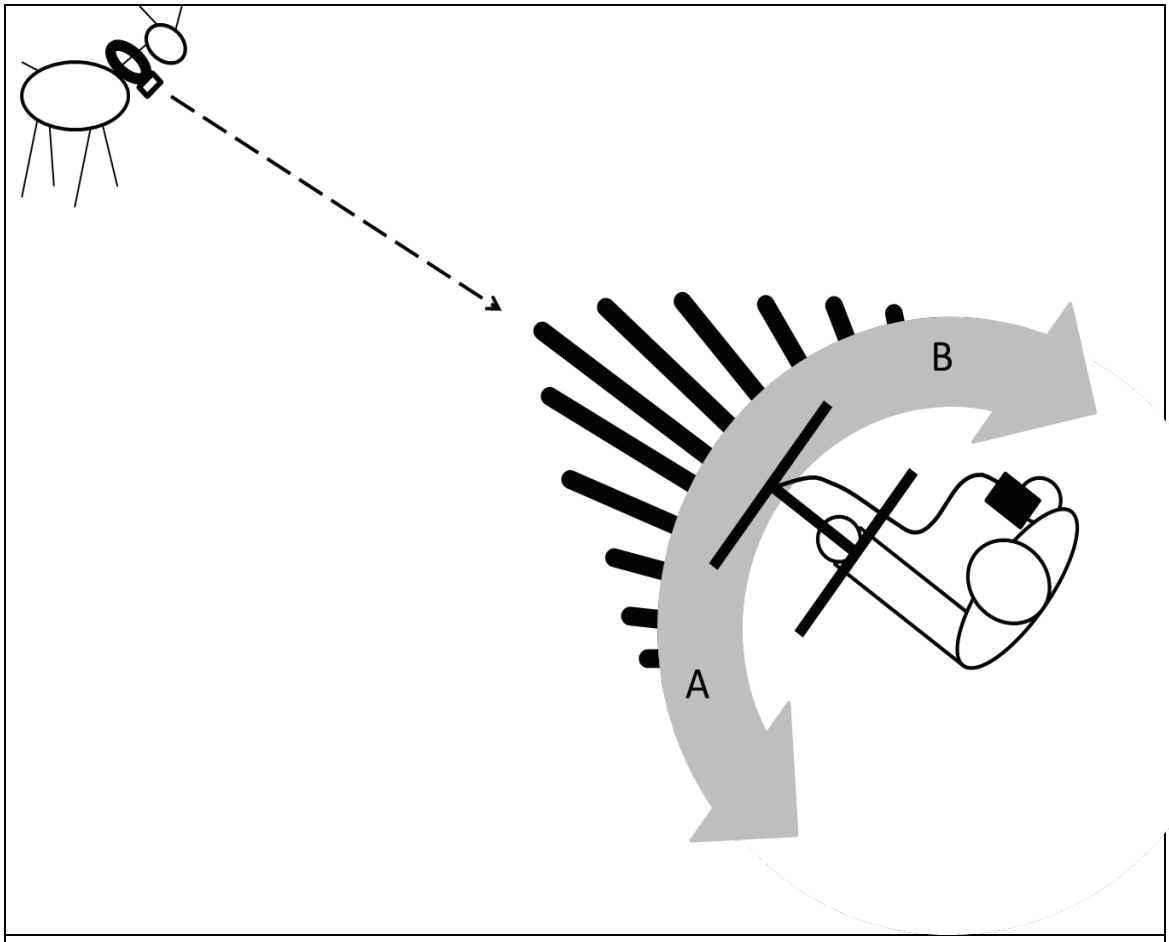

Figure 5.3. Identifying the true bearing to an animal using telemetry equipment. An observer sweeping a directional antenna back and forth, repeatedly, will be able to discern a distribution of signal strengths indicated by the loudness of beeps. Signal strength (black bars) will build uniformly toward the peak signal then decrease uniformly along the sweep. On either side of the sweep, the signal disappears at points A and B.

Why is simply relying on the loudest signal often wrong? Because terrain can have a strong influence on how radio signals "flow" across it—the loudest signal is often coming from a different direction than where the animal actually is. Fortunately,

paying attention to all the auditory cues produced by a telemetry receiver can indicate when terrain is having a strong influence on a signal. Suppose there is a hill separating the observer from a collared animal; that hill can “bend” the signal such that the loudest signal (Figure 5.4, thick dotted line) is not coming directly from the animal (the true bearing; Figure 5.4, thin dashed line). Paying attention to the other information provided by sweeping the antenna and listening carefully can help. A signal bent by terrain tends to make the buildup in signal strength on either side of the beep asymmetrical, so that the beeps are softer toward where terrain is blocking a direct signal, then much louder where the terrain is no longer in the way (Figure 5.4). This also influences the points along the sweep where signals disappear, with point A occurring closer to the peak signal than point B (Figure 5.4).

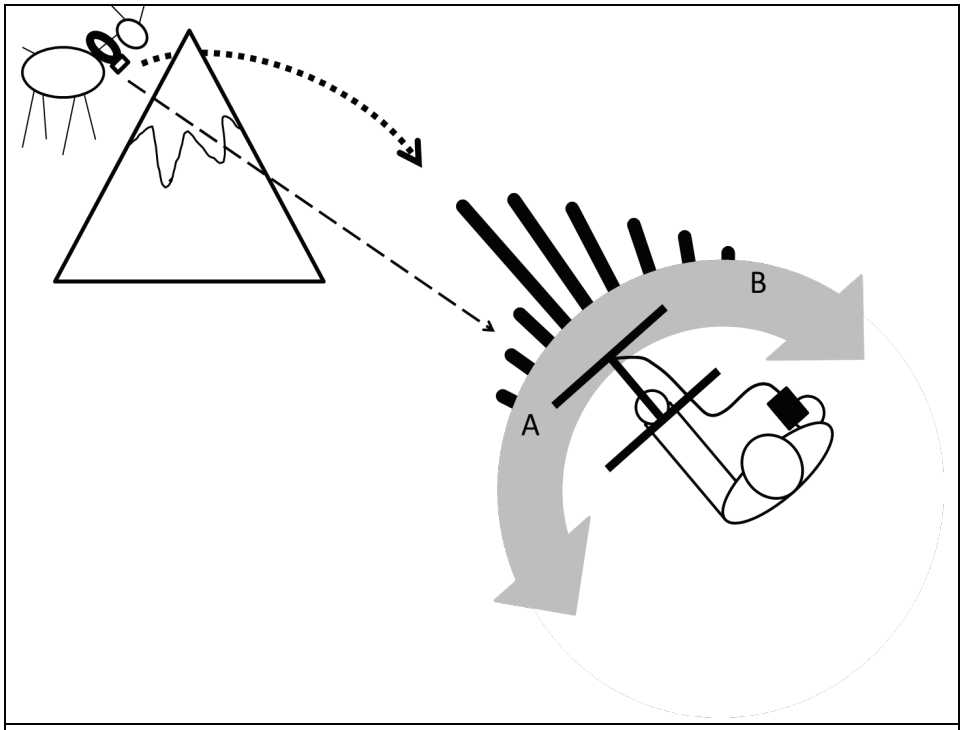

Figure 5.4. Effect of terrain on a radio signal received using telemetry equipment. The true bearing (thin dashed line) is partially masked by the hill, “bending” the signal picked up by the telemetry receiver thus creating a false peak signal (thick, dotted line). This results in an asymmetrical buildup of signal strength on either side of the peak, including where the signal disappears (A and B).

Some terrain (e.g., large mountains, particularly those with bare rock faces) has the ability to bounce VHF signals. This can result in a bewildering number of peak signals along an antenna sweep. Only time and effort can allow the observer to

distinguish a true signal (Figure 5.5, thin dashed line) from a bounced one (Figure 5.5, thick dotted line). Bounced signals can be particularly problematic because they can be very, very strong (Figure 5.5, black bars), so relying solely on the loudness of beeps coming through the receiver to identify the location can be very misleading. Fortunately, along the antenna sweep bounced signals tend to have very little build-up in signal strength on either side of the peak, and points A and B are very close to the peak (Figure 5.5). Quite often in this case, the true bearing to the animal will have a softer peak but can be discerned by the symmetry of the build-up in signal strength around that peak Figure 5.5, light gray bars). Bounced signals constitute one of the toughest challenges in mastering VHF telemetry in mountainous landscapes; time and experience are required to develop the necessary skills.

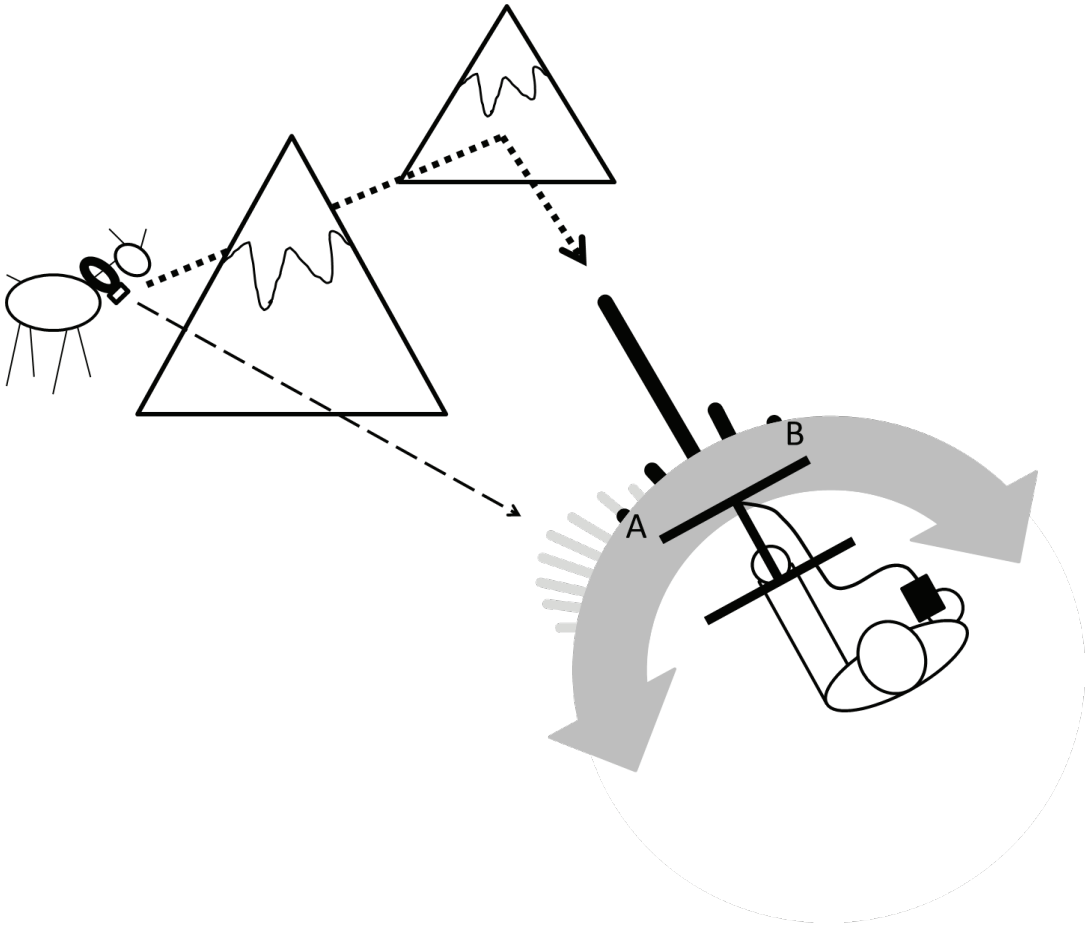

Figure 5.5. Some forms of terrain can "bounce " VHF signals, resulting in very loud peak signals with narrow build up of signal strength on each side (A and B). The true signal (thin dashed line) will have the symmetry of signal strength along the sweep (gray bars) but may not be as loud as the bounced signal (black bars).

When using telemetry equipment to identify a peak signal, it is important to recall that although directional antennas are most sensitive to radio signals toward the front of the antenna, they can actually receive signals from the rear as well. A “back bearing” on an antenna can sound perfectly credible, exhibiting all the appropriate traits of a good peak signal (symmetrical buildup, etc.), but is still nonetheless as far from the true bearing as possible. Therefore, once the signal from an animal is encountered, and shortly after the observer begins sweeping the antenna, it is wise to swing the antenna a full 360° to ensure the true bearing is not actually behind the observer (Figure 5.6).

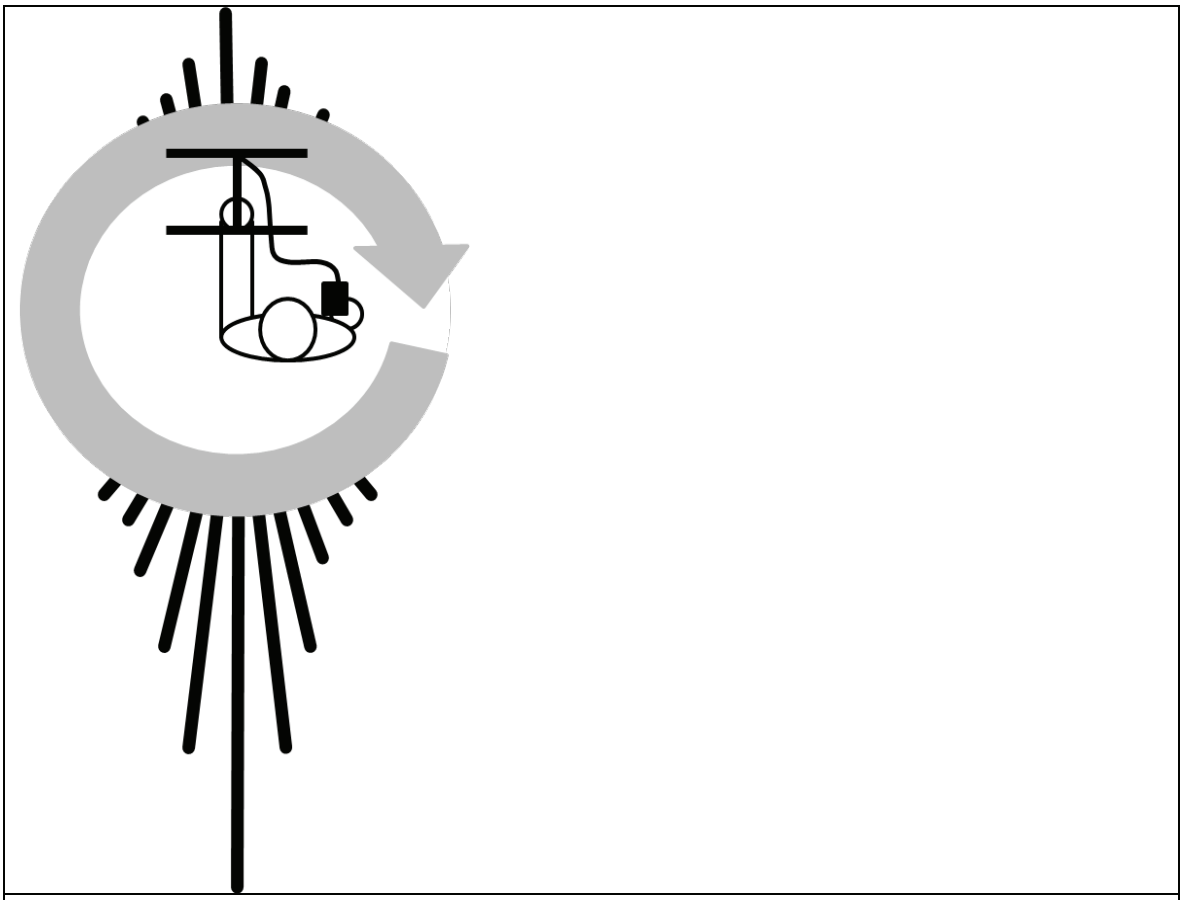

Figure 5.6. Directional telemetry antennas can receive radio signals from both the front and rear, although sensitivity to signals is greatest from the front. To prevent mistaking a “back bearing” for a true bearing, the observer should swing the antenna 360° to ensure the stronger signal is not behind.

With practice, an observer can also improve effectiveness at finding true bearings by adjusting the volume and gain of a receiver. Adjusting the volume of the receiver tends to increase or decrease the amplitude of the signals uniformly across the sweep of an antenna (Figure 5.7).

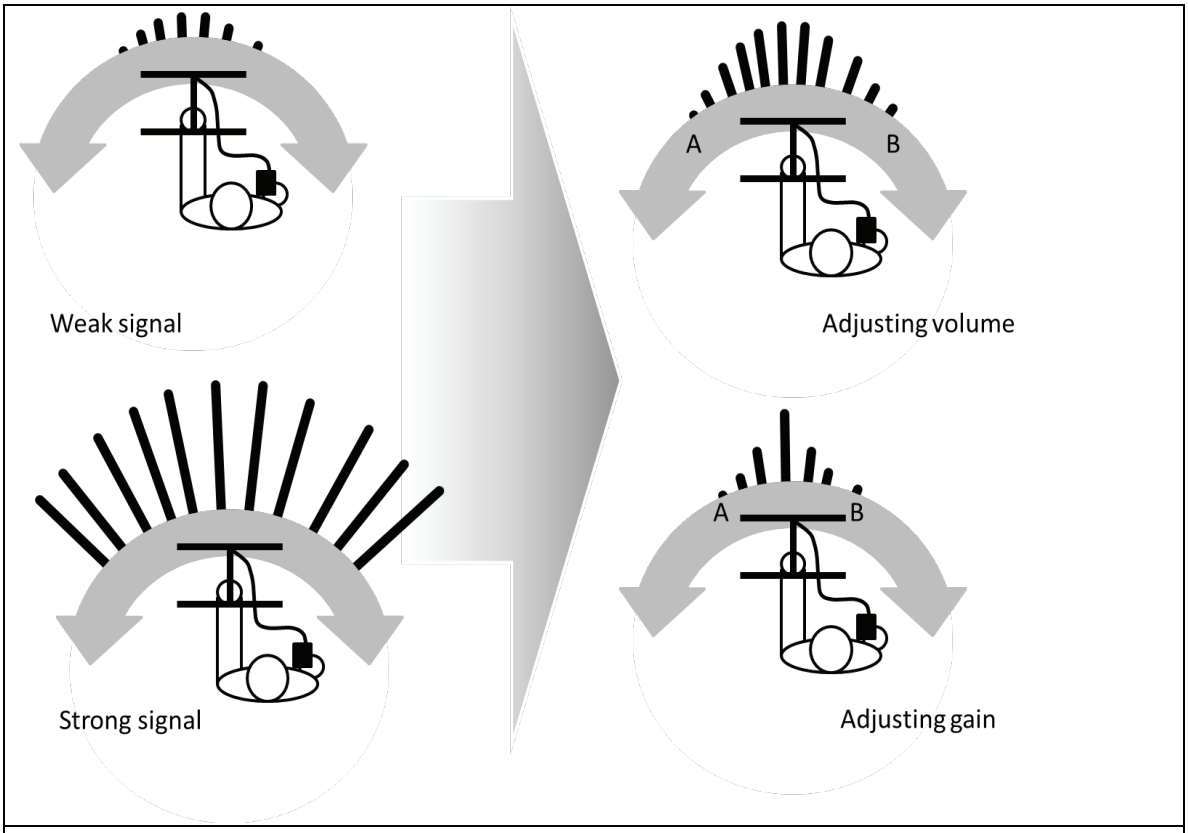

Figure 5.7. The magnitude and shape of the distribution of signal strengths along an antenna sweep can be changed using the volume and gain controls on a telemetry receiver. Volume increases or decreases the distribution uniformly, gain can change the shape of the distribution, to include strengthening the peak and narrowing the distance between A and B.

Adjusting gain affects the quality of the signal, allowing the observer to change the shape of the signal distribution along the antenna sweep (e.g., moving A and B closer together; Figure 5.7). Most observers have strong preferences for the volume, gain, and tone of the beeps they are using to identify bearings, which can only be developed with practice and experience. Some telemetry receivers come with a visual indicator of signal strength that can be used to augment what the observer is hearing; relying solely on the visual indicator, however, is inadvisable for most observers because the ability of the human ear to discern variation in a radio signal tends to be much greater than what the combination of a signal meter and the human eye can accomplish.

Telemetry error

Even the best-trained observers and satellites will not locate animals perfectly. Individual skill, atmospheric conditions, overstory vegetation, topographical idiosyncrasies and animal movements will increase error associated with estimated

locations. It is important to quantify error so that precision of location estimates can be known and used for study design and interpretation of data. For example, an observer using VHF telemetry might use radio bearings to triangulate (Figure 5.8) a location X and Y in 2-dimensional space, implying that there was no error and the X and Y coordinates were known exactly.

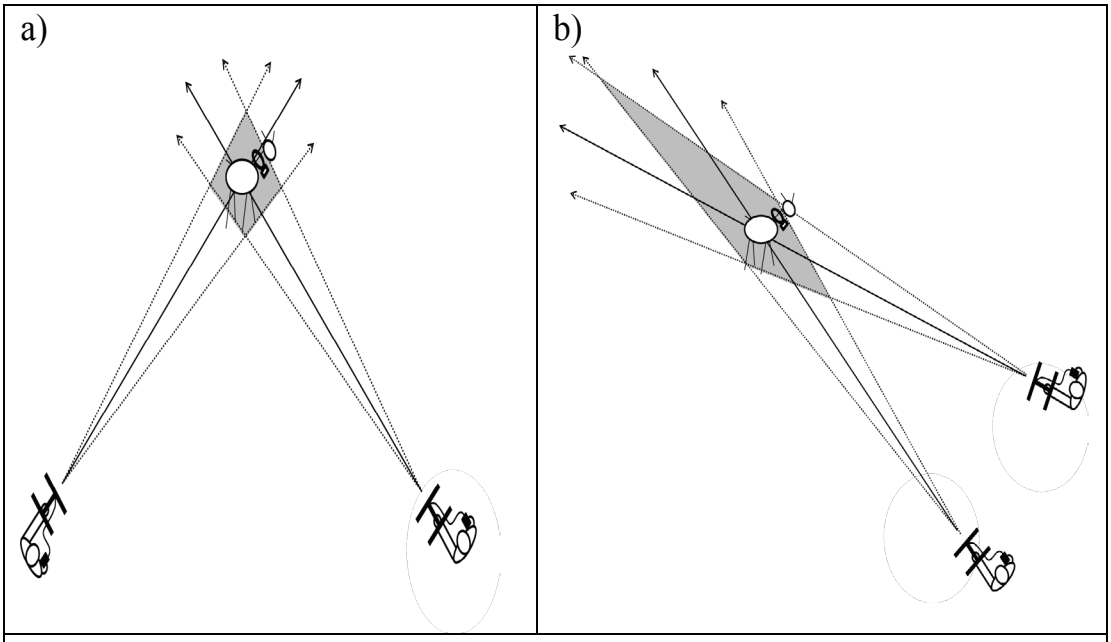

Figure 5.8. Error associated with using bearings collected using radiotelemetry to estimate locations of animals. For each estimated bearing collected by an observer, there is some margin of error on either side of that bearing. Intersections of bearings that approximate 90° (8a) minimize the area of uncertainty (shaded) based on these margins of error, where an observed animal could have actually been located. Bearings that join at acute angles (8b) have much larger areas of uncertainty, and should be avoided if possible.

In reality, this is rarely the case and a more correct expression of the location would be X ± z meters (m) and Y ± z m, with z representing the distance from X and Y where the animal could have been given uncertainty due to all the factors that can influence radio signals. Depending on the animal being studied, the techniques being used, and the topography of the study area, z can be large or small. It is important to quantify z, however, because it will define the area around X and Y where the observer is confident of the animal's location. This area defines the "grain" of subsequent analyses, or the smallest spatial unit at which data can be depicted. For example, if z were ±250 m for a given set of telemetry locations, it would be inappropriate to associate telemetry locations with habitat data that were mapped at the Landsat standard of 30x30 m pixels; thus, if we know an animal's location at ±250 m, we cannot know if it was in an area of habitat mapped

at ±30 m. Therefore, the grain of the habitat maps would need to be coarsened to 250x250 m to match the certainty of animal locations.

For observers homing in on an animal using telemetry, z is likely to be small because the observer can (often) visually identify an animal's location. For an observer using triangulation to estimate locations, this is not the case because error in estimating the true bearing to an animal will influence those estimates. A good way to visualize error associated with collecting radio bearings is to imagine a few degrees to either side of the observer's best estimate of a peak signal that represent how much the observer could be wrong (Figure 5.8). Good observers will have a small margin of error, inexperienced observers will likely have more. Even a small margin of error, however, becomes magnified with distance from the observer; there is likely to be more error associated with locating an animal far away than one that is near. To minimize the effects of telemetry error on triangulation, it is a good idea to ensure at least some of the bearings collected intersect at roughly 90° angles because the area covered by the margins of error for the bearings is as small as possible (shaded area, Figure 5.8a). Where angles of bearings are acute, the margin for telemetry error increases considerably (shaded area, Figure 5.8b).

Many software packages offer the ability to estimate telemetry error based on the premise illustrated in Figure 5.8, but the statistical formulas used for these approaches often overestimate error. Because telemetry error determines the resolution at which analyses of telemetry locations can be performed, a good estimate of error is critical. The best way to estimate telemetry error is to place a "test" transmitter in the study area at a location unknown to the observers (but known with accuracy by somebody!), then have each observer collect bearings for that test transmitter every time he or she collects data, just as if the transmitter was on a study animal. When observed bearings to animals are triangulated to estimate locations, the locations estimated for test transmitters can be compared to the known location, with the difference in linear distance between the 2 representing the error of each estimated location. Average error for test transmitters over the course of a study can provide an excellent and unbiased estimate of telemetry error (Zimmerman and Powell 1995). For long field studies where observers might discern the locations of test transmitters through multiple attempts to locate them, it can be a good idea to move the test transmitters from time to time (without the knowledge of the observers; Mills and Knowlton 1989).

Two types of errors can potentially bias satellite location data: missed location data and location error. Both errors can lead to incorrect inferences about the studied

wildlife population. The first type of error, missing location data, results from unsuccessful fix acquisitions. Canopy cover and terrain can affect fix acquisition because signal transmission decreases in areas with dense canopy cover and rugged topography (Frair et al. 2004, Cain III et al. 2005). Animal behavior (e.g., sleeping influences collar positioning) and the frequency at which the collar collects locations may also influence fix acquisition (Cain III et al. 2005, Hebblewhite et al. 2007). It is possible to develop correction factors and apply them to a location dataset to counter biases associated with missed locations. To estimate correction factors, trials are conducted where GPS collars are placed across a range of conditions (e.g., rolling hills to rugged mountains, open to forested areas, etc.). The effects of these environmental conditions on the probability of a GPS collar successfully collecting a location are modeled and the best-supported model is used to estimate a correction factor (Frair et al. 2004, Hebblewhite et al. 2007).

The second type of error, location error, can lead to misclassification of habitats used or biased estimates of movement paths. Similar to fix acquisition, location error can be affected by canopy cover and terrain as well as atmospheric conditions because signal transmission decreases in areas with dense canopy cover, rugged terrain, and thick cloud cover. Location error can be minimized by screening out 2D locations (location estimated using 3 satellites) and/or locations with high positional dilution of precision (PDOP) values (a measure of satellite geometry; Lewis et al. 2007). Two-dimensional locations may be screened out because they are generally less accurate than 3D (locations estimated using 4+ satellites) locations. Higher PDOP values can be screened out because they indicate narrower satellite spacing potentially increasing triangulation errors and resulting in less accurate locations. There are, however, trade-offs between eliminating inaccurate locations and retaining the maximum amount of location data. Screening out 2-D locations at a specific PDOP cut-off has been found to be a suitable compromise between reducing large location errors and minimizing data reduction (Lewis et al. 2007).

Plotting bearings

Bearings collected by someone homing in on a collared animal may not need to be recorded because the observer is simply using them to home in on an animal's location, which is then recorded once visual contact is made. For observers intending to use a series of collected bearings to triangulate an animal's location, however, a careful record must be kept of the time a bearing was taken, the location from which a bearing was taken, signal strength, animal activity level, and the bearing itself. The location from which a bearing was taken (i.e., a "listening point") can consist either of the name of a permanently identified location for

which spatial coordinates are known, or it can be the grid coordinates for an ad hoc listening point. The strength of the signal can be determined subjectively by each observer (e.g., poor, fair, good, very good), depending on how loud the signal was and how easily its peak could be identified. The bearing should consist of a magnetic heading (preferably taken with a good compass, not a GPS hand-held unit) from the listening point in the direction of the peak signal observed.

Most VHF and GPS collars have a "mortality" switch that changes the frequency of the beeps if the transmitter is motionless for a period of time. This allows the observer to distinguish 2 activity levels for the animal being observed: motionless and active. As noted above, the mortality switch is essential for studies of survival, as it allows the investigator to walk in on the location where death occurred to retrieve the collar and attempt to determine cause of death.

For plotting bearings, it is extremely useful to have a map of one's entire study area, preferably attached to a solid surface (e.g., plywood) and covered with transparent plastic. If permanent listening points are planned for collecting bearings, these can be plotted on the map. Otherwise, sufficient information (e.g., UTM grid lines) should be on the map to allow the plotting of ad hoc listening points. Using this map, and as shortly after the bearings were collected as possible (to ensure freshness of memory), the observer who collected the bearings should plot them and evaluate their relative merits.

Figure 5.9 shows an example of data plotted for a hypothetical animal wearing a transmitter. The observer collected bearings from 4 locations, A–D, and plotted them on a map of the study area. Clearly, bearings from A, B, and D are suggestive of where the animal could have been; triangulation based on these 3 bearings alone would put the animal at the intersection formed by the 3 bearings. The bearing from C is potentially problematic, however, because it does not converge well with the other bearings. Most observers are tempted to automatically discard C from further consideration, but extreme care should be exercised before doing so, requiring evaluation of the ancillary data also collected with the bearing. For example, if the signal strength from C was poor, then terrain may have been influencing the signal; note how, if an animal were at the intersections of bearings from A, B, and D, the broad ridge between this area and C could easily have altered the bearing (note: bearings pointing directly at a large mountain peak can be strongly suggestive of a bounce). In this case, the observer could reasonably conclude the bearing from C was more likely to be erroneous than the other bearings and exclude it from triangulation. But what if D was the last point sampled, signal strength at D was good, and the animal was very active whereas it

was motionless at A, B, and C? Here, it is very possible that the animal could have been resting, then got up and moved rapidly during the sampling period. In this case, the bearing from D might be discarded because the animal was exhibiting 2 different behaviors and the observer wanted the location for the behavior that predominated during the sampling period; however much a location based on A, B, and D might be visually appealing, the actual location of the animal is probably better estimated as the point in the center of the triangle formed by A, B, and C. Alternatively, no convincing evidence may exist that any one bearing should be discarded, arguing for retention of all. In this case, the animal's location could be appropriately estimated as the average of all bearing intersections, but the observer needs to consider how the intersection between B and C (not shown in the Figure, but a long distance away from other intersections) would influence this estimate; if the observer does not believe the distant intersection between B and C was accurate, then that intersection should be excluded from triangulation to avoid biasing the estimated location of the animal. Finally, the animal could have been very active, very distant, or located in obscuring terrain throughout the entire sampling period, making it very tough to locate, a fact reflected in some bewildering bearings that fail to converge convincingly. In this case, the observer must carefully evaluate whether, indeed, the animal was truly located during that sampling period. As difficult as it is to do so, an observer occasionally must decide that no reliable location was collected during a sampling session and exclude all bearings from triangulation.

While plotting, the observer should keep good notes on which bearings to exclude from further analysis. Bearings the observer chooses to retain can then be entered into a computer for triangulation analysis. Clearly, plotting is a subjective process, but so is identification of peak signals. In the end, it is up to the observer to make a judgment call on which bearings are credible and thus useful, which are not. It is important to note here that observers should also plot and make decisions about bearings to keep for location of "test" transmitters; they will be the ultimate measure of the observer's abilities to collect good bearings and to make good decisions about which to believe!

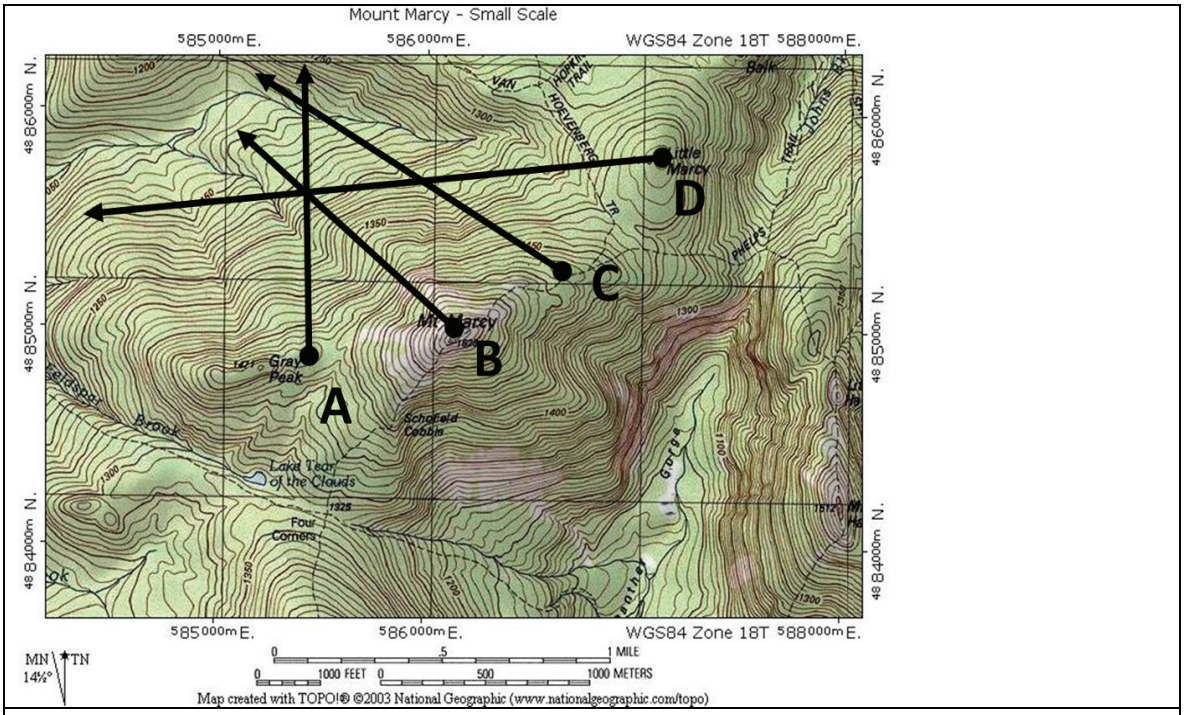

Figure 5.9. Plotting bearings collected using radiotelemetry. A-D represent different listening points at which the observer identified peak signals. Black lines indicate magnetic bearings associated with the peak signals observed at each listening point.

Locating Collared Animals: Satellite

Sampling design

When designing a satellite telemetry study the researcher must determine 1) how many locations are needed per animal, 2) the time period over which locations should be collected, 3) how many animals need to be collared, and 4) which sex/age classes are preferred. The number of locations collected by a collar depends on its fix interval (FI; i.e., the user-programmed frequency for GPS fixes). The FI depends on the study's objective. Is the objective to accurately estimate animal movement, resource use, predator-prey dynamics, home range use, or survival? The FI also depends on the length of time over which the user wants to collect locations. Will locations be collected for 6 months, 1 year, or 2 years? As short a FI as possible is recommended when looking at fine-scale animal movement. This reduces the operational life of the collar's battery but it provides a better representation of fine-scale movement patterns. Short FIs are also recommended for predator-prey studies. With short FIs locations where a predator has potentially made a kill and is feeding can be identified by looking for clusters of satellite locations. Longer FIs (e.g., every 3+ hrs) are generally adequate when

looking at resource selection, survival, or home range use. Longer FIs result in fewer locations but the operational life of the battery is longer allowing these locations to be collected over a longer period of time. Research has found ≥ 50 locations per animal per season of interest are needed to accurately estimate resource selection or survival and 50–200 locations per animal to accurately estimate home range. Regardless of the study objective, it is always important to collect locations over the entire duration of the study that are relatively evenly spread across the times of day or year specified by the research questions.

The operating life of a satellite collar is determined not only by the FI but also by the user-programmed VHF and/or Argos schedules. As the number of hours the VHF beacon is active and the frequency and duration of Argos transmissions increases, the battery pack's operational lifetime decreases. Programming the VHF beacon to turn off during periods when communication is unlikely saves battery life. Programming the Argos uplink to transmit locations infrequently (e.g., once every 2 weeks) and for a short duration (e.g., 6 hours) is optimal for the collar's battery life but likely will result in the user only receiving a sample of the satellite locations until the collar is recovered. As the FI increases, the frequency and duration of the Argos transmissions needed to send all of the satellite locations also increases.

The number of animals that need to be collared depends on the study population and the budget of the study. For a large study population ≥ 20 collared animals are needed to make inferences about the entire population. For small populations (e.g., an endangered felid) fewer collared animals may be needed. Due to the high start-up costs of satellite telemetry studies and how difficult it is to catch some study animals it is rare to find a study with ≥ 20 collared animals.

Programming collars

Specific instructions on how to program satellite collars and the computer software package needed to do so are provided by the collars' manufacturer. Programming a collar generally involves configuring the collar, setting the collar's time and date, and creating a GPS fix (i.e., collection of a latitude/longitude location), VHF beacon, and/or Argos schedule. For all schedules the user enters a start and end date. It is advised that the end date for the Argos schedule is several years past the project's expected end date. For the GPS fix schedule, the user selects a cyclic frequency for GPS fixes (e.g., take a fix every 2 hours), sets specific times within a 24 hour period that a GPS fix will be taken, or sets a "rollover rule" (e.g., the collar will record a location every 4 hours and 15 minutes) such that daily location times are staggered around the clock. For the VHF beacon schedule, the user sets the time interval(s)

within a 24-hour period that the VHF beacon will be active. For the Argos schedule, the user sets the frequency and duration of Argos transmissions. It is possible to program collars so that the GPS fix, VHF beacon, and Argos schedules change throughout the duration of the study.

After programming collars it is critical they are tested to ensure they are functioning and ready to be deployed on animals. To test a collar, attach the battery pack (following instructions from the collar's manufacturer) and place the collar outside in direct view of the sky. Use a receiver to verify the VHF beacon is active, clearly audible, and coincides with the programmed schedule. Leave the collar outside long enough to collect several GPS fixes (the VHF beacon should become inactive when the collar is attempting to collect a fix) and if applicable, to have ≥ 1 Argos transmission. After the appropriate period of time, bring the collar back inside and download the GPS fix data to your computer. Verify that GPS fixes were collected coinciding with the programmed schedule and that the latitude and longitude locations were correct. Next, verify that the Argos transmission was successful by retrieving GPS fixes from the internet-accessible Argos database. If the VHF beacon, GPS, and Argos are all functioning then the collar is ready to be deployed.

Downloading data

To retrieve location data from a GPS store-on-board collar it is connected directly to a computer and the data is extracted using the software provided by the collar's manufacturer. To retrieve location data from a GPS store-on-board collar with remote download capabilities a HCU is used. Users position themselves in the field so they can hear strong VHF beacons from the collars. While scanning the area with an antenna the user commands the HCU to attempt to communicate with any registered collars in the area. A list of the collars the HCU was able to communicate with appears on its screen. The user then selects a collar from the list and commands the HCU to download GPS data from that collar. Once all of the GPS data has been downloaded to the HCU it is connected to a computer and the software provided by the collar's manufacturer is used to extract the data.

Data from collars with an Argos uplink may be received in an e-mail or downloaded from the web. Receiving e-mails is more convenient but there is an additional charge for this option. If the user chooses to download data from the web they must do it as frequently as possible at a maximum of 9 days apart; the Argos system only keeps a history of data received for up to 9 days.

Preparing Data for Analysis

Data collected using VHF telemetry are generally easy to manage because of their low volume, with much of the labor involving simply entering data collected in the field (listening points, bearings, etc.) into a computer database. For locations collected using homing, this is a relatively straightforward process that could be as simple as entering animal identification, location (i.e., X and Y coordinates), and date/time into a spreadsheet. Where field data consist of bearings collected from listening points, 2 approaches are available. First, the observer can use results of plotting to identify a spot on the map he or she believes is the most likely location, derive coordinates for this location from the map, and enter those. This is a very coarse approach however that can inject further subjectivity into estimates of animal locations. A better alternative would be to use computer software to triangulate locations from collected bearings. This requires entering collected bearings into a database that is formatted properly for triangulation software. Software packages vary and, with the advent of satellite telemetry, are becoming increasingly rare as demand for VHF-based software declines. A common program currently available for triangulating field data is Locate III (Pacer Computing, Tatamagouche, Nova Scotia, CA) which will run on personal computers or hand-held personal digital assistants (PDAs) that are convenient for taking into the field. Field bearings can be loaded into Locate III, which will then provide triangulated estimates of locations based on the bearings, as well as estimates of telemetry error (though see caveat regarding computer-estimated telemetry error, above), suitable for subsequent analyses.

Data collected using satellite telemetry are in many respects much more straightforward than those collected using VHF because estimated locations are automatically provided by the collars or satellite service. On the other hand, satellite telemetry provides orders of magnitude more locations than VHF telemetry, requiring very careful management to ensure that locations are not lost, duplicated, or assigned to incorrect animals. Managing satellite data requires the creation and maintenance of computer data files that are carefully scrutinized and updated as more data become available. The first step when creating such a data file is to delete all locations from when the collar was being tested prior to attachment to an animal, from before the animal awoke from anesthesia, and from after the collar was dropped or the animal died. Next, not all attempts of a collar to locate itself are successful; these events will be recorded in the data downloaded from the collar or received from the satellite service and will need to be deleted. With Argos collars, duplicate records of individual locations are sometimes recorded, therefore it is also important to identify and delete duplicated locations.

Finally, ensure that the data file contains a column labeled “latitude” and a column labeled “longitude” and verify that the data included in these columns are in UTM or decimal degrees.

Analysis of Location Data

This section will present an overview of analytical approaches to interpreting locations collected using radiotelemetry. Exercises at the end of the chapter are designed to provide detailed familiarity with the general concepts presented here.

Geographic Information Systems

Nearly all approaches to analyzing location data require the use of a Geographic Information System (GIS). A GIS consists of computer maps and the tools used to analyze them. With the right data, GIS can visualize elevation, forests, political boundaries, population density, land use, and thousands of other things in any part of the world. GIS can be used to answer a vast array of ecological questions including: What is the home range of an animal? What are the migratory patterns of a species? What resources does a species select for?

A GIS map is made up of layers. Each layer represents a different part of the landscape such as animal locations, streams, or roads. Layers may be downloaded from the Internet or created from tables. Each geographic object in a layer (e.g., a city, a stream, a road) is called a feature. In GIS, all features are represented using 3 geometrical forms: polygons, lines, and points. Polygons represent areas large enough to have a boundary such as home ranges or countries. Lines represent linear features that are too narrow to have a boundary such as streams or roads. Points represent individual locations such as villages or animal locations. Points, lines, and polygons are all vector data. Vector data are defined as discrete objects with defined shapes and boundaries.

Unlike vector data, things such as elevation and temperature do not have a defined shape and boundary. Instead, they have a measurable value for any location on earth’s surface. These types of continuous data are represented by rasters. Rasters consist of a matrix of identically sized cells. Each cell represents a unit of surface area (e.g., 10 m^2 or 10 km^2) and contains a measured or estimated value for that area. For example, each raster may contain an elevation or a mean winter temperature.

The map projection is how GIS information is changed from a spherical earth to a flat surface and the coordinate system is the units and origin point used to locate features on a map. All vector and raster layers used in a GIS map must be in the

same projection and coordinate system. When they differ, they may not draw on top of each other in the map or may result in incorrect analysis results.

Numerous GIS software packages exist, though one of the most commonly used programs is ArcGIS (ESRI, Redlands, California, USA), which we will focus on in this chapter. ArcGIS includes 3 applications: ArcMap, ArcCatalog, and ArcToolbox. Most data visualization and analysis is done in ArcMap. ArcMap contains a table of contents listing the layers in the map, a display area for viewing the map, and tools for working with the map and performing analyses. ArcCatalog is used to view and manage all data files, databases, tables, and ArcGIS documents. ArcToolbox is a collection of processing, data conversion, and analysis tools.

Two software extensions for ArcGIS especially useful for analyzing telemetry data are Geospatial Modeling Environment for ARC10 (GME; formerly Hawth's Tools) and Home Range Tools (HRT). GME was designed to help answer questions about, for example, movement, resource selection, or predator prey interactions. GME includes analysis, sampling, animal movement, kernel, raster, sampling, table, vector editing, and specialist tools. HRT allows users to analyze home ranges of animals. HRT includes 2 home range analysis models: minimum convex polygons and kernel methods. HRT is superior to other home range estimator software because within this single software all home range analyses can be carried out on small to very large (i.e., the 1000's of locations that are collected by satellite collars) datasets.

Estimating home ranges

Home range estimates use telemetry locations to portray places important to an animal, including places the animal could have visited but for which no locations were collected. The result is a summary statistic, in many respects, of how an animal behaved over time, and should not be confused with the biological reality which is being summarized, i.e., what the animal considers home. Consider this: if an observer were to describe everywhere you went over the course of a year, would he or she then understand *why* you went where you did or understand completely what you considered your home and what was not? Probably not, and we have the same limitations in understanding an animal's true home range using location data. Nonetheless, a home range estimate gives us a glimpse into the behavioral processes that result in an animal exhibiting site fidelity.

The simplest home range estimator is the minimum convex polygon (MCP). Intuitively, it describes the smallest possible area that could contain observed telemetry locations (Figure 5.10).

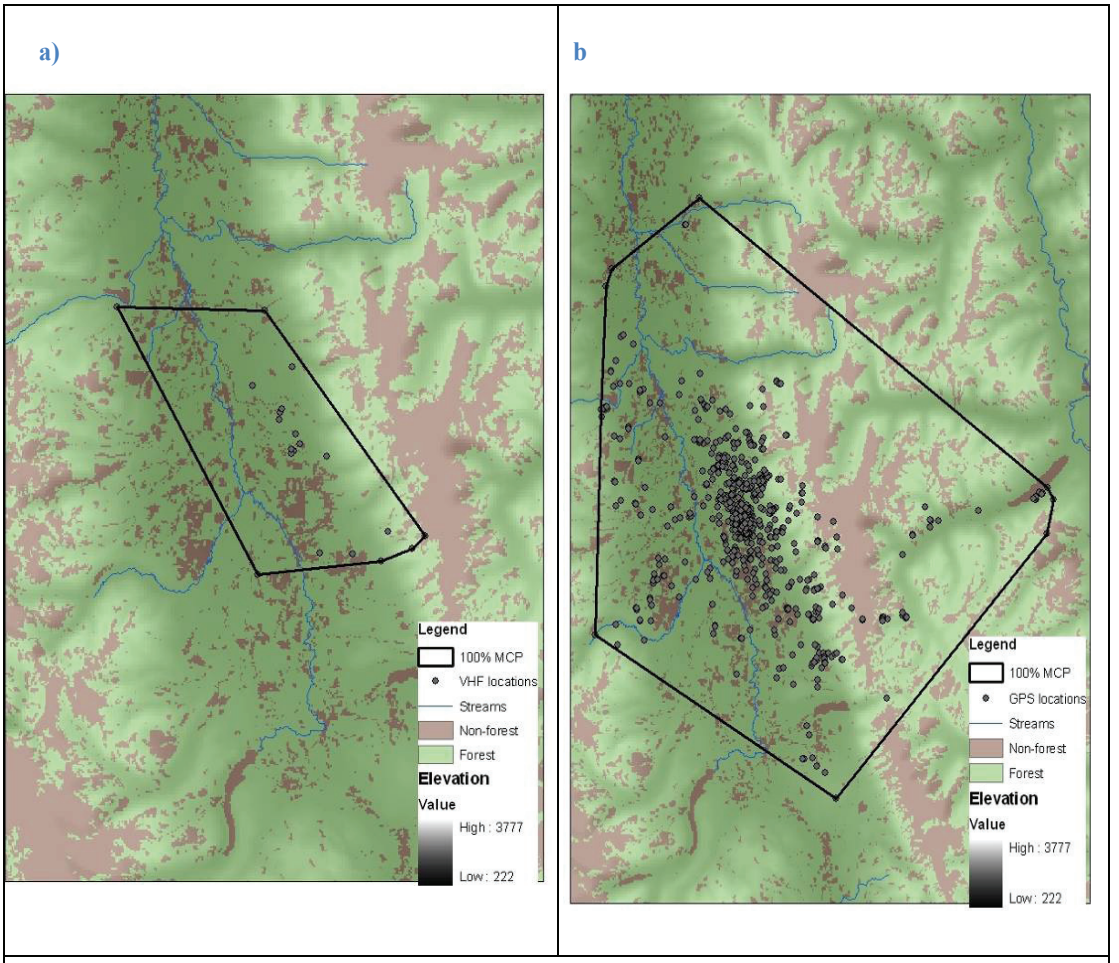

Figure 5.10. Minimum convex polygon estimates of home ranges estimated from location data for a) locations collected using a VHF collar, and b) locations using a satellite collar. Estimates use 100% of the locations; if a smaller proportion (e.g., 95%) were used, outlier observations would have less of an influence on home range area.

MCPs can be drawn to include different proportions of locations; it is customary, though by no means essential, to base MCPS on 95% of the locations, eliminating outlier locations that could represent "occasional sallies" under Burt's (1943) definition. MCPs are useful for getting a coarse idea of where an animal lives and the area of its home range, but they also have strong limitations. First, MCPs simply outline an animal's possible home range and offer no information on behavioral dynamics (e.g., where animals spend a lot of time, where they spend a little) within it. Second, home range outlines from MCPs are highly susceptible to outlying locations (even 95% MCPs), where a distant location or a transient sally can be responsible for adding a large area to a home range estimate, for which there is little evidence of use by the animal (i.e., the area contains no other locations). Because of this fact, estimates of home range area based on MCPs are unstable.

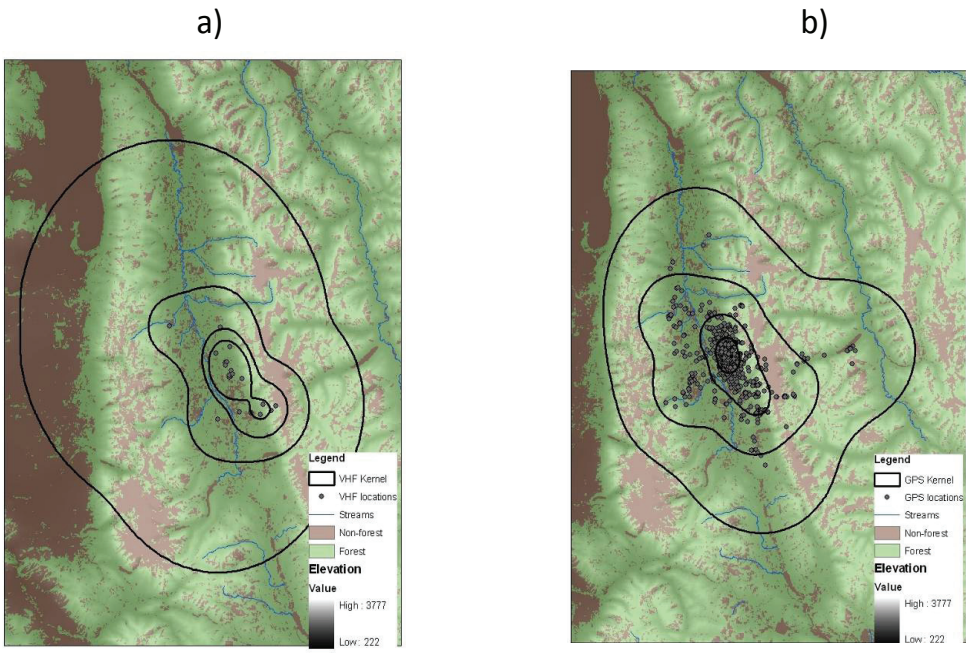

Figure 5.11. Fixed kernel home range estimates derived from the same data in Figure 5.10, including a) locations collected using a VHF collar, and b) locations using a satellite collar. Isopleths represent different proportions of the total distribution of kernel values. The smallest isopleth contains 50% of the kernel values, commonly considered the core of an animal's home range. The next 2 largest isopleths contain 75% and 95% of the kernel values, respectively. The largest isopleth contains 100% of the kernel values.

The most common alternative to MCPs is the kernel home range estimator. The idea behind the kernel is pretty simple—around every location there is a probability distribution (i.e., kernel density) that describes where the animal could have been, given that the location estimate is imperfect. Where locations are close together, their overlapping kernel densities are added, indicating a higher probability of an animal being in the area than a single location would suggest. Across all locations for an animal, this results in a distribution of probabilities that reflect the likelihood of finding an animal at some point in space (also called a "utilization distribution," Figure 5.11). As with the MCP estimator, a 95% kernel home range estimate excludes outlier locations and is commonly considered one representation of an animal's home range. The kernel estimator has 2 distinct advantages over the MCP estimator. First, the distribution of kernel values within the utilization distribution offers tremendous insights into portions of the home range that are both important (high kernel densities) and unimportant (low kernel densities) to an animal (Figure 5.10). Researchers who use kernel estimators commonly evaluate not just 95% utilization distributions, but also 75% and 50% estimates to discern the central areas of importance to animals within their home ranges, commonly referred to as

"cores" (Figure 5.10). Second, estimates of home range area are more stable than those of MCPs as more locations are added, because they include much less area where locations are absent (Seaman and Powell 1996).

Kernel estimates can be classified as "fixed" or "adaptive" and the user must identify which is preferred. The 2 approaches differ in how they "smooth" kernel estimates across locations in areas of low location density. Fixed kernel estimates use the same smoothing factor for all locations, irrespective of their density. Adaptive kernel estimates allow more smoothing where location densities are low (Worton 1989). Adaptive kernel estimates make sense in many ways because there is more uncertainty about animal behavior where location densities are low. Unfortunately, research has suggested that adaptive kernel estimates tend to overcompensate for this uncertainty, particularly for GPS data, thus greatly inflating the apparent size of home ranges. By contrast, fixed kernel estimates tend to be more representative of low-use areas within an animal's home range (Seaman and Powell 1996). For both fixed and adaptive kernel estimates, the degree of smoothing across locations is determined by the smoothing parameter (or bandwidth, commonly designated as h). There is no single best way to select h; much depends on the observers interpretation of whether the resulting home range estimate looks biologically credible and useful. Most software packages that include kernel estimators offer the ability to estimate the value of h that is statistically appropriate to the location data (commonly called the reference bandwidth, or h_{ref}) using least squares cross validation. Where animal locations are highly clumped (and particularly for high volumes of satellite locations; Kie et al. 2010), h_{ref} has been shown to over-smooth the kernel distribution, resulting in excessively large utilization distributions. Where h_{ref} does not yield biologically credible home range estimates, the user can specify different values of h.

Utilization distributions generated by a kernel estimator do not take into account the sequential nature of locations, treating them instead as if they are independent. Even where such independence is statistically true, as we mentioned earlier we know that estimated locations sample a continuous behavior exhibited by an individual animal. To address this biological shortcoming, the Brownian bridge estimator has been developed. Conceptually it is similar to the kernel estimator, except that the probability kernel is estimated between 2 sequential locations, rather than a single location. The Brownian bridge kernel thus looks like a dumbbell shape with circular probabilities around 2 sequential locations connected by a linear probability distribution between them, reflecting the probable path the animal took between the 2 locations (Horne et al. 2007). Where locations are statistically independent, a utilization distribution generated using a Brownian

bridge estimator is not different from one generated by a kernel estimator. Where locations are not statistically independent (which is often the case for high volumes of locations typical of satellite telemetry), Brownian bridge estimates reveal different dynamics within the utilization distribution, reflective of the fact that animals must travel between sequential locations.

There is no way of estimating a home range from location data that will be universally useful and correct. Much depends on the questions that are being asked and the data available to answer them; thus, careful consideration is required before selecting an estimator. That said, utilization distributions generated using the kernel estimator presently appear to be the most biologically informative, the most analytically flexible, and are subject to fewer pitfalls associated with sample size, autocorrelation of data, etc.

Estimating habitat preferences

Telemetry locations can be used to understand habitat associations for animals. This simple assessment answers the question "what kind of environmental features (e.g., forest cover, agricultural land, etc.) does an animal use?" To answer the question, a researcher can assign kernel values of utilization distribution or raw locations to the various habitat "types" within the animal's home range and evaluate what proportion fall in each. For example, an animal might spend 20% of its time (measured as number of locations or summed kernel values) in agricultural land, 30% in sub-alpine forest, and 50% in riparian areas. This can be useful information to know, at face value it suggests that the animal prefers riparian areas, then sub-alpine forest, then agricultural land. But it can also be misleading, depending on the availability of the 3 habitat types to the animal. What if the proportions of the 3 habitat types within the animal's home range were 20% agricultural land, 30% sub-alpine forest, and 50% riparian areas? Then that means the animal was using the habitat types in proportion to their availability. This is exactly what could be expected from an animal with no habitat preferences whatsoever randomly wandering across the landscape. By contrast, what if the proportions of the 3 habitat types within the animal's home range were 60% agricultural land, 30% sub-alpine forest, and 10% riparian areas? Now, the animal is clearly using agricultural land much less than it is available, indicating relative avoidance; riparian areas much more than they are available, indicating selection; and the animal's use of sub-alpine forest in proportion to its availability reflects relative indifference.

To really understand why an animal uses a landscape the way it does, and therefore inform good conservation of that landscape, a researcher must place habitat use by

an animal within the context of habitat availability. There are a number of analytical approaches that accomplish this, generally called use/availability analyses. In all approaches, use is defined by some measure of time spent by the animal (i.e., summed kernel densities or number of locations) in different habitat types of interest. The definition of availability, on the other hand, differs depending on the scale at which questions are being asked.

Johnson (1980) defined 4 nested scales, or "orders" at which use/availability questions can be asked. Analysis of first order habitat selection seeks to understand how habitat determines the geographic range of a species (e.g., why tigers are distributed as they are throughout Asia). Here, availability is defined as all the possible habitats available throughout Asia and beyond (including those with no tigers). Analysis of second order selection seeks to explain how habitat determines the distribution of home ranges of animals within some portion of their geographic range (e.g., why the home ranges of tigers within Royal Manas National Park are distributed as they are). Here, availability is defined as all the possible habitats within the area of interest, including those with no home ranges of tigers. Analysis of third order selection assesses how animals choose to spend their time among the variety of habitat "patches" available to them within their home ranges (e.g., why tigers spend more time in some portions of their home ranges than others). Here, availability is defined as all the habitats included within an animal's home range. Finally, analysis of fourth order selection seeks to understand how animals behave within each habitat patch they select within their home range (e.g., why tigers forage actively and extensively in some parts of their home ranges and rest in others). Here, availability is defined as resources available within a given habitat patch.

Most researchers using radiotelemetry focus on second and third order selection because first and fourth order questions are either intractable or inappropriate for particular conservation applications. Defining availability is easiest for third order questions because only the habitats within home range boundaries need be considered. Defining availability for second order selection, on the other hand can be challenging because no biologically or statistically objective way of doing so currently exists—conceivably, what is available to animals to establish their home ranges could be anywhere from the immediate vicinity in which they live (e.g., a park surrounded by human development) to a very broad region that crosses international boundaries. Researchers therefore generally must arbitrarily choose some spatial extent beyond the home ranges of the animals they are studying and claim it represents availability. This can be highly problematic because the spatial extent chosen will determine the availability of habitats, which will strongly

influence the outcome of a use/availability analysis. Because analytical outcomes of second order selection are a function of a generally arbitrary decision for defining availability, the decision needs to be as biologically justifiable as possible. The researcher interested in second order selection must choose a spatial extent beyond the home ranges of the animals being studied that he or she best believes represents the full range of possibilities to the animals establishing home ranges in that area.

Numerous analytical techniques for estimating second- and third-order habitat preferences are available; outlining them all is beyond the scope of this chapter. Two potential approaches are worth outlining, however, because they represent the spectrum from simple to complex, and they also show how use defined as summed kernel densities or raw locations can be used. The first is Ivlev's electivity index (Ivlev 1961) that defines habitat selection as:

$$\frac{\text{Use - availability}}{\text{Use + availability}}$$

For each habitat type x within a defined area of availability, use can be defined as the proportion of summed kernel densities within habitat type x compared to the total sum of kernel densities within the utilization distribution of an animal, and availability is the proportion of habitat x within the defined area of availability. The index for each habitat type can be plotted and values can be compared graphically (Figure 5.12).

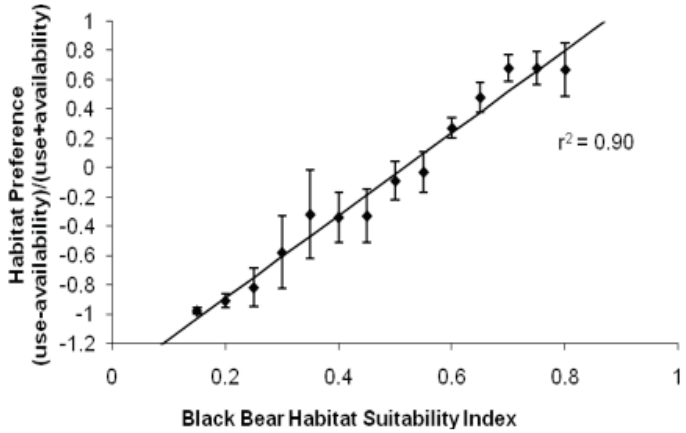

Figure 5.12. Ivlev's electivity index plotted for different classes of a habitat suitability index for black bears in the southern Appalachian Mountains of the US. Habitat suitability classes range from 0 (poor habitat quality) to 1 (excellent habitat quality). Values of the index >1 indicate selection, values <1 indicate avoidance (from Mitchell et al. 2002).

The second approach is the resource selection function (RSF). RSFs are use/availability analyses that make use of locations, not kernel densities, to estimate habitat selection. RSFs use logistic regression to compare locations (used habitat) to at least an equal number of random locations (unused habitat) placed throughout the identified area of availability. The result is a formula that predicts probability of selection across habitat types evaluated:

$$\widehat{w} * (x) = \frac{e^{\beta X}}{1 + e^{\beta X}}$$

where $\widehat{\boldsymbol{w}}^*(x)$ is the probability of selection as a function of the habitat variables, and βX is the vector of the coefficients $\hat{\beta}_1 x_1 + \hat{\beta}_2 x_2 + \ldots + \hat{\beta}_n x_n$ estimated from fixed-effects logistic regression across the "n" different habitat types (Manly et al. 2002). This can be a powerful analysis for several reasons. First, the signs of the beta coefficients (β) from the formula indicate whether habitat type x is preferred (positive β) or avoided (negative β). The beta estimates themselves indicate the strength of selection for habitat *x*. Confidence intervals on beta estimates can indicate whether selection is statistically significant or not, i.e., confidence intervals that include 0 indicate no statistical evidence of selection or avoidance. Finally, RSFs are easily mapped, providing useful visual representations of how habitat selection by animals is distributed on a landscape (Figure 5.13).

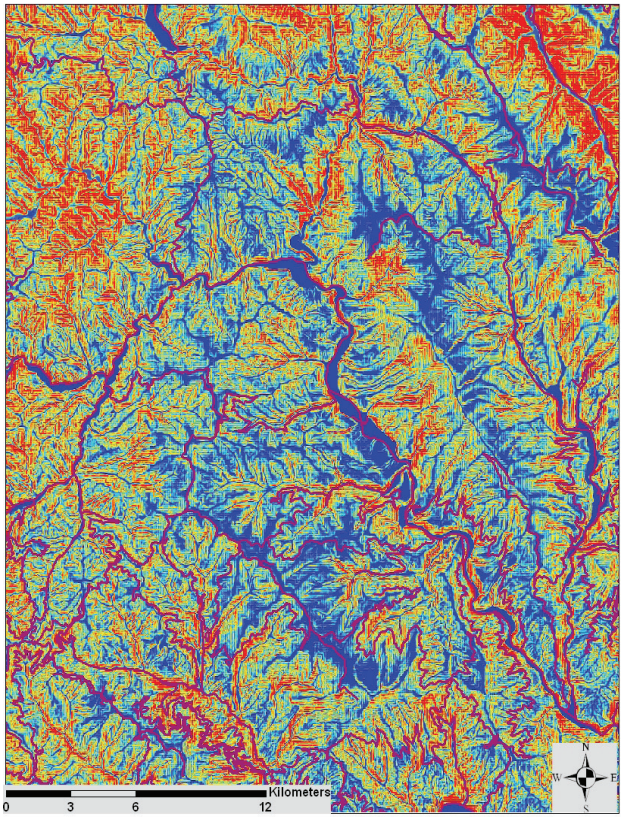

Figure 5.13. Resource selection function (RSF) showing habitat selection of male and female black bears in the Coeur D'Alene Mountains of northern Idaho, USA. Red indicates high probability of use, blue indicates low probability of use.

Analysis of movements

Within the broad categories of migration, dispersal, and day-to-day behaviors, movements can be analyzed in a wide variety of ways. Analysis of movements need not be complex, it could be as simple as identifying how an animal traversed a landscape to determine dispersal corridors or areas that facilitate connectivity. In such a case, simple description may suffice. More complex analyses seek to identify *why* an animal moved as it did; in such cases, measured aspects of an animal's movement are compared to features of the landscape or habitat characteristics hypothesized to have caused the animal to move as it did. Measuring aspects of animal movement requires standard terminology to ensure clarity of thought and communication. Turchin (1998) provided the following:

> Movement: the process by which individuals are displaced over time.
>
> Path: the line of travel of an animal indicated by the telemetry locations collected during a period of observation (e.g., where an animal went during a 12-hr period).

Path shape: the geometric nature of a series of telemetry locations, often defined using terms like tortuosity, sinuosity, or circuitry (e.g., was the animal's movement along a path straight or highly convoluted, with many turns?).

Route: a path that is used regularly or repeatedly.

Move: the segment of path contained between 2 consecutive stopping places (e.g., how an animal moved between 2 patches in which it spent time foraging).

Step: the segment of a path between 2 locations recorded within a fixed period of time (e.g., how the animal likely travelled between 2 telemetry locations).

Bout: that portion of a path characterized by a single behavior (e.g., foraging, moving, etc; note, a bout is very hard to define without observing the animal directly).

Displacement: the distance between any 2 telemetry locations.

In addition to these measures, movements can also be assessed for their duration, direction, distance, and speed.

To be relevant to conservation, measures of movement are typically related to spatial properties of populations or habitat characteristics. For populations that are distributed patchily in space, movements of animals between sub-populations should be sufficient to ensure genetic and demographic viability. It is therefore reasonable to evaluate whether movements of animals are sufficiently long, direct, or frequent enough to maintain connectivity among sub-populations. Because habitat can facilitate or impede movements of animals (whether they are migrating, dispersing, or going about daily living) correlating measures of movement with habitat features can offer insights into critical habitat, movement corridors, and migration routes. For example, rapid movement rates might indicate habitat that is of little value to an animal whereas slow movement rates could indicate habitat containing resources that are important (e.g., food upon which the animal forages, slowing its movements). Similarly, important corridors for connecting sub-populations, critical habitats, or foraging patches within a home range could be indicated by strongly linear movements, whereas movements with more turns could indicate habitat where an animal spends time searching for important resources such as food.

Estimation of survival

Survival estimation using telemetry uses what are typically described as "known-fate" or "complete follow-up" models. Much of the description of these models in this section is adapted from Mills (2007), which you could also refer to for examples.

The simplest way to think about survival estimation using known-fate models is to start with the ideal case where the surviving animals *(x)* relative to the total number *(n)* are known unambiguously, and we have just one time interval with constant survival. The estimated proportion surviving is like a coin-tossing game (with life and death on each side of the coin):

$$\hat{S} = \frac{x}{n}$$

The estimated variance (based on a binomial probability model) is:

$$\widehat{var}(\hat{S}) = \frac{\hat{S}(1-\hat{S})}{n}$$

For several reasons this bionomial model is too simple for most real-world applications. First, we usually want to estimate survival over not just one, but rather multiple time intervals (say, weeks, or months or even years). Second, we don't always know the fate of individuals, because the transmitter stops working or falls off, or a marked animal wanders away so the signal is lost. In these cases we must "right-censor" those individuals, not counting them as either dead or alive. Third, this approach assumes that all individuals were marked at the same time and so have endured the same mortality risks; in the real world, however, we usually mark animals a few at a time (staggered entry of new individuals).

The binomial model can be generalized to deal with these complications by taking advantage of failure-time or hazard methods used in engineering (estimating the lifetimes of machine components) and medicine (estimating survival time of patients). One of the most widely used failure-time models is the Kaplan-Meier method (Pollock et al. 1989, Wintestein et al. 2001). The Kaplan-Meier approach accommodates multiple intervals of sampling, limited right-censoring, as well as staggered entry whereby animals are released gradually into the sample over time. The estimate of survival for *t* units of time from the start of the study is essentially the survival multiplied across the *t* time intervals (remember that ∏ means product):

$$\hat{S}(t) = \prod_{i=1}^{t} \left[1 - \left(\frac{number\ of\ deaths\ at\ time\ i = d_1}{number\ at\ risk\ at\ time\ i = r_i}\right)\right]$$

where the number at risk in each time step (r_i) includes everybody tagged, alive, and not censored at the start of an interval.

The variance is:

$$var\left[\hat{S}(t)\right] = \frac{\left[\hat{S}(t)\right]^2\left[1 - \hat{S}(t)\right]}{r_t}$$

An example of the Kaplan-Meier estimator for bobwhite quail *(Colinus virginianus)* with both censoring and staggered entry is given in Mills (2007).

Like all estimates of survival based on marked animals, the Kaplan-Meier method assumes that marked animals are representative of the population (e.g., across sex and age classes, habitat types, or other characteristics), and that the mark does not affect survival. Also, it is assumed that whatever reason caused the animal to be censored is not related to their fate; this assumption will be violated if, for example, radios are destroyed only when animals are killed. The final assumption is that survival times are independent for the different animals (one animal dying does not affect others). If this assumption is violated—for example when predators tend to kill off entire litters of newborn radiocollared snowshoe hares (*Lepus americanus*; O'Donoghue 1994)—the Kaplan-Meier estimate of survival is not biased but the variance will be too low.

Notice that survival does not need to be constant among individuals or over time, making this method a good choice when survival probabilities change due to hunting pressure, weather, and other events. In fact, we often are interested in statistically testing how survival differs among treatments (e.g., harvest compared with no-harvest). Likewise, we often want to know how survival is affected by explanatory variables, either in the environment (e.g., weather, predator type, disease status) or inherent to the animal (e.g., sex, age, dispersal status). These covariates can be associated with survival estimates in a number of ways (White and Garrot 1990, Murray 2006).

What about sample sizes? How many telemetered animals do you need for good survival estimates? Well it depends on the question, of course, but as a general rule

for Kaplan-Meier estimates a suggested minimum is 25, and preferably 40–50, marked animals in the population at all time steps (Pollock et al. 1989, Winterstein et al. 2001). For model-based survival estimation with covariates, guidelines from human research are a minimum of 30 death events (so the number of telemetered animals may be much greater) plus 10–15 additional mortalities per variable under consideration (Murray 2006).

Conclusion

Few research tools provide as much useful information about wild animals as radiotelemetry. Using radiotelemetry to study and understand the wildlife of mountainous Asian landscapes offers exciting opportunities and rigorous challenges. With careful thinking applied to the research questions before a single collar is deployed, radiotelemetry can tell us about home ranges, habitat use, movements, survival, and more. Without careful advance thought, however, radiotelemetry has the potential to provide a bewildering variety of data that can be difficult to make sense out of. This chapter was intended to provide the basic foundation for planning and conducting a successful radiotelemetry study. The researcher who makes use of this foundation will quickly discover that more detailed knowledge is generally needed to fully realize the potential of a radiotelemetry study. Though by no means comprehensive, references throughout this chapter (and listed below) should provide much of the additional needed information, as well as useful leads to the constantly growing body of literature available on radiotelemetry.

Acknowledgments

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CHAPTER 6

Carnivore Diet Analysis from Scat

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Introduction

Diet analysis is a vitally important component to any research program seeking to understand the basic ecology and life history traits of target carnivore species. Examples from the literature include investigations of food habits (Garla et al. 2001, Nunez et al 2000), prey selection (Karanth & Sunquist 1995), food resource partitioning between sympatric species (Azevedo et al. 2006, Wang & MacDonald 2009), and the role of species in both natural and disturbed ecosystems (Chavez & Gese 2005). The most commonly used method for diet analysis of terrestrial carnivores is by examination of the indigestible prey remains in scat. Alternative methods exist, such as examination of stomach contents of harvested animals, direct observation of feeding animals, and genetic identification of prey items in scat remains. If animals aren't regularly harvested, if the species of interest is cryptic, and/or if budgets preclude genetic analysis, macro- and microscopic analysis of scat may be the best choice.

Fortunately, nature is full of scats. Scat is the most easily retrievable, abundant biological sample left by free-ranging wildlife in the natural environment. Carnivore scats often contain the bony and keratinous structures from prey items that were not digested by the individual. Thus examination of the teeth, bones, claws, scutes, hooves, and hair in a scat sample allows a researcher to identify what animals were consumed.

The objective of this chapter is to provide an overview of methods to identify the prey species remains in the scats of free-ranging carnivores. These methods are inexpensive and relatively simple to perform, even in a field setting without electricity!

Preliminary Considerations

Safety

When handling the scats of carnivores and other free-ranging wildlife it is important to first consider safety. Wild animals, especially top carnivores, can and

do carry a wide range of pathogens. Some of these pathogens are transmissible to humans and other animals through contact with scat. Thus a researcher should take special precautions to protect him or herself from pathogens present in wild animal scat when collecting, processing, and analyzing scat samples for diet analysis.

Any time a scat is handled surgical gloves should be worn. Never smell a scat directly. The fecal matter in dried scats can be chalky which can facilitate the transmission of helminth oocysts through inhalation. Wear a mask when breaking apart dried scats. Parasites of the genus *Echinococcus* are a specific concern when handling canine and domestic cat scats. Canid species (and domestic cats) are definitive hosts of these parasites whose larvae cause hydatid cysts in humans and can be lethal. The mode of infection in humans is ingestion thus researchers should wash hands before and after work, clean implements and work area regularly with 10% bleach and never eat, drink, or smoke around scat samples or equipment. Oocysts can be killed by heating the scats in an oven or pressure cooker until they reach 85°C (185°F) or by freezing at -80°C for 48 hours (pers. comm. Bill Granath).

BOX 6.1. A Note About Scat Odor

Scat odor *can* aid in predator species identification because the scat of some species may have distinctive smells. Even so, human olfaction is so limited that is not a reliable diagnostic tool. We do not recommend directly smelling a scat because the observer runs the risk of inhaling parasites or inadvertent contact between skin and scat. Moreover, it could lead to erroneous conclusions about species identification.

Sometimes smelling a carnivore scat while collecting it is unavoidable, especially if it is fresh. Scat odor can be an indicator of the freshness or age of a scat. For example a fresh scat might have a particularly strong odor or an old scat may have a strong earthy or moldy smell. Scat odor can be a predictor of DNA amplification success (Wasser 2004) thus it is useful to record this information, especially if the sample will undergo genetic analysis.

Scat collection, preparation and storage

Scat collection begins when a sample is encountered that is suspected to have originated from the target species. Much can be learned about an animal, its habits, and even its physiology by careful observation of the scat in its environment. Before handling the scat, take thorough notes about scat placement, proximity to other scats, the habitat, slope, on/off trail, presence of blood, mucous, worms, mold, size, shape, contents, color, odor, and any other detail of interest.

Take a photo of the scat with a standard size reference, such as a tape measure, in the frame. Record the suspected species and any evidence, such as tracks, to support the assertion. Photograph the evidence as well, with the tape measure in place if possible. *With a gloved hand* collect the whole scat. Collecting the whole scat will ensure that all of the different types of prey remains that might be present in the sample are represented in your findings and permit prey biomass calculation (see "Metrics for analysis" section below).

Preparing scats for analysis and subsequent storage can be as simple as washing the fecal material from the prey remains under running water in a fine-mesh sieve and drying in open air or in an oven at low temperature (80°F/27°C) until you are confident that there is no moisture left. An alternative method for washing scats can be attempted if you have access to a washing machine and electricity. Place each scat in a nylon stocking and wash on a gentle cycle (Chavez & Gese 2005), dry as above. Though the latter method is convenient and reduces the investigator exposure to pathogens, an appreciable amount of sample can be lost during agitation (>21% recorded by Gamberg & Atkinson (1988)).

Using either of the washing methods above you may find that some carnivore scats, especially aged ones, can be very compact and hard to break apart for analysis. It is important to break apart all of the sections of the scat. Bones and other hard parts are often found packed within a scat, surrounded by dense hair and fecal matter. It is conceivable that the prey remains of one entire small prey item get packed into one segment of the scat. If this segment is not examined then the prey item could be missed in your analysis.

Scats that have been washed and *thoroughly* dried can be stored in plastic bags at room temperature indefinitely. Similarly, unwashed scats can be stored in plastic bags indefinitely but are messier, take up more storage space, and must be thoroughly dried or kept frozen to prevent molding.

BOX 6.2. Determining Species Identity

Positive predator species identification of the scat sample is imperative for inclusion in a diet study. Where sympatric carnivore species occur it is easy to misidentify the species of the individual that left the scat. For example, a recent study reported a 0% accuracy of three trained observers in identifying mink scats in northern Scotland (Harrington et al 2010). If you have adequate freezer space and reliable electric supply or dessicant for genetic sample collection, the safest route is to collect all possible target scats but only include in the analysis those whose species ID has been confirmed by associated tracks and sign (Núñez et al. 2000, Scognamillo et al. 2003, Azevedo 2008), thin layer chromatography

(Taber et al. 1997), or genetic analysis (Farrell 2001). The samples not fitting this criterion can be analyzed in the future if higher resolution methods of diagnostic predator identification (e.g., DNA analysis, thin layer chromatography) become attainable.

Reference library

A prey reference library of bony and keratinous structures (e.g., bones, hair, claws, scutes, teeth) must be assembled from possible prey animals *whose species identification has been confirmed* before conducting diet analysis with unknown scats. Species identification from prey remains in scat is not possible without voucher specimens of potential prey items for comparison to the unknown prey remains.

Assembling a reference library that represents known specimens of all potential prey species in your study area might be the most challenging part of your study. To compile this reference library, collect tufts of hair, claws, hooves, and teeth from as many individuals of each putative prey species from the study area as possible. There are several different types of hair on the body of mammals. For prey species identification guard hair is most commonly used, though some argue that underfur, the insulating bottom layer, is more diagnostic. It is likely that most hair types will be in the scat of a carnivore that has consumed an animal. Try to represent as many hair types as possible in the reference library, sampling from multiple places on the body. Importantly, the hair should be plucked from a specimen rather than cut or shaved to be representative of the complete structure of the hair, including the proximal end and hair follicle. Possible sources of specimens for the reference library include museums, farms, road kill, kill-sites, and collaboration with researchers that capture animals. Be sure that all reference samples are identified to species with absolutely no doubt, well labeled, and catalogued in an electronic database if possible.

As a first step in Bhutan, we recommend establishing a centralized electronic database to house digital photographs of reference samples from across sites in Bhutan. The literature also offers some photo reference libraries of microscopic hair properties to aid in prey species identification (e.g., see Oli 1994 for snow leopard prey). Because photos alone cannot replace having actual samples in a reference library, the digital photo database for prey species should only be a framework for collecting actual samples from known prey species.

Prey Species Identification: Techniques

This section provides an overview of tools available to field ecologists for diet analysis of carnivore scats. All of the methods presented here can be executed at a

field station without specialized equipment or even electricity. A list of suggested equipment is presented in Box 6.3.

BOX 6.3. Suggested Equipment for Diet Analysis

Optical microscope; ≥400X magnification
Digital camera* or Digital Microscope Eyepiece Camera**
Measuring tape
Tweezers
Hand lens; ≥2X magnification
Paper plates
Glass slides & *Plastic* cover slips
Binder clips (all-metal)
Toaster oven
Clear nail polish
Acetate strips and roller & hot plate
Hydrogen peroxide (H_2O_2)
Mounting oil (e.g. paraffin, clove)
Razor

*camera lens should be complementary size to the microscope eyepiece
**requires computer for download (no memory)

Macroscopic techniques

Macroscopic characteristics of the bony and keratinous structures (prey remains) found in carnivore scats are useful for prey species identification. Of the techniques for species diagnosis that will be discussed, macroscopic comparison of an unknown sample to the reference library of known samples is the most expedient. For example, teeth found in scats can be particularly diagnostic for small and medium sized mammal species. Figure 6.1 shows the significant diversity of tooth morphology between small mammals of the suborder Hystricomorpha (Myers 2006). Similarly hooves, claws, and scutes can significantly narrow the selection of potential prey species.

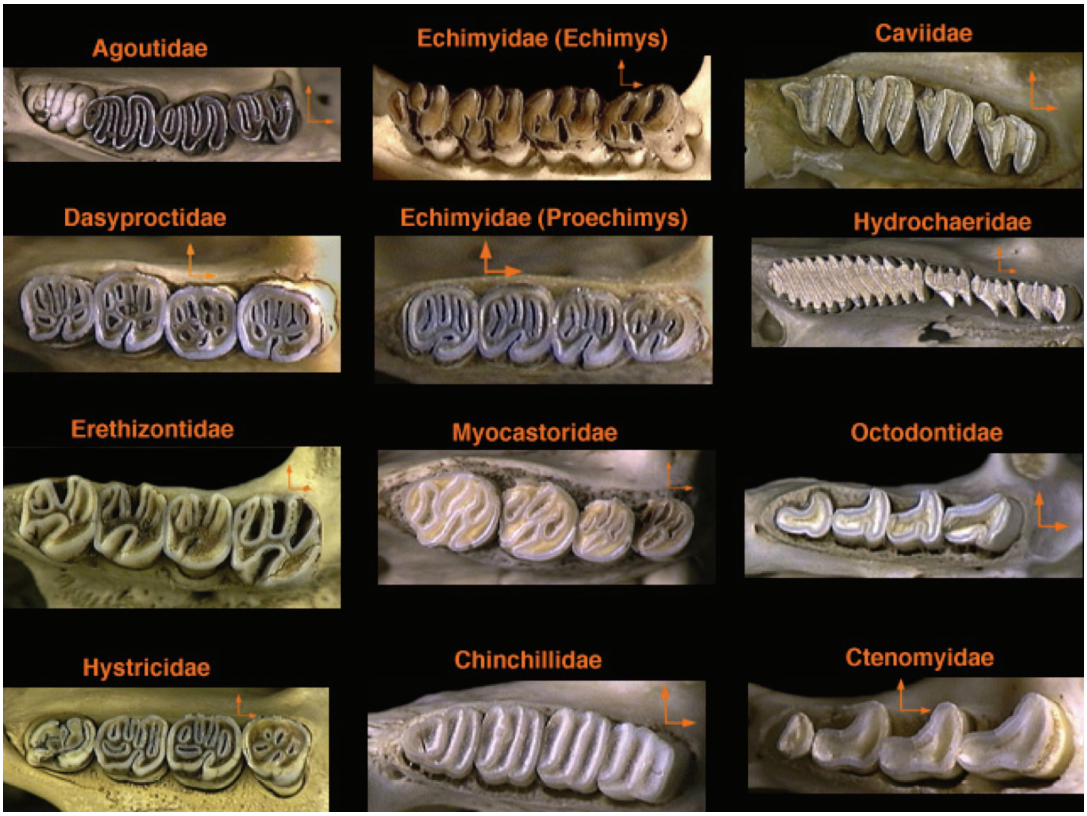

Figure 6.1. Teeth of individuals from the suborder Histricomorpha. Photo credit: Phil Myers

Take the scat presented in Figure 6.2 for example. This is a photograph of the prey remains in a jaguar scat after it had been washed and dried. Hooves are present. Thus we know that at least one hoofed species is present in the scat. In this study area this evidence narrows the choices down to deer species, tapir, domestic livestock, and peccary species.

To fine-tune the identification we must examine the hair. Macroscopic hair characteristics provide another means to narrow the selection. The length, diameter, banding pattern and general morphology lend clues to the identity of the species of the consumed mammal. In the example (Figure 6.2), relatively long, very coarse, banded hair shafts indicate that our unidentified prey species is a peccary, and not deer, livestock, or tapir.

Figure 6.2. Processed jaguar scat showing hair, large bone fragments, and hooves. Photo credit: Julie Betsch

Macroscopic hair properties for prey species identification, as noted in this example, are convenient and less time-consuming than microscopic methods discussed in the next section. In general however, these properties alone may be inadequate for confident species diagnosis from prey hair. Macroscopic hair structure can be misleading as very different species can share very similar hair morphology.

Microscopic characteristics of prey hairs found in carnivore scats are widely used by researchers for diet analyses (Hausman 1920, Oli 1993, Chavez & Gese 2005). This owes to the fact that, in general, the microstructure of mammalian hair is unique to each species. The series of microphotographs in Figure 6.3 from Oli 1993 (modified here with permission) provides a few examples of useful diagnostic features of hair. Next we will briefly describe these properties and techniques to visualize them.

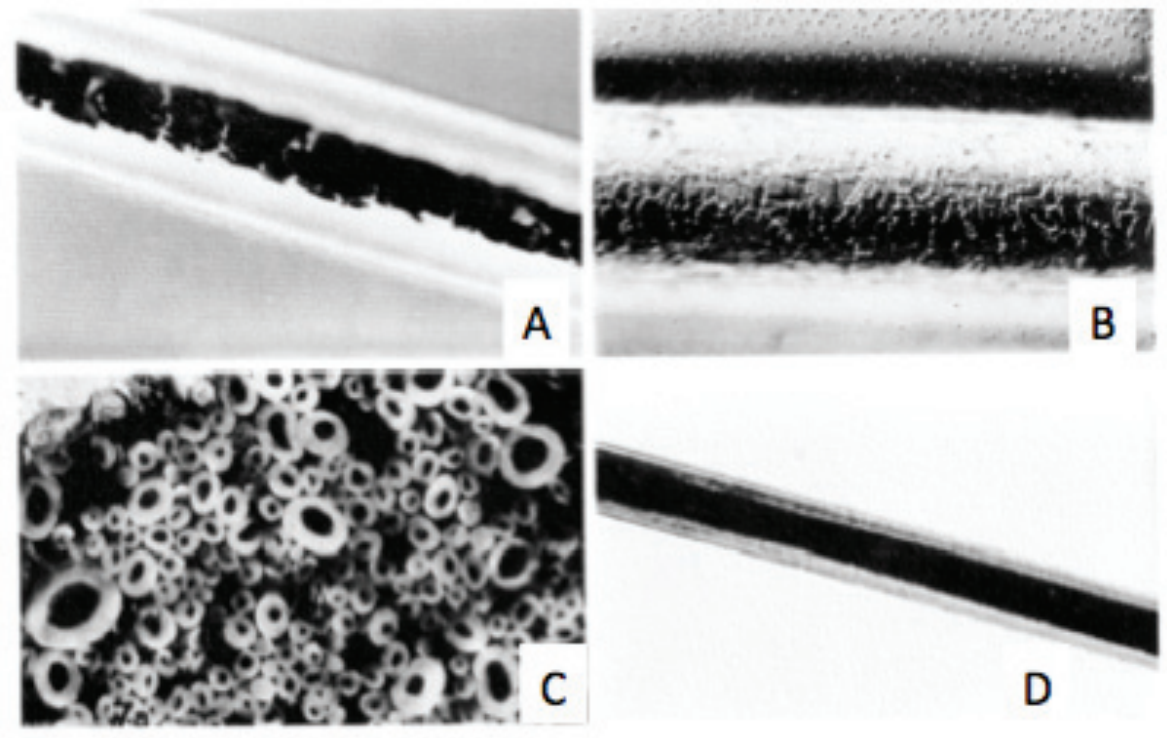

Figure 6.3. Microscopic characteristics that aid in species identification. A. and D. Medullar patterns of horse and snow leopard, respectively; B. Predominant cuticle scale pattern of horse hair; C. Cross-section of snow leopard hairs.

The coat of a mammal is comprised of different types of hair, e.g., guard hair, underfur, fibrissae. When comparing the properties of hair found in scat to that in the reference library it is important to consider the same type of hair because the microscopic properties discussed will vary between hair types. Furthermore these properties vary even along the hair shaft itself. Scales near the distal end may be damaged or "distorted". Scales near the proximal end may be crowded. A final consideration to bear in mind is that hairs of individuals might vary between age classes and seasons. I have obtained relatively consistent results analyzing the medulla, pigment, and cuticle scales of guard hairs at a distance roughly one third of the length of the hair from the follicle. The hair type and section to be examined is up to the investigator but should be consistent across samples and references.

A hair is made up of four parts—the cuticle, the cortex, pigment granules, and the medulla (Hausman 1920). The cuticle is comprised of flattened, dead cells that surround the outside of the hair shaft like scales on a snake (though not as uniform). The shape and configuration of these scales have been generally considered unique for each species and thus can provide some basis for species

identification. The bulk of the hair shaft is made up of cortex which is made of densely packed cells, at the center of which runs the medulla. The configuration of the medulla and the ratio of medulla to cortex can further aid in species identification. Finally pigment granules can be found in the cortex either in granular form or dispersed. These properties taken together—cuticle scale pattern, medulla configuration, ratio of medulla to cortex, and pigment distribution—are useful diagnostic tools for species identification.

Cuticle scale impression

The cuticle scales that comprise the outside of the shaft of the hair are not readily visible even with a light microscope. To visualize the cuticle scale pattern an impression must be made in some clear, pliant medium. A cuticle scale *impression* is easily viewed under a light microscope (Figure 6.3B).

The most useful methods for making a cuticle scale impression produce clear results in a medium that will harden and can be catalogued for future use. For this there are three materials commonly used: plastic cover slips, acetate strips, and clear fingernail polish. Plastic cover slips and acetate strips probably produce the best results (cleanest impressions) but require an even heat source. Clear fingernail polish requires less equipment, does not require electricity, and can produce adequate results. If these methods are performed smoothly, there should be no damage to the hair or scales and you can return the hair sample to your reference library.

Briefly, to obtain an impression on a plastic cover slip, place one or a few hairs (with separation between hairs) on a glass slide, cover the hair or part of hair to be analyzed with a plastic coverslip, place another glass slide on top and bind the resulting "sample sandwich" with an all-metal binder clip. Place the sample in an oven and experiment with duration of heating and oven temperature to maximize the quality of the impression that is left in the plastic (a good place to start is 160° C for 2 minutes). The cuticle scale impression in Figure 6.4 was made using this method.

Bowyer & Curry (1983) describe the use of acetate strips and a roller press to make cuticle scale impressions. Simply heat two acetate strips on a hotplate, place hairs between the strips, and then insert into a roller press. Both acetate strips will have a cuticle scale impression and can be catalogued for future use.

Finally, a cuticle scale impression can be made in clear fingernail polish by applying a stroke of polish on a glass slide and placing the hair(s) in the polish (Figure 6.3B).

Allow the slide to dry and remove the hair by rubbing your finger across the hair perpendicular to the length of the hair.

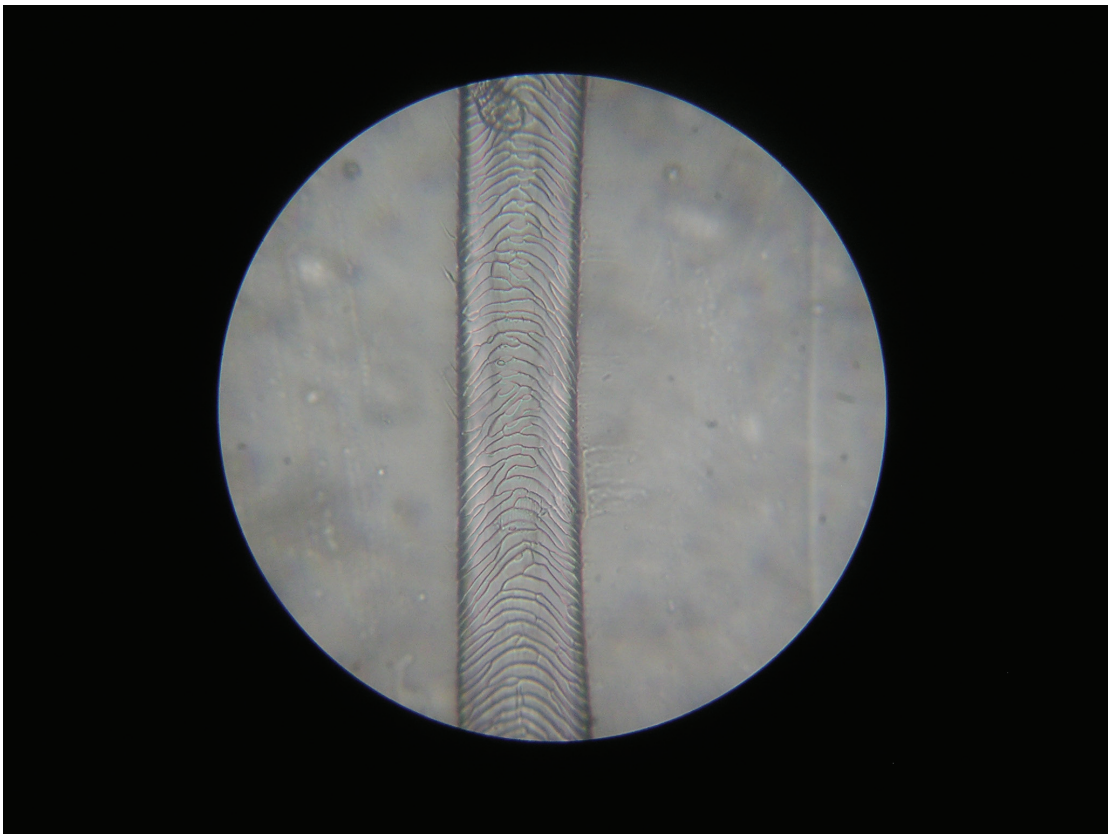

Figure 6.4. Cuticle scale pattern of a Proechimys longicaudatus guard hair: Photo credit: Julie Betsch

Regardless of methods used, the impressions should be observable under 400X (or greater) magnification, can be photographed and electronically archived for ease of use and distribution, and the impressions themselves can be labeled and archived.

Cross-section

Diagnostic properties of the medulla and pigments are visible by taking a cross section of the hair or hairs (Figure 6.3C). From the resulting view of the hair it is possible to characterize the shape of the medulla, the amount of medulla relative to the cortex, and the distribution of pigments within the hair shaft.

One method for taking a cross-section requires a tuft of hair, a short piece (2 cm) of electrical wire insulation, fishing line, and a razor. Thread the fishing line through the electrical wire insulation, form a loop by sending the end of the line back through the insulation. Place the tuft of hair through the loop and pull the ends of the fishing line so that the loop and hair are drawn into the insulation. Stop pulling

just as the loop emerges from the opposite end. Cut a fine slice off of the very end using a sharp razor and discard that slice. Then take an extremely fine slice off of the end and mount in oil on a glass slide for viewing. Photo archive the results.

Slide mount

A longitudinal view of the hair shaft allows an examination of the distribution and arrangement of both pigments and the medulla embedded in the cortex. These features vary from species to species and can thus be an indicator of species identification. A wet mount of the hair for examination provides a good perspective (Figure 6.3A, 6.3D).

The technique can be as easy as adding a drop of oil and hair sample to a glass slide with a coverslip. This simple treatment renders the cuticle scales invisible. The cortex is generally translucent and the pigment granules (if granulated) and medulla will be exposed. In some cases the pigment in the cortex is so heavy as to obscure the medulla. These samples should be treated for 15 minutes with hydrogen peroxide (H_2O_2) to reduce the pigment then mounted in oil as above.

Metrics for analysis

This section provides a brief introduction to commonly used metrics for describing the food habits of carnivores.

Frequency of occurrence

The percent frequency of occurrence (FO) is a measure of the proportion of scats that contain a specific prey item. Very simply, the number of scats containing prey item *x* divided by the number of total samples.

$$FO = (s_x/N) * 100;$$

where s_x is the total number of scats containing prey item *x* and *N* is the total number of scats (Vanak & Gompper 2009). Frequency of occurrence is possibly the most frequently reported statistic relating to a carnivore's diet. It accounts for all prey species present in the samples equally, including small and/or rare items that may not contribute significantly to the nutritional demands of the individual. As such, the metric may be an indicator of the effect that a species has on other organisms in the community, but does not adequately advance an understanding of what is important to the predators' metabolic needs. If taken alone, frequency of occurrence overestimates the importance of small prey items in the diet and underestimates the large items (Weaver 1993).

Percent relative occurrence

The percent relative occurrence (RO) of a prey item is more indicative of the relevance of each prey item in the diet. The calculation is very similar to that of frequency of occurrence.

$$RO = (p_x/T) * 100;$$

where p_x is the number of occurrences of prey item x and T is the total number of occurrences of all the prey items in all samples (Vanak & Gompper 2009).

Percent relative occurrence is still limited however because it does not reflect the proportion of the diet comprised by each prey item. Again, a small prey item may occur often relative to the total number of occurrences of all prey items but still not contribute significantly to meeting nutritional requirements (e.g., grass). Moreover, the importance of small prey items might be overrepresented because they have a high surface area to volume ratio, which 1) increases the number of scats created from indigestible parts from one feeding event (relative to large prey) (Núñez 2000) and 2) increases the likelihood that the prey will be identified (Garla et al. 2001). An alternative metric, biomass, is thus used to correct for this caveat.

Biomass

Prey biomass is a key metric in understanding the feeding ecology of specific carnivores as it reflects the proportion of each prey item (or prey category)[2] in comprising the diet. The charts in Figure 6.5 reveal the difference in the importance of each prey item when assessed as frequency of occurrence or biomass consumed.

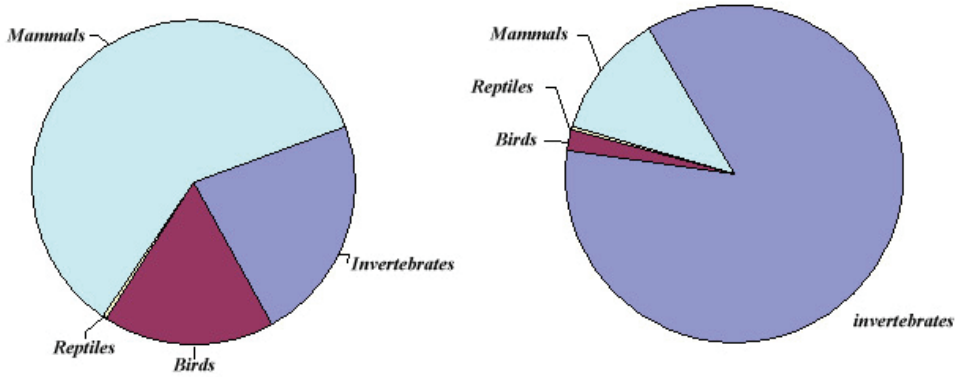

Figure 6.5. Prey composition of the Little Owl: frequency of occurrence (left) and biomass consumed (right)

[2] Some analyses focus on the importance of weight classes of prey items rather than on the influence of individual species.

Calculating biomass relies on the use of a correction factor to explain the relationship between the body weight of the living prey and the scats that are produced from its consumption (Ackerman 1984). This relationship and concomitant correction factor is dependent on the digestive capabilities of each predator and presumably unique to species. To derive this correction factor requires captive feeding trials that are generally impractical or impossible to perform for each diet study. Thus researchers commonly use published correction factors for their species of interest or those derived for closely related taxa (Garla et al. 2001, Stoen & Wegge 1996). Details on computing biomass and numbers of prey killed from field data using correction factors can be found in Ackerman et al. (1984) and Rühe et al. (2008), respectively.

Niche breadth

Niche breadth refers to the diversity of prey items taken by the predator species of study in relation to the frequency of prey items available. This metric has been evaluated using a number of different approaches (Colwell and Futuyma 1972, Krebs 1999, Ray & Sunquist 2001, Loveridge & Macdonald 2003).

Niche overlap

Niche overlap describes the degree to which two or more predator species share a resource. In this circumstance we consider food, though the metric can be used to describe the joint use of other resources such as time or space (Colwell & Futuyma 1972). It is the most common metric used when comparing the feeding ecology of sympatric carnivore species, such as leopard, tiger and dhole in Wang & MacDonald (2009). Like niche breadth, the literature offers many approaches for calculating niche overlap (Pianka 1973, Colwell & Futuyma 1972,see Mueller & Altenberg 1985 for a brief overview).

Interpretation

The analyses chosen by the investigator will be driven by the specific research question of interest. Regardless of which analyses are used, there are a couple of things to bear in mind when interpreting the data. First, the items present in a scat sample only represent species that were consumed and does not always imply depredation. Many carnivores will scavenge or steal prey from other predators that they have not killed themselves. Another thing to consider is that a large prey item from a single predation event can be represented in many scats. One might consider combining multiple scats that are collected in close proximity to each other, that contain the same prey remains and appear to be the same age, as one data point so as not to over represent the contents in the final analysis (Garla et al.

2001). The decision of whether or not to combine samples should be informed by the natural history of the carnivore species (could the scats be from different individuals of the target species?) and the objective of the analysis.

Conclusion

Hopefully this chapter has provided some basic tools that can be implemented even in a rustic field station to conduct a diet analysis of carnivore scats. This is not an exhaustive list of techniques for species identification; investigators have successfully executed other methods for diet analysis. A few strategies are presented here, though anyone interested should consult the literature and experiment themselves for best results.

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CHAPTER 7

Priorities And Protocols For Freshwater Monitoring

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Importance of Understanding and Monitoring Freshwater Systems

Freshwater systems, including streams, rivers, lakes, and wetlands, are home to diverse aquatic species and important resources for the terrestrial species that drink from ponds and lakes, travel along streams and rivers, and obtain fish and other prey from all of these habitats. In addition to the intrinsic value of freshwater habitats and species, there are two key reasons for studying and protecting streams, rivers, lakes, and wetlands. First, freshwater systems provide important ecological services that are the foundation of healthy and happy human cultures. These services include supplying clean water, taking up and retaining pollutants, and providing both food and recreational resources. Second, by integrating and responding to habitat conditions in upstream watersheds, freshwater systems can be extremely useful for monitoring the ecological health of large areas. The use of small streams as sentinels of ecological health in the larger landscape may be especially valuable in Mountainous landscapes, where such streams are abundant, and where rugged terrain and the lack of roads limit access to large areas. Waters from mountains served as the headwaters for major rivers, therefore its headwaters and rivers are the source of water for a broader landscape in the plains and low lands.

Of particular importance in the coming years are the upcoming changes to these systems associated with increased population, improving the standard of living including providing electricity through microhydroelectric plants in smaller watersheds and the construction of dams, and continued stocking (historically of brown trout and currently of rainbow trout). Denser populations in communities have larger impacts on water quantity (greater withdraws for human use and irrigation) and quality (increased wastes and nutrients released into the systems). As populations increase, ensuring the needs of water for the communities are considered in water allocation is critical. In addition, understanding the nutrient processing capacity of aquatic systems and potential water quality issues is critical to incorporate into community planning at the watershed level. The construction of

dams for power generation has three main effects on the wildlife of streams and rivers. First, dams block movements that may be crucial to survival and reproduction in aquatic species. Second, dams change the natural flow regime of streams and rivers, which can negatively affect aquatic species by eliminating life history cues and creating a mismatch between the timing of life history stages (e.g., dispersal, metamorphosis, reproduction) and the flows needed to complete those stages. Third, dams create reservoirs, which can become hotspots of sedimentation, nutrient pollution, and invasive aquatic species. Understanding how hydroelectric dams may influence the aquatic communities in order to be able to mitigate potential damage is critical.

As we consider future monitoring and research needs, the top priority needs include developing the tools for and performing general biodiversity surveys for aquatic invertebrates and fish. The first steps would involve developing "Fishes" and "Aquatic Invertebrates" identification guides. The development of these guides would be the basis for a region-wide database to begin collating a biodiversity inventory. As this initial step is being completed, we recommend training field staff and local biologists in aquatic sampling to increase the coverage of this biodiversity survey, which would ultimately be housed on-line in a spatially explicit database. We would recommend both stratifying by ecoregion to encompass the broadest array of potential habitats, as well as prioritizing watersheds where hydroelectric is planned to develop a baseline for those systems.

Understanding the baseline conditions is necessary to detect change in the future. This chapter is a first draft at developing potential methods.

Overview for Designing Sampling Protocols for Freshwater Research

The first step in designing a monitoring or assessment program is considering questions regarding the reasons for the program. Specifically addressing: Why do you want to monitor, and what are your goals? What parameters or characteristics are essential for meeting these goals? How will you do this, and are the necessary tools available? Where and when will you sample to achieve reliable data in a useful manner?

Even though this appears obvious, deciding on the goal behind the sampling protocol is essential before taking the first measurement. There are major differences in how you would set up a sampling plan depending on your goals or objectives.

a) **Baseline studies** to characterize the initial ecosystem health with which you may compare to changes in this and similar sites in the future

associated with landscape or climate changes. It can also provide information needed to examine whether there are potential problems that need more investigation. In this type of design you are examining data over time for a trend analysis. To better detect the shifts in the ecosystem, sampling occurs in the same area, same habitat type, similar season, and with the same methods, to minimize variation in observation and systems. If you are conducting a baseline study, select reaches of different stream orders or use an index site as an indicator of the upstream watershed.

b) **Biodiversity surveys** where you are interested in surveying all of the potential different habitats and regions to provide as complete a dataset as possible. In this type of design you would spread your sites across different season, times, areas, and habitats to ensure that your sampling encompasses the variation present across the landscape to pick up differences in species present across all ecoregions, habitats, and seasons.

c) **Impact assessment** where there is a potential degradation or problem and you want to assess whether it is indeed a problem and to what degree. If you are evaluating an impact on a stream, you might want to sample upstream of the impact, immediately downstream of the impact (or within the impact zone), and even further downstream to evaluate the extent of damage or degree to which the stream can recover from the impact. You would want to have a control (or a similar, relatively un-impacted stream or reach) with an equivalent sampling scheme to be sure the changes observed are associated with the impact and not a natural landscape pattern. For example, wider, lower gradient reaches of a stream often have more fine grained sediment (they are naturally more silty) than more constrained, higher gradient reaches and naturally an invertebrate community more indicative of organic pollution tolerance. This design is a control and impact (CI) study. If you know of a potential impact that will occur, you can improve your inference strength by incorporating sampling before the impact, then after the impact in a Before-After-Control-Impact (BACI) design.

d) **Research questions** require different protocols that may help answer a specific question of interest. For example, what is the minimum flow required to allow passage for a particular species. What impacts may current stocking of rainbow trout have on native fish communities? What are the impacts of dams on the aquatic communities?

Once you have chosen an objective, determining a sampling frame is necessary. A sampling frame is the unit over which you hope to make inferences, and from which you pull your reaches to sample. Often in aquatic sampling our sampling frame is the watershed. Unless there are unlimited resources or you are working in a very small system, it is impossible to survey the entire system or sampling frame (e.g., the entire watershed or length of stream of interest). For the sampling, one must select sections of the stream or river system to monitor. Each section that is evaluated is referred to as a stream reach. Often these reaches are 100–200 m to ensure that they are representative of that area.

General Sampling Protocols

Temperature

Introduction

Water temperature can have strong impacts on the composition of aquatic communities. Because they are ectothermic animals, many aquatic organisms only survive or thrive within a limited temperature range. Physiological functions are commonly influenced by temperature, such as metabolic rates, development time, and growth. Temperature also impacts key aquatic ecosystem processes, such as rates of material decomposition and productivity. The importance of temperature has resulted in temperature being one of the most commonly measured aquatic parameters in the U.S. and has been useful in characterizing habitat for different species, as well as describing impacts of climate change, disturbances to riparian vegetation, and/or evaluating water temperature increases with water diversions and subsequent low flow conditions. Water temperature can be strongly influenced by dams (which often have warm surface waters and cold bottom waters). Depending on what layer of water the dam release is from, the downstream may increase or decrease substantially and alter the natural daily and seasonal patterns.

Water temperature varies with time of day, season, water depth, and location. Diel variations depend on the size and mobility of the water body, e.g., heat gains and losses occur more rapidly in streams, which are typically shallow and mobile compared with lakes, which are deeper, more stable water masses. Water temperature of a system is dependent upon solar radiation (of area and site-riparian shading), air temperature (climate and elevation), the dimensions of the river (e.g., width, depth, and gradient), human impact, the temperature and volume of groundwater entering the system, and the water source (rainfall, snowmelt, glacial fed). The substantial variation in temperature over the course of a day, season, and across sites result in very limited utility of point-in-time (or single measurements with a thermometer). But it is typical to add general point-in-

time measures to a general survey of systems. If this temperature is recorded with date, site location, time of day, and weather, it can be useful to begin to characterize a system. Or if you are working with point-in-time protocol (a calibrated thermometer) to describe an impact or look for a trend, be sure to measure the temperature on at least a monthly basis, at the same time of the day each month.

Measurements over months and years can result in an understanding of the thermal regime. The best approach for collecting temperature data are thermal recording devices that can be left in the field for long periods of time reducing the necessary site visits needed to characterize temperature, but the data loggers are more costly than a thermometer. These devices allow one to examine maximum temperatures, summertime average temperature, and seasonal shifts in temperature (e.g., growing season). Comparisons of these summary statistics are often used in trend analyses, quantifying habitat for different species, or for evaluating riparian impacts.

Equipment

- Data loggers, cable, and station for downloading to computer
- Material for housing a data logger and material for anchoring the logger in place
- Datasheet, markers (stake or flagging), hand-held thermometer, and GPS

There are many manufacturers for thermal data loggers that report all of the relevant specifications. Before ordering loggers, you need to consider the following issues: accuracy, precision, memory capacity, durability, and programmability. When properly functioning, most data loggers are accurate and capable of relatively precise (±1°C or less) temperature readings. Memory capacity is more important if temperatures are to be recorded for long periods (for example, more than 6 months) or short sampling intervals (for example, less than 30 minutes). Most data loggers manufactured today have a minimum of 8 kb of memory, which allows deployment of 165 days with data recorded at 30-minute intervals. The interval for which the recordings should be recorded depends upon the measure of interest (minimum or maximum daily temperature or average daily temperature) and the system (the diel fluctuation). The larger the diel fluctuation, the higher the resolution necessary to capture accurate temperature profiles. Dunham et al. (2005) provides a nice graphical example of missing the maximum temperature with different sampling intervals. Often a sampling interval of 30 minutes to 1 hour

provides a good estimate if the diel fluctuations are less than 8–10°C (Dunham et al. 2005).

Durability of temperature loggers relates to both whether the loggers themselves are waterproof and type of housing to use to hold the logger in place. Data loggers that are submersible should be placed in flow-through housings (for example, heavy duty, UV resistant PVC pipe or an equivalent type of material) to protect them from physical impact and direct solar radiation. Testing housings before placing them at a site is useful as fine screens or small holes may get fouled with algal growth or filled with debris and fine sediment. Reducing exchange of water through the housing can result in biases in the temperature readings. Often waterproof temperature loggers are more expensive than those that are not waterproof. Loggers that are not submersible can be placed in waterproof containers (e.g., sealed, water tight containers) and then placed in the water. This can work quite well, except in streams with large rapid temperature fluctuations (as there is often a lag time in the containers resulting in an overestimate of minimum temperatures and an underestimate of maximum temperatures). In all cases the user needs to be careful to consider the color of the housing as solar radiation can create a bias (e.g., black and clear containers can create biases in temperature readings) and keep the logger out of direct sunlight.

Temperature loggers should be calibrated before and after deployment. This can be as simple as starting the temperature logger to read every minute, putting the temperature loggers in a freshwater, well-mixed, ice bath (e.g., a large cooler with ice water) for one hour. Ensure that the temperature of the bath is 0°C with a regular thermometer and check that the thermal data loggers level out at 0°C.

Field site selection

Typically we are interested in characterizing a representative temperature of a reach, but spatial variability occurs in water bodies of all sizes. A good field site is an area that is deep enough not to go dry at base flow (e.g., low wintertime flow), well-mixed, and constantly flowing. Typical site choices may be the main channel or thalweg and avoiding setting a logger near a tributary junction or known upwelling area (e.g., spring). If you are setting a logger for a longer-term study in an unfamiliar area, probing the area with a thermometer to explore variation in temperature is a wise approach. In addition, avoid areas that are obviously actively scouring or aggrading (accumulating sediment) as a buried logger could provide some bias. During the retrieval process it is important to ensure that the person collecting the data loggers indicates the placement of each logger collected, specifically was it in or out of the water, was it buried by sediment, etc.

Relocating an undamaged logger is a critical step to ensuring data collection. After site selection, it is important to be sure that you can relocate the logger (take a GPS location and mark it on a map), take detailed field notes using distances from permanent markers (possibly even include a detailed hand-drawn map of the reach with the logger including its placement), and if possible mark the logger placement on the stream bank (stake, flag, etc). Natural or human disruption may also prevent relocating a logger. Placement of the logger out of the way of human, livestock, and wildlife high use areas is often necessary to avoid logger loss. Additionally, signs and discussion of the loggers with local people and landowners can help avoid unintentional disruption of data collection.

Natural disturbances cannot be controlled but seasonal disturbances are predictable. The most common issue is high stream discharge. Drag at high flows and movement of substrate and debris can damage or dislodge loggers. Unless measuring at high flows is necessary, deploying loggers after high flows have subsided and retrieving loggers before high flows become problematic is the safest approach. Regardless of the flow, to avoid a dislodged logger, anchoring the temperature logger to a large rock or tree is advisable.

Data processing and analyses

First examine all data for errors, including temperature readings obviously out of the range of measure (< -5°C or >30°C). Then plot the readings and examine the daily fluctuations. Substantial jumps in temperature or increases in the daily fluctuations can indicate that the logger was out of the water. Flagged observations should be considered with field notes and personnel familiar with the system to determine if they should remain in the analyses.

Often a variety of statistical summaries are used to describe the temperature regimes. The focus may be minimum temperatures, maximum temperatures, mean daily temperatures, or some type of summertime average. Maximum temperatures may be the absolute daily maximum, weekly mean maximum temperature, or average summertime maximum temperature. Often in the U.S. water quality criteria for western cold water fish are based on maintaining enough water in a system to remain below critical thresholds of maximum temperature to avoid damage to aquatic organisms. Daily average temperatures are often used to estimate habitat quality or growth rate projections and development time for aquatic organisms.

Archiving temperature data is useful for ensuring that the baseline data is maintained and available for future impact assessment or trend analyses.

Obviously the approach depends upon the software available, but should include information on the following: the logger type and model, serial number, calibration information, watershed and stream name, GPS coordinates, detailed information on field site location and habitat characteristics, time of deployment and settings, and any notes from logger retrieval.

Best references for measuring water temperature in lotic systems:

http://fresc.usgs.gov/products/papers/1431_Dunham.pdf

Potential companies to purchase temperature loggers:

http://www.onsetcomp.com/products/data-loggers/temperature-data-loggers

Substrate

Introduction

The stream bottom is important to stream organisms as it impacts the growth of benthic algae, fungi, and bacteria. In addition, the community composition of bottom-dwelling macroinvertebrates is strongly influenced by bottom substrate. Many macroinvertebrate Orders (stoneflies, mayflies, and caddisflies) that are important prey for fish require stream bottoms of gravel and cobble without lots of fine sediment and silt. Several fish and amphibian species may lay their eggs in the gravel and interstitial spaces can be important for egg development and juvenile rearing.

The stream bottom is composed of organic matter and silt, clay, sand, gravel, cobble, and larger rock referred to collectively as substrate. The expected substrate on the stream bottom varies over the length of a stream (from headwaters to the mouth) and among habitats (from pools to riffles), and between seasons (high flow and low flow seasons). High gradient, high flow systems move much of the smaller sized substrate downstream leaving large gravel, cobble, and larger rock. In areas with lower velocity much of the fine substrate falls out to the bottom and accumulates. Typically riffles have larger substrate than pools, and high-gradient headwater stream sections have larger substrate than lower, larger streams near their mouth. Excessive amounts of fine sediment can decrease clarity (increase turbidity) and prevent sunlight from reaching algae, fish from finding food, and clog the filter feeding apparatus of macroinvertebrates. In addition, excessive amounts of fine sediment can bury and suffocate fish eggs or smother macroinvertebrates. Sources of excessive sediment are often associated with erosion from road building

or other construction, large-scale clear cutting, or high density travel of livestock or humans across and within streams.

There are multiple approaches to measuring substrate including cobble embeddedness or quantifying the size structure of the sediment. Cobble embeddedness is the extent to which the cobble are surrounded or covered by fine sediment. In western U.S. stream systems, when cobble embeddedness reaches 30–40%, macroinvertebrate production and salmonid spawning success is reduced. We often may also quantify the size frequency distribution of the substrate by either doing a Wolman pebble count or taking McNeil core samples that are then dried, sieved, and weighed. Below we will describe both cobble embeddedness and Wolman pebble counts because of their ease and lack of reliance extensive equipment. The high flow season will move substrate and scour pools and as a result we often sample substrate before the winter or high flow season.

Assessment of size-frequency distribution: pebble counts
The Wolman pebble count method (Wolman 1954, modified by Potyondy and Hardy 1994) is a widespread technique for assessing substrate.

Equipment: hand ruler, caliber, data sheet, pencil, clipboard, (possible field tape measure)

At the selected study reach, set up approximately 12 diagonal (zig-zag) transects with bank intersection points spaced apart twice the typical stream width (this can be measured or approximated). Sample approximately 10 evenly spaced particles per transect, resulting in over 100 measurements per reach. With this technique, it is important to sample a minimum of 100 particles but no disadvantage (other than time) to recording more observations (often up to 200 observations). It is key to minimize bias in particles chosen, thus ensuring an even distribution of samples across the stream bed and collecting an unbiased particular along transects is necessary. Ensure the sampling plan (distances between samples on transects) is clear and as data collectors proceed along transects they pick the particle at toepoint (the first particle felt by the finger at the toe of the boat of the data collector). Once the particle has been collected, the data collector then measures the intermediate axis in millimeters using a ruler or caliper. The intermediate axis is neither the longest nor shortest of the perpendicular axes of the rock. The intermediate axis is the dimension of the particle that controls whether or not it could pass through a sieve (from Harrelson et al. 1994; picture). If boulders are too big to pick up, take an approximate measurement in the field by holding a ruler

above the boulder assuming the two largest axes are visible. For small particles (under 2 mm on the intermediate axis), record the size as less than 2 mm.

Sort data points into rank order and plot every 10^{th} percentile point to plot a cumulative frequency line. This plot has particle size on the x-axis and cumulative % on the y-axis. Certainly examining the differences in these plots or comparing indices such as the 50% size range, median, and range of particles at impacted (wildfire, grazing, roads) and reference sites have helped explore how the impact may be altering the substrate composition.

Assessment of embeddedness

Embeddedness is a substrate attribute indicating the extent to which the larger particles (larger boulder, cobble, pebble) are buried. Having space and water flow in the interstitial spaces between larger particles are important for supporting benthic invertebrates, small overwintering fish, and some species' eggs. Two measures help describe cobble embeddedness. First, visual assessment of the amount of fine sediment (i.e., silt and sand) in a known area (i.e., sampling hoop or square) and, second, the percent to which it is buried by fine sediment.

Equipment: hand ruler, hoop or grid, data sheet, pencil, clipboard, (possible field tape measure)

Training observers to distinguish between gravel, cobble, and boulders is important. If possible, having a reference jar of each size class or doing some pre-field training on size classes and measuring particles is useful. At the each sampling location (each of 5 or 6 replicate habitats, such as riffles) visually assess substrate within a single randomly located (i.e., blind toss) 60 cm diameter steel hoop (or sample grid). Classify the area within the hoop into one of five embeddedness categories (Platts et al. 1983). Fine sediment includes material less than 2 mm in diameter such as silt, sand, and clay. The categories are numerical classes ranging from 1 to 5 corresponding to embeddedness levels of gravel, cobble, and boulder particles of over 75, 50–75, 25–50, 5–25, and less than 5% of their surface covered by fine sediment, respectively (see picture).

In addition to the spatial extent of fine sediment, evaluating the extent that larger particles are buried in fine sediments is measured. Randomly select a cobble from the hoop and remove it from the streambed, retaining its spatial orientation as you pick it up. Estimate the percent of the cobble's height that is embedded by finer sediment. If it appears buried one-half of the way down, it is 50% embedded (see picture). Usually you can see the point at which it protrudes from the bottom and is exposed. The buried rock is relatively clean while the exposed rock is covered with

bacteria, algae, and fine sediment. The percent of the cobble's height that is embedded can be estimated directly or the observer can measure the embedded height (*De*), or the vertical height of the particle that was embedded in fines prior to removal; and the total vertical height of the particle (*Dt*) which are used to calculate embeddedness as $[(\sum De)/(\sum Dt)] * 100$.

Useful References

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Available: http://elerdc.usace.army.mil/elpubs/pdf/sr36.pdf. (September 2004)

Whitman, M. S., E. H. Moran, and R. T. Ourso. 2003. Photographic techniques for characterizing streambed particle sizes. Transactions of the American Fisheries Society 132:605–610.

Wolman, M. G. 1954. A method of sampling coarse riverbed material. Transactions of the American Geophysical Union 35:951–956.

Appendix 7.1: Example Datasheet (Temperature)

Site information: _____
Watershed: _____ Stream name:_____
Site #:_____
GPS coordinates: _____Site noted on map? ____

Detailed site description:

Draw detail of site reach and location of logger (Photo ID reference:_____)

Habitat type of placement (riffle, run - remember well-mixed area):
_____
Wetted width at site:_____ Depth of logger:_____
Tethering or anchoring method:
_____
Comments:

Logger information:
Logger type: _____ Serial number: _____ Sampling interval: _____
Date placed in stream: _____ Time placed in stream: _____

Retrieval information:
Date logger retrieved: _____ Time logger retrieved: _____
Habitat type of placement (riffle, run - remember well-mixed area):
_____
Wetted width at site:_____ Depth of logger:_____

Comments on status of logger (e.g., buried):

Appendix 7.2: Example Datasheet (Substrate Pebble Counts)

Data Collectors: _____

Date: _____ Time: _____

Watershed: _____ Stream Name:_____

Site No:_____

GPS coordinate: _____Site noted on map? _____

Detailed site description:_____

_____

Sample	Size (mm)	Sample	Size (mm)	Sample	Size (mm)	Sample	Size (mm)	Sample	Size (mm)
1		21		41		61		81	
2		22		42		62		82	
3		23		43		63		83	
4		24		44		64		84	
5		25		45		65		85	
6		26		46		66		86	
7		27		47		67		87	
8		28		48		68		88	
9		29		49		69		89	
10		30		50		70		90	
11		31		51		71		91	
12		32		52		72		92	
13		33		53		73		93	
14		34		54		74		94	
15		35		55		75		95	
16		36		56		76		96	
17		37		57		77		97	
18		38		58		78		98	
19		39		59		79		99	
20		40		60		80		100	

Appendix 7.3: Example Datasheet (Substrate Embeddedness)

Data Collectors: _____
Date: _____ Time: _____
Watershed: _____ Stream Name:_____
Site No:_____
GPS coordinate: _____Site noted on map? _____
Detailed site description:_____
_____

Dt = Total height of cobble
De = Embedded height

Habitat type: _____ Sample #: _____
Embeddedness level category (1-5):_____

Dt: _____ De: _____ OR % embedded: _____
Dt: _____ De: _____ OR % embedded: _____
Dt: _____ De: _____ OR % embedded: _____
Dt: _____ De: _____ OR % embedded: _____
Dt: _____ De: _____ OR % embedded: _____
Dt: _____ De: _____ OR % embedded: _____

Habitat type: _____ Sample #: _____
Embeddedness level category (1-5):_____

Dt: _____ De: _____ OR % embedded: _____
Dt: _____ De: _____ OR % embedded: _____
Dt: _____ De: _____ OR % embedded: _____
Dt: _____ De: _____ OR % embedded: _____
Dt: _____ De: _____ OR % embedded: _____
Dt: _____ De: _____ OR % embedded: _____

Habitat type: _____ Sample #: _____
Embeddedness level category (1-5):_____

Dt: _____ De: _____ OR % embedded: _____
Dt: _____ De: _____ OR % embedded: _____
Dt: _____ De: _____ OR % embedded: _____
Dt: _____ De: _____ OR % embedded: _____
Dt: _____ De: _____ OR % embedded: _____
Dt: _____ De: _____ OR % embedded: _____

Habitat type: _____ Sample #: _____
Embeddedness level category (1-5):_____

Dt: _____ De: _____ OR % embedded: _____
Dt: _____ De: _____ OR % embedded: _____
Dt: _____ De: _____ OR % embedded: _____
Dt: _____ De: _____ OR % embedded: _____
Dt: _____ De: _____ OR % embedded: _____
Dt: _____ De: _____ OR % embedded: _____

Field site:

Classify the embeddedness of the channel in five or more of the representative habitats (riffles, runs, pools) on the mid-stream location (thalweg). Report the modal or most common rating for the site.

APPENDIX: CASE STUDY

Assessing Distribution and Abundance of Three Small Felid Species in Royal Manas National Park, Bhutan

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Introduction and Study Objectives

Wild felids are among the most biologically threatened taxa on earth, and many species are believed to be threatened with extinction in their natural environment (Swanson 2005). Although it is a small country, Bhutan is home to 11 of the world's 36 felid species (Wang 2008, Wangchuk et al. 2006). Tigers (*Panthera tigris*), leopards (*Panthera pardus)*, snow leopards (*Uncia uncia*), clouded leopards (*Neofelis nebulosa*), and leopard cats (*Prionailurus bengalensis*) are warranted full protection as Schedule 1(A) species in Bhutan (MoA and RGoB 2002). In contrast, Bhutan's six other felid species have not yet been granted conservation status due to lack of information on their distribution, abundance, and population trends.

This case study draws on many of the topics from this book to develop an example of how one might actually implement a scientific study of Bhutan's small felid species. The objectives of this example case study proposal are: 1) compare three non-invasive methods—remote camera trapping, scat collection (for genetic analysis), and hair collection (also for genetic analysis)—for surveying small felid species potentially differing in abundance, home range area, and habitat use; and 2) determine the distribution and abundance of three small felid species—leopard cats, marbled cats (*Pardofelis marmorata*), and golden cats (*Pardofelis temminckii*)—in Royal Manas National Park, Bhutan, where camera trapping recently confirmed their presence.

In the rest of this Appendix chapter, we will write as if this were an actual proposal to do this work, providing a "hands-on" example for how the topics of this book might be applied to conduct wildlife research in Bhutan. Objective 1 will be addressed in a pilot study on one intensive 5X5 km survey unit. In this pilot study we will use capture-mark-recapture techniques to determine abundance of the three small felid species, and compare probability of detection, species- and habitat-specific biases, and relative cost of each survey method for achieving study objectives. For Objective 2, we will use an occupancy modeling framework to determine probability of occupancy and proportion of area occupied across the landscape for each target species within the Park.

Study Area

Covering 1057 km^2, Royal Manas National Park is Bhutan's largest representative habitat of tropical and sub-tropical ecosystems (Figure A1). It is located in south central Bhutan and is bordered on the north by Jigme Singye Wangchuck National Park (1723 km^2) and on the south

by India's Manas Tiger Reserve World (360 km²). RMNP's broad altitudinal range (129 m–2124 m above sea level) encompasses a diversity of ecosystems, including sub-tropical moist broadleaf forests, warm broadleaf forests, cool broadleaf forests, subtropical dry chir pine habitats, temperate meadows and grasslands, and freshwater and wetlands ecosystems. The Park is home to thousands of animal and plant species, many of which are globally endangered. Royal Manas National Park is not only the most biologically diverse protected area in the Kingdom of Bhutan, but is also a hotspot for global biodiversity (Tempa et al. 2013).

The Park is administratively divided into three blocks—Gomphu, Manas and Umling—to ensure effective delivery service to people residing within the Park. The blocking is not based on habitat types and each block has a separate staff for research with research priorities being the same in all blocks. The park is inaccessible during the summer months (July–September) due to heavy rains and frequent roadblocks.

Target species

This study will evaluate the distribution and abundance of three small felid species known to occur in Royal Manas National Park: the leopard cat, marbled cat, and golden cat. Information on habitat, home range, and behavior are provided in Table A1 and images of the species are provided in Figure A2. To date, there have been no studies on these cats within Bhutan.

Timeline

This study will establish guidelines for implementing non-invasive studies of small felid species in similar habitats of Bhutan and will generate baseline data for long-term monitoring of population trends for three small felid species in RMNP. Prior to implementing a pilot study, we will conduct reconnaissance and mapping of major foot and game trails and access points in RMNP. A comprehensive trail map will facilitate planning and implementation of the Park-wide occupancy survey and subsequent long-term monitoring. Potential target species scat and hair samples collected during reconnaissance will be used, in conjunction with known-species zoo samples, to optimize extraction and amplification of DNA for species (and potentially, individual) identification.

In Year 1, we will conduct a pilot study (Objective 1) to evaluate the effectiveness of three non-invasive survey techniques for achieving study objectives. The pilot study for abundance/density estimation will be implemented at one intensive minimum 5X5-km survey unit located in an area of high trail density within RMNP (see Figure A1 for suggestion). We suggest a spacing of 1 km between cameras but admit that survey size may need to increase to 1.5 km spacing if we do not achieve enough captures and recapture of different individuals. The pilot study will be conducted over two 3-month sessions, e.g., April–June (Session 1) and October–December (Session 2), with results compared between sessions to explore for seasonal differences in detections and abundance/density estimates. The October-December time frame is probably best due to the weather and hence the first survey should begin at this time.

We will use findings from the pilot study to inform design of the larger-scale occupancy survey, which will be implemented in Years 2 and 3. The occupancy survey will be conducted by RMNP

administrative block, beginning with the Manas block. In total, we plan to survey 35-40, 3X3-km units, 3-4 times each, distributed over the three administrative blocks over a 2-year period. Occupancy estimates from surveyed units will be combined with GIS landscape and field-collected habitat data to predict occurrence of target species across the Park.

Pending outcome of the initial 3-yr study, we suggest expanding, in Year 4, to conduct annual abundance/density surveys via remote camera traps for monitoring long-term population trends in the original (pilot) target block noted in Figure A1 and in at least 2 other blocks for comparison to the original survey block. These survey sites should be similar in size and will be conducted once per year. This long-term monitoring program will allow the accumulation of data that will enable estimation of trends in abundance through time (i.e., stable, increasing, or decreasing) and eventually allow for calculation of yearly survival rates for the felids. This information, currently unknown for these wild felid species, is important for assessing population status and health of the felids. Positive population trends and high survival rates could indicate healthy or persistent populations while negative population trends and poor survival would indicate a potential problem with long term species survival.

Used in combination with the occupancy surveys (see below), we can determine where across the park the small cats are present, and then, using our estimates of density from the long-term monitoring, extrapolate density estimate to estimate total number of felids on a park-wide basis.

Pilot Study

The pilot study will evaluate feasibility and cost-effectiveness of three detection methods (remote cameras, hair rub pads, and scat transects for DNA sample collection) for the Park-wide occupancy study. Specifically, for each survey method, pilot study results will be used to: 1) optimize survey protocols, e.g., determine appropriate spacing of camera and hair rub pad stations; 2) estimate target species 'capture' rates; 3) identify species-habitat relationships that may warrant habitat stratified sampling; 4) identify seasonal biases in species detection and occurrence; 5) calculate minimum cost-per-unit for occupancy detection; and 6) estimate minimum cost-per-unit for estimating abundance of common target species.

Remote camera trapping

We will follow standardized camera trapping protocols developed for other species of small cats such as ocelots (Dillon and Kelly 2007, 2008), Geoffrey's cats (Cuellar et al. 2006), and bobcats (Heilbrun 2006). Cameras will be placed in a grid-like formation with a spacing of 1–1.5 km between camera traps based on the small home range sizes of the leopard and marbled cats. This spacing should ensure no holes in the grid large enough for an entire home range and hence each individual should have a probability of being captured (Otis et al. 1978). We do note however, that this spacing may be too close together for the golden cat, which has a much larger home range and therefore our grid may not be large enough to accurately assess golden cat abundance (Maffei and Noss 2008).

We will use a minimum of 25 camera stations and each station will have 2 cameras mounted on opposite sides of a road or trail to photograph both flanks of the passing animal for positive ID.

Cameras will be placed in areas that are natural funnels (e.g. trails, roads, newly cut trails, etc.) at 20–30 cm in height and will be operative for 24 hours per day. Cameras will be checked for proper functioning, downloading images, and for battery and memory card levels approximately every 10 days. Stations will be operational for 70–90 days to ensure enough captures and recaptures for mark-recapture analysis.

Both leopard cats and marbled cats have unique coat patterns allowing for individual identification necessary for mark-recapture analysis. Golden cats have subtle markings that may allow estimation of abundance following the methods of Kelly et al. (2008). We will compile capture histories for each individual animal and analyze the mark-recapture data for each species in Program CAPTURE (Rexstad and Burnham 1991) to estimate abundance. We will also estimate density using the classic ½ mean maximum distance moved (½ MMDM) method originally developed for small mammals (White et al 1985) and modified for tigers (Karanth 1995, Karanth and Nichols 1998). Finally, due to the recent development of spatially explicit models for analyzing camera-trap, mark-recapture data, we will also estimate density directly through Program DENSITY (Efford 2004; 2007). Both CAPTURE and DENSITY are available as free software downloads.

For species that are not individually marked (i.e., the golden cat or any prey species we are interested in), we will calculate trap success as the number of photo captures per trap night. A photo capture will consist of any distinct individual photo captured within a 30-minute time period. Trap success can be used as an indication of activity level at each particular camera station, and has been used as an index of abundance (O'Brien 2003) but this is controversial (Carbone et al. 2001, 2002, Janelle et al. 2002). At the very least trapping rates can identify areas in the study site with high versus low animal activity and animals "captured" can be used to compile a species inventory for the park (as in Tempa et al. 2013).

DNA collection

Hair rub pads

We will examine the effectiveness of hair rub pads for detecting target species. In the pilot study within the remote camera trapping grid, we will place rub pad sets along surveyed game and foot trails, halfway between camera sites. A rub pad station will consist of 4 rub pads spaced along the trail at 10 m intervals. Rub pads will be nailed to trees at target species' shoulder height, with visual attractants (pie pans) overhead. A detailed description of hair rub pad construction, set-up, and choice of scented lures can be found in Long et al. (2008). Rub pads will be checked and rebaited every 10 days while checking camera traps for proper functioning. If rub pads prove to be cost effective for detecting target species, their use as a survey tool of the Park's small felid species will be expanded in the Park-wide occupancy study.

Scat collection

Scat collection will be conducted on foot during the checking of the camera stations and hair rub pads within the pilot study unit. In addition, researchers will explore other likely felid movement corridors such as game trails, ridge lines, and riverbanks. In all cases researchers will note type of trail, weather conditions, scat color, scat degradation category, presence of mold, etc., following Wultsch (2009) (and this book Chapter 3). Researchers will record distance

travelled on scat transects to assess the efficiency of scat collection techniques (scats collected per km walked)—much like a photographic trap success rate, which can also be used later to assess activity levels and potentially relative abundance.

Scat samples are known to degrade due to environmental factors. Data collected regarding scat quality can be compared to amplification success to determine how to identify high quality scat samples in the field for more efficient data collection and analysis. If this approach proves difficult or inefficient, an alternative is to pre-clear scat transects, then resurvey transects at a later date (e.g., 10 days after clearing, concurrent with checking camera and hair rub pad stations) to collect newly deposited scats.

Appropriate sampling duration (i.e., time between clearing a transect and resurveying for fresh scats) depends on 'capture' rate, DNA degradation rate, and logistical issues of site access and coordination with concurrent survey methods. A longer sampling duration may increase sample size, but may reduce DNA quality. DNA degradation rate may vary with season, with faster degradation during warm, wet seasons. If a sampling duration of 10 days yields low genotype success (e.g., successful DNA amplification from <70% of collected scats), a maximum sampling duration for scat transects can be determined by clearing felid scat from transects, and resurveying transects every 2 to 3 days for several weeks. When a new felid scat is found, the date of first discovery should be recorded and a portion of the scat collected during each subsequent survey until no sample remains. Thus, each scat will yield samples of various ages (i.e., exposure to DNA-degrading field conditions) for estimating season-specific DNA degradation rates. These data can be used to determine the relationship between number of days passed and number of new target felid scats deposited per km transect, and DNA amplification success as a function of sample age.

Individual identification

The DNA from both the scat and hair collection techniques can be used to identify individuals through microsatellite analysis, but identifying individuals from genetic samples is more costly and time-intensive than identifying species (Chapter 3). If funding permits individual identification, mark-recapture statistics can be used following similar methodology as for remote camera trapping (see above and previous chapter this book) to estimate abundance and density within the pilot camera trapping grid. This would provide tremendous insight into which technique is most efficient and economical for abundance estimation.

Habitat assessment

For the three methods described above, capture rates can be linked to specific habitat variables collected at the camera station level (or for scat, within a specified radius of scat locations) to enhance understanding of habitat features that influence animal presence and activity across the grid (Davis et al. 2011). Therefore, we will collect habitat data surrounding each station/scat location following Davis (2009) and Davis et al. (2011). Please see Chapter 4 for habitat data collection protocol surrounding camera stations. Similar measures can be taken surrounding each scat sample. In addition, if detailed GIS maps are available, habitat variables can be extracted from GIS layers in circular buffers surrounding camera traps or scats following Holub and Kelly (2008) to further examine relationships between capture rates and landscape

features. If the pilot study identifies some target species as habitat specialists, subsequent surveys will use a stratified sampling design for detection surveys.

Occupancy Survey

After completion of the pilot study, we will expand our approach through the use of detection/non-detection surveys to predict occupancy of target felid species across the entire Park (see Chapter 2). We will use a combination of all three types of detection methods for this study. Typically, repeated visits to a site are used to create a detection history for each site (like a capture history for each individual animal in mark-recapture) and to estimate detection probability, site occupancy, proportion of area occupied, and to model the covariates that influence occupancy. Occupancy data can be analyzed in the free software Program PRESENCE (MacKenzie et al. 2006).

For our study, we have placed a 3X3 km grid across the entire RMNP (Figure A3). We suggest surveying a random (or stratified random) subset (30 to 40) of these cells. The final sampling protocol for the occupancy survey, particularly the most cost-efficient combination of methods and their implementation (number and distribution of stations, sampling duration, etc.), will be based on results of the pilot study. For example, we may use 5 remote cameras for 2 weeks in each grid cell placed in likely locations for our target species. During initial set-up of camera stations and the follow-up site visit to retrieve photos we may survey a minimum of 5 km of transects for scat collection within each grid cell. If a particular target species proves elusive to camera trapping and scat surveys but effectively detected by hair rub pads, we may distribute hair rub pads in habitat types frequented by that species. Each cell will be surveyed a minimum of three times to create a detection/non-detection history for each grid cell.

For each searched grid cell we will extract GIS information on important habitat and landscape features to be used as covariates in predicting occupancy across the landscape. Such covariates may include slope and elevation (ruggedness), habitat type, % available water, distance to nearest road, road density, distance to nearest village, human use pattern, etc. In this way, these patch-occupancy models (MacKenzie et al. 2006) will allow us to use detection/non-detection surveys, combined with spatial modeling, to estimate and predict species occurrence across a landscape. As an example, Linkie et al. (2006) conducted repeated sign surveys for tigers (tracks and scat) in Sumatra, combined with data layers from GIS, to model tiger presence and predict probability of occurrence across the landscape. They found that tiger occurrence was predominantly influenced by distance to public roads, and identified four core areas for tigers.

Anticipated Results

We anticipate estimating abundance and density for the leopard cat, marbled cat, and possibly the golden cat. We will also provide a species inventory for all species captured via remote camera photographs providing a baseline of information on predators and prey species. We will assess factors such as optimal trap spacing and optimal combination of detection devices resulting in the largest number of species detections. Habitat data collection surrounding each camera trap station will be used to model the factors that influence trap success giving us valuable information on habitat preferences. In addition, trap success of predators relative to

other predators, prey, and humans will give us insight into other biological factors influencing target species activity rates within the camera grid.

We also anticipate completing a feasibility study designed to evaluate the cost-efficiency of the three survey methods for achieving project objectives. Criteria for comparison will include (scaled to method-specific costs): 1) number of each target species detected as a function of survey duration; 2) time to first detection of each target species; 3) proportion of each target species detections accounted for by each device type; and 4) rate of trap "failure".

After the initial pilot study, expansion of trapping grids and stratifying by habitat will allow determination of whether abundance/density of target species varies by habitat type. Occupancy surveys will result in estimates of detectability, occupancy rates, and proportion of area occupied. We will assess species occurrence over an entire landscape through sampling only a portion of that landscape. And finally we will determine the landscape factors most important in determining occupancy over a broad scale, giving us tremendous insight into the ecology of the target species.

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