Short communication

Sampling animal sign in heterogeneous environments: How much is enough?

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ABSTRACT

Animal ecologists often use animal sign as a surrogate for direct observation of organisms, especially when species are secretive or difficult to observe. Spatial heterogeneity in arid environments makes it challenging to consistently detect and precisely characterize animal sign, which can bias estimates of animal abundance or habitat use. Piute ground squirrels (*Urocitellus mollis*) and Owyhee harvester ants (*Pogonomyrmex salinus*) live in arid environments and are fossorial, which can make them difficult to observe directly. Their relative abundance can be assessed using sign (i.e., burrows and nests). We implemented an *over-sampling* framework (i.e., recorded an excessive amount of information) with two observers to 1) identify a sampling intensity that balanced precision with our resource constraints, and 2) assess classification and detection of squirrel burrows and ant nests across vegetation conditions. We sampled 20 1-ha plots for ground squirrel burrows and ant nests using six 4 m × 100 m belt transects. Analyses of precision and sampling effort indicated that three belt transects covering 1200 m² per ha provided sufficient precision, while minimizing effort. Regardless of vegetation conditions, counts by two observers were strongly correlated for ground squirrel burrows ($r = 0.99$, $P < 0.001$, df = 18; slope = 0.92) and harvester ant nests ($r = 0.99$, $P < 0.001$, df = 18; slope = 1.01) indicating observer consistency and perhaps high detection probability. These findings illustrate an approach for evaluating sampling designs in many ecological contexts.

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1. Introduction

Accurate estimates of a species' distribution and abundance across landscapes are fundamental to animal ecology (Wilson and Delahay, 2001; Morrison et al., 2006). However, obtaining reliable counts of secretive (e.g., nocturnal), cryptic, or fossorial animals can be difficult, particularly when sampling extensive landscapes. As a surrogate for direct observation, wildlife biologists often rely on animal sign such as tracks (e.g., Bonesi and Macdonald, 2004), scat (e.g., Hodges and Mills, 2008; Alves et al., 2013), or burrows (e.g., Lara-Romero et al., 2012). Detection and enumeration of this sign is then used to produce estimates of occupancy, true density or abundance, or relative abundance. In some cases, counts of animal sign may not correlate with occurrence or true abundance because

of imperfect detection, mis-match of scales, animal behavior, or a disconnect between sign deposition and individuals (MacKenzie et al., 2002; Pollock et al., 2002; Bonesi and Macdonald, 2004). Ensuring sign surveys are applied appropriately is essential. For instance, if the research objective is to assess factors influencing relative abundance, it is important to evaluate detection of sign and ensure sampling is sufficiently precise to identify changes in these metrics (Engeman, 2005; Morrison et al., 2006). Understanding what influences the detection and precision of values derived from animal sign is especially important when temporal or spatial variation is high.

Many terrestrial environments, including arid lands, exhibit spatial heterogeneity reflecting variation in soil, topography, climate, and plant competition for limited resources (e.g., Hunter and Price, 1992). Animal distribution, habitat use, and abundance are often associated with this variability because of resource needs such as food, water, and shelter (Morrison et al., 2006). Sampling animal populations in variable environments can be challenging,

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often resulting in a trade-off between precision of parameter estimates and sampling effort (Wilson and Delahay, 2001; Hodges and Mills, 2008). Effort can be defined by number of sampling units or sub-samples, time used for each sample, or financial cost. Evaluating the precision versus effort trade-off is important to identify a protocol that maximizes detection and statistical precision, yet balances financial and logistical constraints. Few studies evaluate the performance of sampling protocols (Morrison et al., 2006), which is surprising considering the influence of sampling design on statistical analyses and research objectives.

We sought to implement an approach that could be used by others to evaluate relative abundance or habitat use of animals using sign surveys. We include relative abundance and habitat use together because counts per unit area can be interpreted as both, depending on the relationship between sign and individuals. Our objectives were to assess 1) how precision of abundance estimates varied as a function of sign abundance and variation, and sub-sampling intensity, 2) how sampling effort varied with abundance of sign, and 3) if and how sign classification and detectability changed across vegetation conditions and sign abundances. We implemented an *over-sampling* design (i.e., recording an excessive amount of data relative to logistical constraints) with two observers in the sagebrush-steppe, a heterogeneous and arid landscape. We surveyed for recently active burrows of Piute ground squirrels (*Urocitellus mollis*; hereafter ground squirrels) and nests of Owyhee harvester ants (*Pogonomyrmex salinus*; hereafter harvester ants). Ground squirrels and harvester ants are ecologically important because of their role as ecosystem engineers (Laundré, 1993; MacMahon et al., 2000), prey (Marti et al., 1993), and seed consumers (Van Horne et al., 1998; Anderson and MacMahon, 2001). Our over-sampling approach allowed us to identify a precise and efficient sampling protocol characterizing ground squirrel and harvester ant relative abundance. This framework could be applied by other investigators assessing sampling designs in spatially heterogeneous environments.

2. Study area

The Morley Nelson Birds of Prey National Conservation Area (BOP) is a 1962 km^2 region within southwestern Idaho (Latitude: 43.283, Longitude: 116.200), and captures a gradient of ground squirrel and harvester ant densities. The Snake River canyon in the BOP provides nesting habitat for one of the most abundant and diverse assemblages of raptors world-wide (Olendorff and Kochert, 1977), which primarily forage on small mammals including ground squirrels (Marti et al., 1993). The BOP is an arid (110–350 mm annual precipitation) sagebrush-steppe environment that is managed under a multiple-use framework by the Bureau of Land Management. The BOP landscape has been increasingly fragmented over the last 100 years or so, primarily due to exotic grass invasion, human use, military training, livestock grazing, and increased fire frequency (Yensen et al., 1992). These factors have contributed to a gradient of non-native plant invasion and successional stages within vegetation communities, including big sagebrush (*Artemisia tridentata*), native perennial grasslands, and exotic annual grasslands (i.e., primarily cheatgrass, *Bromus tectorum*).

3. Methods

We collected data within 100 m × 100 m (1 ha) plots ($n = 20$). Each plot was selected based on a stratified random design within areas that were historically sagebrush. To capture a gradient of successional and non-native plant invasion stages, three strata were delineated using the best available GIS data: 1) areas that were previously burned and seeded (aerial or drill seeded with

native and non-native rangeland perennial plant species), 2) areas that were burned and not seeded, and 3) unburned areas (i.e., fires absent for at least 50 years). We randomly selected sampling plots in each stratum to capture a representative distribution of burrowing animal relative abundance.

Two observers visited the sampling plots in late March 2013, and surveyed for recently active ground squirrel burrows and harvester ant nests. The end of March coincides with the emergence of all ages of ground squirrels (i.e., high squirrel activity), and substantial ant activity within our study area. At each 1-ha plot, both observers independently counted burrows or ant nests within six 100 m × 4 m belt transects spaced ≥16 m apart in a north-south orientation (i.e., 6 sub-samples per plot). To ensure survey consistency, we extended a 100 m tape that we used as our transect center, used a 2 m long PVC pipe to determine if burrows or nests were within belts, and recorded counts in 50 m increments. Additionally, we surveyed half of the belt (i.e., 2 m wide) at any one time to increase detection probabilities. We classified ground squirrel burrows as recent if the entrance was approximately 6–12 cm wide (Laundré, 1989), >5 cm deep, and had ≥2–3 mm of fine soil at the burrow entrance. We classified harvester ant nests as active if we observed individuals in or on the nest. If we observed no ants on a nest, we disturbed the surface to observe if individuals responded.

3.1. Data analysis

We first examined how the precision of plot-level estimates of burrow and nest counts changes as a function of the number of transects surveyed within each plot. To capture the maximum heterogeneity between transects, we selected sub-samples (i.e., 2–5 transects) from the six 4 m (wide) × 100 m (long) belt transects in each plot that maximized the spatial distance between transects (i.e., ≥16 m apart). For each sub-sample, we calculated the mean number of burrows or nests and associated *SD*. We plotted the means and *SD*s for all sub-samples across plots to identify the range that encompassed most of the means and *SD*s we observed. For ground squirrels, the range of means and *SD*s was 1.00–15.00 and 1.00–7.00, respectively. The median number of burrows per transect and *SD* across plots and sampling intensities was 8.33 ± 3.48. For harvester ants, means ranged between 0.10 and 3.00, and *SD*s were 0.10–1.25. The median number of nests per transect and *SD* (excluding means of zero) across plots and sampling intensities was 0.50 ± 0.58.

We then built a matrix for each sampling intensity across plots with means as rows and *SD*s as columns, and populated the matrix with calculations of relative standard error (RSE = *SE*/mean). We used RSE rather than relative *SD*, or coefficient of variation, because *SD* describes the variation within a sample, whereas *SE* indicates the reliability of estimated means; that is, *SE* describes the variation within the sampling distribution. To examine patterns of the RSEs, we plotted RSE as a function of burrow or nest abundances, *SD* of abundances, and sampling intensity. We were interested in identifying a sampling intensity that reached a RSE ≤ 30% (e.g., McCune and Grace, 2002) near the overall (i.e., across plots and sub-samples) median number of burrows or nests per transect and median *SD*. We chose this criterion because, on average, we wanted our precision to be at least 30% of our average abundances.

Second, we assessed how sampling duration (i.e., person-hours) changed as a function of plot-level abundance of ground squirrel burrows and sampling intensity to determine the level of sampling that maximized time efficiency. We calculated sub-sample duration by multiplying a fraction to the total time spent sampling (i.e., six 4 m × 100 m transects on each plot). For example, the duration of sampling for three transects was estimated by multiplying the total