

Abstract

Bailey, Larissa Lynn. Estimating detection probabilities for terrestrial salamanders in Great Smoky Mountains National Park. (Under the direction of Theodore R. Simons.)

Recent worldwide amphibian declines have highlighted a need for more extensive, rigorous monitoring programs. Investigators must make decisions about which state variable to monitor based on the monitoring program's scientific or management objectives, while considering economic and logistical constraints. Two sources of variation; spatial variation and variation in detection probability constrain the inferences drawn from these monitoring programs. Our research focused on estimating detection probabilities for three state variables commonly used in terrestrial salamander monitoring programs: population size, proportion of area occupied, and species richness.

Approximately 10% of the world's salamander species are found in the southern Appalachian region and they are a high priority taxon in Great Smoky Mountains National Park (GSMNP). We used Pollock's robust design in a 3-year capture-recapture study at 15-20 replicated sites in a single watershed in GSMNP. We used competing models to estimate detection probability parameters for plethodon salamanders, determine the importance of temporary emigration (i.e. the probability of being absent from the sample area), and explored temporal and behavioral effects on conditional capture probabilities. Models that included random temporary emigration were chosen four times more often than models with no temporary emigration. Models that contained behavioral effects in capture probabilities were preferred over models with only time

effects, but there was evidence that behavioral and time effects together influenced capture probabilities.

We used the ‘best’ robust design model to test *a priori* hypothesis about spatial and temporal variation in salamander detection probability parameters. We explored the effects of 3 large-scale habitat characteristics (disturbance history, elevation, vegetation type) and found vegetation type and elevation were significant covariates in temporary emigration, conditional capture probability, and surface population size estimates. All detection probability parameters increased over the 3-year study, but estimates of surface and superpopulation (total population) did not change.

We estimated the proportion of area occupied (PAO) and species detection probability for 7 salamander species using other sites within the same watershed. We tested whether the type of sampling method, the number of sites sampled, or the number of sampling occasions per site affected PAO parameter estimates. We also investigated *a priori* hypotheses about temporal and spatial variations in PAO parameter estimates associated with four large-scale habitat characteristics (covariates). Both PAO and species detection probability estimates varied among species, sampling method, and year. In general, the accuracy and precision of PAO and detection probability estimates were better using natural cover transects rather than coverboard transects. Reducing the number of sampling occasions or the number of sites sampled reduced PAO precision. Average species-specific detection probabilities showed consistent patterns over our 3-year study (no species x year interaction), but within year detection patterns varied among species. PAO methods were capable of revealing differences in species’

distribution types (clumped or widespread) as well as potentially important species-specific habitat covariates.

Finally, we explored the effectiveness of estimating species richness using two methods, but neither method performed well, primarily because there are not enough different species at each site to allow meaningful comparisons across time or space.

This study represents the largest mark-recapture study on terrestrial salamanders and is the first to estimate a suite of potential state variables and related detection probabilities. We found strong evidence that detection probabilities change over time, space, and among species. Therefore, we discourage using unadjusted count data to make inferences about the status of amphibian systems without estimating or eliminating differences in detection probabilities.

**ESTIMATING DETECTION PROBABILITIES FOR TERRESTRIAL
SALAMANDERS IN GREAT SMOKY MOUNTAINS NATIONAL PARK**

by

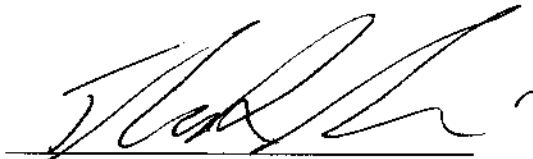
LARISSA LYNN BAILEY

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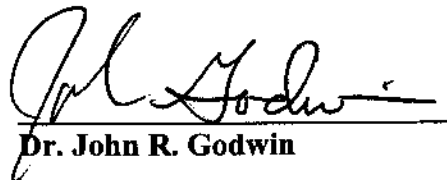
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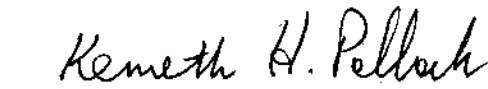
**Dr. Theodore R. Simons
Chair of Advisory Committee**



Dr. Nick M. Haddad



Dr. John R. Godwin



Dr. Kenneth H. Pollock

Dedication

I dedicate this dissertation to my parents for their 40th wedding anniversary.

Biography

I grew up in the mountains and deserts of western Colorado. When my family and I weren't in school, we were at our cabin, splitting time working on one of the grandparent's farms. We hauled our own water, had no television, phone, one radio station, a small library, natural air conditioning and the great outdoors as entertainment – in retrospect I realize my childhood was unique and unusual for someone of my generation. I have been blessed with a family of mentors, especially female mentors, who have challenged and encouraged me to seek out and take advantage of opportunities available to me. Academics and athletics have dominated my life and allowed me to travel widely.

I chose to stay near home for my undergraduate studies at Mesa State College in Grand Junction, CO, but spend summers and semesters abroad at the University of Hawaii at Hilo, Puerto Rico, and around the world with the Semester at Sea program. My undergraduate interests were broad and I had trouble deciding on a graduate field. I spent 2 years pondering the possibilities while employed in a very rewarding job as the Coordinator of the Academic Advising Center at Mesa State College. I visited North Carolina State University, while scouting undergraduate schools for my younger sister. I was impressed by NCSU's Biomathematics program - its quality, its mission, its faculty, staff, and students, and I was thrilled when I was accepted to the program a year later. After completing my masters degree, I wanted to move back into a more biological field. It was the people of NSCU and their commitment to their environment and their profession that kept me here; it was a very good decision.

Acknowledgements

I believe the success, experience, and enjoyment that a student gains during their graduate career is a direct reflection of their graduate committee. I had a fantastic committee – Ted Simons, Ken Pollock, Nick Haddad, and John Godwin. These gentlemen each brought a unique perspective to the project; they were encouraging and involved in every aspect of the research (only John missed helping me in the field); and they serve as examples of the type of mentor I hope to be someday, given the opportunity. I especially thank Ted Simons and Ken Pollock who entrusted a large salamander project to a kid from Colorado, a state with only one salamander species. I was fortunate to be a part of two outstanding labs while in the Zoology Department and I thank the following faculty and students for their support, advice, opinions, and impromptu brainstorming: Martha Groom, Jim Gilliam, Erin Johnson Hyde, Susan Shriner, Kendrick Weeks, Jeremy Lichstein, Juan Manuel (Pajaro) Morales, Roxana Aragon, Todd Preuninger, Ursula Valdez, Andrea Poldosky, Bill Pine, Garrick Skalski, Ellen Damschen, and Ashlee Rowe. One of the things that attracted me to NCSU was the communication and cooperation among departments - my research was enhanced by David Dickey and Marcia Gumpertz (Statistics), George Hess (Forestry) and Steve Ellner (Biomathematics).

I learned a great deal from the two professors I taught under: Dr. Dick Lancia and Dr. Phil Doerr. Over my 7 years at NSCU, Phil has served as my teacher, my advisor, my mentor, my collaborator, and my friend. The administrative and support staff I interacted with have spoiled me for any other job I might hold in the future – thanks to Wendy Moore, Susan Marschalk, Thurman Grove, Kayde Brownlee, Dollie Moore, Jan

Fites, Mary Connors, Ginger Howell, Jim Gilliam, and many others. I also thank the research staffs at Great Smoky Mountains National Park and North Carolina Museum of Natural Science for their logistical and administrative assistance. The Environmental Protection Agency PRIMENet, the U.S. Geological Service, North Carolina Herpetological Society, and the U.S. National Park Service provided funding for this research.

I had the privilege of working with a extraordinary group of field assistants, many of which are far better natural historians than I am; I learned as much from these folks as they did from me: Hillary Stephenson, Jolene Csakany, Samantha Marcum, Raoul Bain, Wendy Ward, Matt Beall, Lisa Klein, Cris Hagen, Iwalani Ching, Michael Kuntz, Lorraine McInnes, Kate Montieth, Melinda Wilson, Karen Whitehead, and especially Thomas Lossen and Marke Ambard, who helped integrate us into the local community.

Probably the most valuable asset I obtained in my time at NCSU is an incredible, talented group of friends: Dewayne, Celia, Louise, Alan, Salinda, Nate, Kirsten, Walt, Mike, Kim, Julie; NC Fish and Wildlife Coop Students; Russell, Liz, Brian, and the Biomath Crew; Randy, Lori and my teammates; and all aforementioned folks, all of which I consider my friends.

Finally, it has been said that persistence and determination are as important to obtaining a Ph.D. as intelligence. It is the underlying love and support of my family that is my foundation and strength. I am proud of each and every one of them: Mom, Dad, Grandma, Tiff, and three people who have become family – Dave, Kate, and Andrew.

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**Chapter 1. Estimating detection probability parameters for Plethodon salamanders
using the “robust” capture-recapture design**

Larissa L. Bailey¹, Theodore R. Simons¹, and Kenneth H. Pollock²

¹ Cooperative Fish and Wildlife Research Unit, Department of Zoology, North Carolina
State University, Campus Box 7617, Raleigh, NC 27695-7617, USA

² Department of Statistics, Biomathematics, and Zoology, North Carolina State
University, Campus Box 8203, Raleigh, NC 27695-8203, USA

ABSTRACT

Recent concern over global amphibian population declines has highlighted a need for more extensive, rigorous monitoring programs. Two sources of variation; spatial variation and variation in detection probability make the design and implementation of effective monitoring programs difficult. We used Pollock’s robust design in a 3-year capture-recapture study to estimate detection probability and temporary emigration for plethodon salamanders in Great Smoky Mountains National Park. We used 12 competing models to determine the importance of temporary emigration and explored temporal and behavioral effects on conditional capture probabilities. Models that included random temporary emigration were chosen four times more often than models with no emigration. The two ‘best’ models contained random emigration and were selected twice as often as any other model. Models that contained behavioral effects in capture probabilities were selected more often than models with only time effects. When

we included Markovian emigration, the probability of emigrating from the surface was usually less than the probability of remaining an emigrant (69% of site-years). Markovian emigration estimates were often similar and always had overlapping confidence intervals, thus the Markovian model was rarely chosen over the random emigration models (only 7.7% of site years). Our study is the first to formally estimate temporary emigration in terrestrial salamander populations, and our results verify that significant proportions of terrestrial salamander populations are subterranean. We determined that the probability of capturing salamanders on the surface and surface population sizes varied temporally within a sampling season. Therefore, we caution against using unadjusted count indices to compare salamander populations over time or space unless detection probabilities are estimated. Temporary emigration models will improve abundance estimates when a large proportion of the population is unavailable for capture during a given sampling period.

Key words: capture-recapture, detection probability, Great Smoky Mountains National Park, MARK, model selection, plethodontid salamanders, Pollock's robust design, population monitoring, temporary emigration.

INTRODUCTION

Concern over amphibian populations has increased steadily in recent years with evidence of global-scale declines (Houlahan et al. 2000 , Alford et al. 2001) and the unexplained disappearances of entire groups of species (Wake 1991, Blaustein et al. 1994). These declines have highlighted a need for more extensive and rigorous monitoring programs to detect and determine the causes of population declines (Heyer et

al. 1994). Numerous organizations are attempting to document, measure, and monitor amphibian populations, especially those believed to be in decline (e.g. Amphibian Research and Monitoring Initiative, North American Amphibian Monitoring Program, Partners in Amphibian and Reptile Conservation, Declining Amphibian Populations Task Force, and US State and Federal agencies). Amphibians can be categorized into 2 broad classes: aquatic (both pond breeding and streamside) or terrestrial (those with direct larval development or those that breed in small terrestrial water sources such as bromeliads). In this paper we describe methods for estimating detection probability for terrestrial amphibians, specifically plethodon salamanders. However, the methods could be applied to many aquatic amphibians or other species in which the population available to sampling is a subset of the total population inhabiting a given area.

Plethodon salamanders have recently been promoted as excellent indicators of biodiversity and forest ecosystem integrity (Welsh and Droege 2001). They are relatively long-lived, slow to mature, and have lower fecundity than most anurans (Petranka 1998). They are susceptible to a variety of natural and anthropogenic perturbations (see Welsh and Droege 2001 for a review) in part due to their permeable skin, which is used for both respiration and osmoregulation.

The lack of long-term population studies and a generally poor understanding of the precision and accuracy of salamander sampling methods have hindered efforts to establish effective, large-scale monitoring programs (Hyde and Simons 2001, Pollock et al. 2002). There are two sources of variation that must be incorporated into a good monitoring design: spatial variation and detectability (Yoccoz et al. 2001, Pollock et al. 2002). A good spatial sampling design involves selecting sample units in a manner that

permits inference about the entire area of interest (e.g. stratified random sample). Additionally, because not all animals are detected in a sampled area, monitoring programs must incorporate methods for estimating or removing effects of variations in detection probabilities (Pollock et al. 2002). While some salamander studies incorporate a spatial design (e.g. Hyde and Simons 2001), few estimate detection probabilities (but see Tilley 1980, Jung et al. 2000, Smith and Petranka 2000, Salvidio 2001). Instead, most studies use a variety of sampling methods that produce relative abundance indices (usually count data) to compare population trends over time or space. Using count statistics as indices of abundance is generally unwarranted (Nichols and Conroy 1996, Yoccoz et al. 2001, Pollock et al. 2002). Two critical assumptions must be met for comparisons of indices to be valid:

- (1) There must be a direct linear relationship between the index and the population size (i.e. the index is directly proportional to population size)

$$E[C] = \beta \cdot N \quad \text{or} \quad \hat{N} = \frac{C}{\hat{\beta}} \quad \text{for a known area}$$

C = number of individuals counted or caught

β = probability of ‘detection’

N = population size

- (2) The probability of ‘detection’ must be constant over time and space

$$E[C_1/C_2] \simeq \beta_1 \cdot N_1 / \beta_2 \cdot N_2 \simeq N_1/N_2 \quad \text{if and only if } \beta_1 = \beta_2 \quad (\text{Lancia et al.}$$

1994)

The assumption of constant detection probability is unlikely to be met for many terrestrial salamanders because detection probability is thought to vary for several reasons. First,

the capture probability for salamanders near the surface may vary spatially due to habitat characteristics or temporally with changing environmental conditions. Furthermore, terrestrial salamander populations are believed to be largely subterranean, with only a few individuals near the surface and available for capture on a given sampling occasion (Taub 1961, Heatwole 1962, Hairston 1987, Petranka and Murray 2001). Site-specific habitat characteristics, environmental conditions, or seasonal behavioral patterns may influence the size of the available surface population.

There are several key elements of salamander detection probability estimation. First, there is a distinction between the surface population and the ‘superpopulation’ of salamanders associated with a sampled area. We define ‘surface population’ as the population of salamanders near the surface and available for capture during a given sampling period. ‘Superpopulation’ refers to the population of salamanders both near the surface (available for capture) and subterranean individuals (unavailable for capture) within the sampled area. Two parameters that influence salamander detection probability are:

- (1) Conditional capture probability, p^*_i , is the probability that an animal is captured given that it is near the surface during sampling period i ($i = 1, 2 \dots k$, $k =$ sampling occasions)
- (2) Temporary emigration, γ^*_i , is the probability that an animal is alive but not available for capture during sampling period i ($i = 1, 2 \dots k$, $k =$ sampling occasions). In our study, we restricted horizontal emigration (see Methods) and assumed that temporary emigration involves salamanders moving temporarily below the surface.

Thus, the probability of ‘detecting’ a given salamander in the superpopulation at a particular time is the product of $(1 - \gamma_i^*) \cdot p_i^*$. This probability of detection, referred to as the effective capture probability (Kendall 1999), is the capture probability reported in most salamander capture-recapture studies (e.g. Jung et al. 2000). Not surprisingly, effective capture probability estimates are usually low (often below 0.10) and result in population estimates with large confidence intervals (e.g., Howard 1987).

The occurrence of temporary emigration often violates key assumptions for both open and closed-population capture-recapture models. Closed-population models assume that neither emigration nor immigration occurs within the sampling area during the study. Open-population models, such as the Jolly-Seber (JS) model (Seber 1982), assume that all emigration from the sampling area is permanent (Pollock et al. 1990). Violations of these assumptions result in biased estimates of population parameters. The presence, severity, and direction of the bias depend on the proportion of emigrants and whether the emigration is completely random or Markovian (Kendall et al. 1995, Kendall et al. 1997, Kendall 1999, Potak-Zehfuss et al. 1999).

In this paper, we briefly review these 2 types of temporary emigration and describe a field study that used a “robust” capture-recapture design to estimate temporary emigration, conditional capture probability, recapture probability, effective capture probability, and surface population size for terrestrial salamander populations. Three years of capture-recapture data from plots in Great Smoky Mountain National Park (GSMNP) were fit to 12 competing models using program MARK (White and Burnham 1999) to test a series of *a priori* hypothesis about salamander population parameters. We predicted a high prevalence of temporary emigration at all sites and explored whether

temporary emigration was random or Markovian. Additionally, we explored whether conditional capture probabilities showed any time or behavioral effects ('trap-shy' or 'trap-happy' response). Finally, we tested whether surface population size estimates varied across primary sampling periods.

STUDY AREA

Great Smoky Mountains National Park (GSMNP) is at the forefront of efforts to develop long-term natural resource inventory and monitoring on National Park Service lands. Located along the Tennessee-North Carolina border, GSMNP is internationally recognized for its rich temperate forest biodiversity. Geography and geology, along with steep, complex topography, create temperature and moisture gradients across the Park's 205,665 ha of contiguous forest. These gradients produce high levels of temperate species diversity in many taxa, including salamanders. Approximately 10% of the world's salamander species are found in the southern Appalachian region (Petranka 1998). They are a high priority taxon for the Park's inventory and monitoring program due to their high diversity, large number of endemic species, and the limited amount of data on the distribution, abundance, and natural history of most species.

METHODS

Types of Temporary Emigration

Completely Random Emigration.--

Completely random temporary emigration implies that animals move into and out of the study area at random such that at any given time the number of animals in the

study area and available for capture is a random sample of a “superpopulation” of animals, N^o , associated with the sampled area:

$$E[N_i / N^o] = (1 - \gamma_i^*) N^o$$

In this salamander study, individuals can move in and out of the study area vertically, but their horizontal movement is restricted (see Field Methods). Temporary emigration refers to an individual’s movement down into the soil, thus temporary emigrants are unavailable to surface sampling techniques. The probability that a salamander is near the surface at time i does not depend on its location at time $i - 1$. If emigration is completely random then parameter estimates from either open (JS) or closed-population models (Otis et al. 1978) are unbiased, although the estimates apply to the superpopulation (N^o) not the surface population (N_i) (Kendall and Nichols 1995, Kendall et al. 1997, Kendall 1999). In this case temporary emigration lowers the effective capture probability and reduces precision on all other parameter estimates.

Markovian Emigration.--

Markovian emigration represents a situation where the probability that an animal is in the study area during primary period i depends on whether the animal was in (or out of) the study area at sampling occasion $i - 1$. The presence of Markovian emigration would imply that the probability that a salamander is available for capture at the surface at time i depends on its vertical location at time $i - 1$. In this case, there are two probabilities to consider:

γ_i' = probability that an animal stays away from the study area in i , given that it was a temporary emigrant in $i-1$

γ''_i = probability that an animal in the study area in period $i-1$ moves out of the study area for period i (Kendall et al. 1997)

It is difficult to form generalizations about the effect of Markovian temporary emigration on either open and closed-population estimates because the potential bias strongly depends on the relationship between γ'_i and γ''_i , the change in this relationship over time, and the available proportion of the superpopulation in the study area prior to the start of sampling (Kendall et al. 1997, Kendall 1999).

Pollock's Robust Design

Recent advances in capture-recapture theory have resulted in models that incorporate and estimate both types of temporary emigration (Kendall et al. 1997, Kendall 1999). Data collected using Pollock's (1982) robust design are most appropriate for these models. Under this design, primary sampling periods, i ($i = 1, 2, \dots, k$) contain l_i secondary sampling periods that are separated by a time interval that is short enough to assume the population is effectively closed (i.e. no births, deaths, immigration, or emigration) (Fig. 1). Primary periods are separated by longer time intervals during which population additions (immigration and births) and deletions (emigration and deaths) are likely to occur (Fig. 1).

Data from secondary samples within each primary period can be analyzed using closed-population models that allow for unequal capture probability (Otis et al. 1978, White et al. 1982). The closed-population models estimate conditional capture probabilities, recapture probabilities, and surface population size for each primary period. Data within each primary period are pooled to estimate survival rates and temporary

emigration rates between primary periods (Fig. 1) (Kendall and Nichols 1995, Kendall et al. 1997, Kendall 1999).

Field Methods

Our field methods were designed to validate several common salamander abundance indices and estimate different components of salamander detection probability. Individual capture histories for all salamanders at each site were used to estimate population parameters from capture-recapture models. We then compared population size estimates derived from these models and compare them to relative abundance indices from the same sites to determine if a constant, linear relationship existed for any of the indices. In this paper we present only the robust capture-recapture results. The validation of relative abundance indices and the comparison among different capture-recapture models is the subject of companion papers (Bailey this thesis, Chapter 2)

From 1999-2001 we sampled salamanders from 15 x 15 m plots within the Roaring Fork Watershed (Mt. LeConte USGS Quadrangle). We sampled 15 plots in 1999 and 20 plots in 2000 and 2001. Plots were located off-trail, but near permanent GPS-referenced census points where both large and fine-scale vegetation and soil information had been collected prior to our study. Plots were located in both disturbed (previously settled or logged prior to the establishment of the Park in 1934) or undisturbed areas between the elevations of 740 – 1070 m. All sites are now completely re-forested but there may be important differences in the plots due to disturbance history.

Each plot was enclosed with a silt fence to inhibit horizontal salamander movement to or from the plot. We raked the perimeter of each plot and buried the bottom edge of the silt fence 10-15 cm into the soil around the perimeter of each plot. The remainder of the fence was raised and stapled to 60 cm tall wooden stakes. We draped the top 15 cm of the fence toward the inside of the plot, creating a lip to make it difficult for salamanders to crawl over the fence and escape. We established 3 parallel transects, following the method of Hyde and Simons (2001), to estimate relative abundances. We established a natural cover transect (15 m long x 3 m wide), 5 coverboard arrays placed 3 m apart along a 15 m transect, and 5 leaf litter search locations (1 x 1 m) placed 3 m apart along a 15 m transect within each plot.

We collected capture-recapture data from each plot during 4 primary sampling periods between early April and mid-June. Each plot was sampled for 3-4 consecutive days (secondary periods) within each primary period (Fig.1). Primary periods were separated by 6-10 days. The sampling order of the plots was rotated so that plots were not searched at the same time each sampling day. Plots were not searched when it was raining.

During each sampling occasion we sampled the 3 transects first, then turned the remaining natural cover, and then searched the inside edge of the fence. This procedure ensured that every salamander on the surface had a probability of being captured. We marked the location of individual salamanders as they were caught and recorded the following information for each individual: species, presence of previous marks, snout-vent length (SVL), substrate under which it was caught, and its age and sex (if possible). All unmarked salamanders over 18 mm SVL were individually marked using fluorescent

elastomer (Northwestern Marine Technology Inc., Shaw Island, Washington, USA). Individuals were uniquely marked by injecting a small amount of elastomer at up to four body locations (base of each limb) using three elastomer colors (yellow, red and orange) (Jung et al. 1997, Hyde 2000). Permutations of colors and position allowed the salamanders to be uniquely identified on all future capture occasions. We sterilized injection syringes with alcohol between each marked salamander. After marking, the animals were released at the marked plot locations where they were caught.

Demographic Closure and Heterogeneity

A variety of models can be fit to data collected using Pollock's robust design. Those that include temporary emigration parameters are detailed in Kendall and Nichols (1995) and Kendall et al. (1997). Most temporary emigration models assume demographic closure over secondary samples, and no heterogeneity in capture probabilities. We used program CAPTURE (Otis et al. 1978, Rexstad and Burnham 1991) to fit our 1999 data to a series of closed-populations models to explore for the presence of heterogeneity and violations of the closure assumption over secondary sampling periods. Program CAPTURE selects a 'best' model from a set of 8 closed-population models where capture probability may vary due to time (t), heterogeneity (h), and trap response (b) in all possible combinations (M_o , M_b , M_h , M_t , M_{bh} , M_{tb} , M_{th} , M_{tbh}) (Otis et al. 1978). In addition, program CAPTURE performs a test for demographic closure using M_h as the null hypothesis (Otis et al. 1978). Other closure tests are available (Stanley and Burnham 1999), but they use model M_t as the null model in the

absence of behavioral effects. We found this model extremely unlikely given our data (see Results).

Kendall et al. (1997) derived an additional ad hoc estimator for random temporary emigration when capture probabilities are heterogeneous. This method requires large numbers of recaptured individuals, thus we used data from one of our best sites to calculate ad hoc temporary emigration estimates and compared them to estimates obtained from the method described below.

Model Description and Selection

We developed 12 models to test our *a priori* hypotheses about salamander population parameters. The models have variations of the following basic parameters:

N_i = available, surface population size during primary period i ($i = 1,2,3,4$)

γ_i = probability of temporary emigration (probability of being absent from the study area) for primary period i ($i = 1,2,3,4$)

p_{ij} = probability that a salamander is captured on secondary sampling occasion j of primary period i given that the salamander available for capture (conditional capture probability)

c_{ij} = probability that a salamander is recaptured during secondary sampling occasion j in primary period i given that the salamander was available for capture.

Conditional capture and recapture probabilities are assumed to be constant over secondary samples, but may vary among primary periods. All 12 models assumed a fixed apparent survival rate over primary periods, $\phi(.) = 1$. Annual survival rates are lacking for most terrestrial salamander species, but are usually estimated to be above 45% (Organ

1961, Tilley 1980, Hairston, 1983). Primary periods in our study were separated by only 6-10 days, suggesting that survival rates should be near 1. Even a conservative annual survival estimate of 30% would translate to $\varphi(.) \sim .965$ between primary periods, whereas a more realistic value of 50% would translate to $\varphi(.) \sim .98$. We substituted an ultra conservative survival rate of $\varphi(.) = .95$ into our ‘best’ model to verify that this level of survival rate reduction had negligible effects on detection probability estimates. We used program MARK (White and Burnham 1999) to fit the following 12 models to the capture histories for each site, each year. A quick reference for the 12 models is provided in Table 1.

Models 1 & 2. – Constant conditional capture probability, $p(.)$; constant recapture probability, $c(.)$; constant surface population, $N(.)$; and either constant random temporary emigration, $\gamma(.)$ (Model 1), or no temporary emigration, $\gamma(.) = 0$ (Model 2). This model is equivalent to the closed-population behavioral model M_b over secondary samples (Otis et al. 1978) and JS open-population Model D for primary periods (Pollock et al. 1990).

Models 3 & 4. – Conditional capture probabilities vary across primary periods, $p(i.)$; constant recapture probability, $c(.)$; constant random temporary emigration, $\gamma(.)$; and either constant surface population, $N(.)$ (Model 3), or time-specific surface population, $N(i)$ (Model 4). These models are equivalent to closed-population behavioral model M_b and JS Model B but with random temporary emigration included.

Models 5 & 6. – Same as Models 3 & 4 but ignoring temporary emigration, $\gamma(.) = 0$.

Models 7 & 8. – Conditional capture and recapture probabilities equal and time specific, $p(i.) = c(i.)$; constant random temporary emigration, $\gamma(.)$; and either constant surface population, $N(.)$ (Model 7), or time-specific surface population, $N(i)$ (Model 8). These

models are equivalent to closed-population null model M_o and JS Model B but with random temporary emigration included.

Models 9 & 10. – Same as Models 7 & 8 but ignoring temporary emigration, $\gamma(.) = 0$.

Models 11 & 12. – Constant and equal conditional capture and recapture probabilities, $p(.) = c(.)$; constant surface population, $N(.)$; and either constant random temporary emigration, $\gamma(.)$ (Model 11), or no temporary emigration, $\gamma(.) = 0$ (Model 12). These models are equivalent to closed-population null model M_o and JS Model D but with random temporary emigration included. Models 11 and 12 are the most restricted models possible and though they are biologically unrealistic, they may serve as suitable null models for comparisons with more general models.

We based model selection on Akaike's Information Criteria corrected for small sample size (AIC_c) (Akaike 1973, Burnham and Anderson 1998). The purpose of using AIC was to select 1 model from a range of alternatives that most adequately described the data with as few parameters as possible. We stress that the models chosen here are only an approximation of reality, chosen *a priori* to compare several hypotheses concerning salamander population parameters. The model selected as 'best' does not necessarily represent all of the biological processes that influenced our salamander populations.

We fit the 'best' model (Model 1) to all combinations of sites and years (site-years) to obtain estimates of random temporary emigration, $\gamma(.)$, conditional capture probability, $p(.)$, and average surface population size, $N(.)$. Because most capture-recapture studies of terrestrial salamanders report the effective capture probability, we used our estimates to derive effective capture probability, $p^o(.) = (1 - \gamma(.)) p(.)$ for each site-year. On rare occasions program MARK was unable to fit parameters reliably, so we

only included parameter estimates when the estimate was less than the standard error of the estimate. Finally, we modified the most commonly selected model to include first-order Markovian emigration. A salamander's presence on the surface is believed to be influenced by seasonal behavioral patterns and environmental factors such as surface moisture and temperature. These influences could result in either random or Markovian temporary emigration.

RESULTS

Demographic Closure and Heterogeneity

Using our 1999 data and program CAPTURE, we checked for demographic closure and the presence of heterogeneity over secondary samples. 41 of 56 possible closed populations (14 sites each with 4 primary periods) contained at least 1 recapture within secondary samples and could thus be used to test for closure and heterogeneity. The closure test was rejected on only 1 of 41 eligible populations. In addition, all but 2 of the 41 closed populations selected either the null model (M_0 , no effects on capture probability) or a model with time or behavioral effects. Model M_0 (no effects) was chosen 'best' 24/41 times and a behavioral effect was the most prominent effect in the remaining populations (included in 12 of the remaining 17 populations). Therefore, subsequent evaluations of robust design models assumed no heterogeneity and demographic closure over secondary samples. In addition, we suspected behavioral (trap-shy) effects to be present in our marked populations. We recognize that the tests to detect heterogeneity will have low power for our field sample sizes but homogeneous

models allow for maximum likelihood estimation and are available in program MARK, and thus contributed to our decision to use homogeneous capture probabilities.

Model Selection and Parameter Estimation

We analyzed 14 sites in 1999 (7 disturbed and 7 undisturbed) and 19 sites in 2000 and 2001 (9 disturbed and 10 undisturbed) for a total sample of 52 site-years. The number of salamanders captured varied widely among site-years ranging from 26 to 481 salamanders.

Overall, Model 1 was selected 'best' more often than any other competing model (Table 2). Models that included a random temporary emigration parameter were chosen more often (80.7%) than models with no emigration terms (19.3%). Both the top 2 models included random temporary emigration (Model 1 and Model 4). None of the remaining models were consistently selected (< 10% of site-years)

Models incorporating behavioral or trap-shy effects (Models 1-6) were selected more often (76.8%) than those without behavioral effects (Models 7-12 = 23.2%). Models with both behavioral and time effects (Models 3-6) were chosen more often (38.4%) than models with only time effects (Models 7 –10 = 13.4%). When behavioral effects were removed, estimates of conditional capture probabilities declined and surface population estimates increased dramatically (Table 3). The second most frequent model (Model 4), contained both time and behavioral effects, and indicated that conditional capture probabilities and surface population sizes may have varied among primary sampling periods. Thus, we found evidence of a strong trap-shy response in capture

probabilities and some evidence of temporal variations in both conditional capture probabilities and surface population sizes.

We explored the possibility of Markovian temporary emigration by modifying our ‘best’ model (Model 1) to include constant Markovian emigration. We found this model likely ($\Delta AIC_c < 2.0$) for 15 of the 52 possible site-years, and it was chosen ‘best’ for only 4 site-years (7.7%). We were unable to fit the model to data from 3 site-years, primarily because the $\gamma'(\cdot)$ parameter failed to converge. Parameter estimates of $\gamma'(\cdot)$ were usually greater than $\gamma''(\cdot)$ estimates (69.2% of site-years), indicating that emigrants at a given time period were more likely beneath the surface during the previous time period than on the surface. In other words, there was a higher probability for an individual to remain beneath the surface than for an individual on the surface to emigrate into the soil. However, estimates of $\gamma'(\cdot)$ and $\gamma''(\cdot)$ were usually similar and their confidence intervals always overlapped, thus the Markovian emigration model was rarely favored over random temporary emigration models.

Conditional capture probability estimates were severely reduced in models without temporary emigration (Table 4). Estimates of conditional capture probability and average surface population size showed no differences among models with random and Markovian emigration because these parameters are fit with the closed-population models across secondary samples (Table 4).

The average estimate of random temporary emigration (using Model 1) across site-years was high (0.87 ± 0.01 , $n = 50$ site-years). The average conditional capture probability estimate was 0.29 ± 0.01 ($n = 50$ site-years). Combining these 2 estimates, $p^o(\cdot) = (1 - \gamma(\cdot)) p(\cdot)$, yielded an effective capture probability of 0.03 ± 0.002 ($n = 50$ site-

years). Changing survival rate to $\varphi(.) = .95$ in Model 1 reduced estimates of random temporary emigration by ≤ 0.03 and other parameter estimates were unaffected.

Temporary random emigration estimates using Kendall's ad hoc estimator (Kendall et. al. 1997) were similar to estimates from Model 1 (Table 5), indicating that individual heterogeneity may not bias results on our sites.

DISCUSSION

We used Pollock's robust design (Pollock 1982) to estimate and test *a priori* hypotheses about temporary emigration, conditional capture probability, and surface population size for terrestrial salamanders. We found strong evidence for temporary emigration on all of our study sites. This phenomenon has been recognized previously (ex. Smith and Petranka 2000, Jung et al. 2000, Hyde and Simons 2001, Petranka and Murray 2001), but it has rarely been estimated. Taub (1961) conducted one of the few studies to address this issue directly. Through experimental field cages she found that between 2-32% of the total salamanders in a given sampling area were on the surface and available for capture during a single sampling occasion. Our results suggest that on average 13% of our salamanders were available for capture during a given sampling period. Furthermore, our study supports that temporary emigration is likely a random process, rather than Markovian. However, we acknowledge our ability to distinguish between the 2 types of temporary emigration is weak because of low numbers of recaptured animals and poor precision in both $\gamma'(.)$ and $\gamma''(.)$ estimates. However, the trend for $\gamma'(.) \geq \gamma''(.)$ is interesting and warrants further investigation of biological mechanisms that would produce such a trend.

Variations in conditional capture probability govern our ability to ‘detect’ salamanders at a given location. Our findings suggest that conditional capture probabilities vary due to a strong trap-shy response, and temporal factors that may reflect changing environmental conditions (e.g. temperature and soil moisture) or seasonal behavioral patterns. Conditional capture probabilities may also reflect temporal variation in the size of the surface populations. The frequent selection of Models 4 and 6, which contain time effects on conditional capture probabilities and surface population sizes, emphasizes the non-independent nature of these parameters.

Evidence of behavioral effects on capture probabilities suggests that estimation methods assuming equal capture probabilities (for example the Lincoln-Peterson, Schnabel, or Schumacher-Eschymeyer methods – see Pollock 1990 for details) may not be appropriate for terrestrial salamanders. These methods are highly sensitive to unequal catchability. Applying them to species exhibiting a trap-shy behavioral response often leads to an overestimate of population size (Pollock et al. 1990). As an example, our Model 8, assumed equal catchability ($p(i.) = c(i.)$) and produced substantially higher surface population estimates and standard errors than models incorporating behavioral effects (Table 3). Models incorporating unequal catchability were selected for most site-years (~77%). Estimator precision is lower due to the trap-shy behavioral response.

Conditional capture probability and temporary emigration are confounded in estimates of ‘effective capture probability’ reported in traditional closed-population capture-recapture models (Kendall et al. 1997, Kendall 1999). We used our temporary emigration and conditional capture probability estimates for each site-year to calculate effective capture probabilities that could be compared to other salamander studies. Our

overall estimate (0.03 ± 0.002 ; $n = 50$ site-years) is within the range of similar studies on terrestrial salamanders (Jung et al. 2000, Smith and Petranka 2000). Random temporary emigration will not bias estimates of effective capture probability, but it will reduce the precision of parameter estimates and it limits populations estimates to the ‘superpopulation’ only (Kendall 1999). This constraint is clearly illustrated by our results in Table 4 where the population estimate under Model 2 ($\gamma(.) = 0$) is 7 times the surface population estimate under identical models that contain temporary emigration terms. The precision of the Model 2 population estimate ($CV = 32.2$; $CV = 1 \text{ SE/estimate} \times 100$) is much less than the surface population estimates for the temporary emigration Model 1 ($CV = 3.7$). The benefits of incorporating temporary emigration into models include the ability to partition the different components of the effective capture probability, allowing more precise estimates of the surface population size.

Pollock’s robust design and temporary emigration models have their own set of limiting assumptions. The models assume demographic closure and no heterogeneity in capture probabilities over secondary samples. These assumptions need to be tested before using temporary emigration models in program MARK (White and Burnham 1999). We tested both assumptions using the closed-population program CAPTURE (Otis et al. 1978). The closure test included in program CAPTURE allows heterogeneity in capture probabilities but is sensitive to the presence of time or behavioral variation (Otis et al. 1978). Other closure tests are available but assume time-specific variation in capture probabilities (Stanley and Burnham 1999). We found time variation to be the least likely of the possible capture probability effects (null, time, heterogeneity, and behavior), and thus chose to use the closure test in program CAPTURE. However, both

types of closure tests are insensitive to temporary emigration when it occurs in the middle of the study and both perform poorly when the number of captured animals is low (Stanley and Burnham 1999).

Heterogeneity of capture probabilities is expected in many wildlife populations due to factors such as age, sex, size or social status (Pollock et al. 1990). Heterogeneity may be present in salamander capture probabilities due to variations among species (Petranka and Murray 2001, Bailey this thesis, Chapter 2) or age or size (Tilley 1980, Salvidio 2001). The model selection procedure in program CAPTURE yielded little evidence of heterogeneity over secondary sampling periods. The null model (M_0) was chosen most often, but this may reflect low recapture rates and a lack of power to reject the null model, M_0 . Thus assumptions of demographic closure and no heterogeneity over secondary samples are supported for our data, but low recapture rates, typical of salamander capture-recapture studies (Jung et al. 2000, Smith and Petranka 2000), make that support equivocal. We were able investigate the potential impact of heterogeneous capture probabilities on one site-year using Kendall et. al's (1997) ad hoc estimator. There was good consistency between the ad hoc and Model 1 temporary emigration estimates, indicating that individual heterogeneity may have a minor impact on temporary emigration estimates at our sites.

Our approach can be applied to a wide variety of organisms and environments. Kendall et al. (1997) explored situations where terrestrial mammals might move out of a study area by temporarily migrating out of the trapping grid or retreating into burrows during a torpor state. Marine mammals may only be visible in certain locations and only when they are near the surface of the water (see Fujiwara and Caswell, in press). Bell

and Plegder (in review) have used temporary emigration models on Pekeka frog (*Leiopelma pakeka*) populations in New Zealand. They found evidence of strong trap-shy behavioral effects and temporal variability in capture probabilities among secondary samples. They concluded that survival rates were constant over time and that temporary emigration varied temporally in some cases, but not in others.

Probably the most common use of temporary emigration models involves situations where only breeding individuals are observable. Temporary emigration models have been applied to snow geese (*Anser caerulescens*) (Kendall et al. 1995), Grey seals (*Halichoerus grypus*) (Schwarz and Stobo 1997), Hawksbill sea turtles (*Eretmochelys imbricata*) (Kendall and Bjorkland 2001), and Gulf sturgeon (*Acipenser oxyrinchus desotoi*) (Potak-Zehfuss et al. 1999) in situations where the available population is composed of breeding individuals. We feel these models have tremendous potential for pond breeding amphibians where breeding populations fluctuate widely with hydroperiod length (Pechmann et al. 1991, Semlitsch et al. 1996). In these situations, it is possible that temporary emigration and available sample populations vary over time, but that size of the superpopulation remains quite stable.

MANAGEMENT IMPLICATIONS

Long-term, large-scale amphibian monitoring studies are currently being planned by many organizations (e.g. Amphibian Research and Monitoring Initiative, North American Amphibian Monitoring Program, Partners in Amphibian and Reptile Conservation, Declining Amphibian Populations Task Force). These programs will likely use relative abundance indices (count data) or capture-recapture methods to

monitor population status. Detection probabilities are likely heterogeneous over time and space in these studies. Our results have 2 important management implications for programs whose objectives include monitoring salamander populations. First, our results indicate that large proportions of terrestrial salamander populations are subterranean and unavailable for capture during a given sampling occasion. Ignoring this temporary emigration will result in reduced estimates of effective capture probability and imprecise population estimates. The ability to estimate temporary emigration and surface populations allows us to examine how these parameters vary spatially and temporally (see Bailey this thesis, Chapter 2). We believe using unadjusted count indices to compare populations over time and space without estimating detection probability is not justified. A second management implication of our results stems from our finding that the capture probability of individual salamanders varies due to behavioral (trap-shy) and time effects. Therefore, we caution against using capture-recapture methods that assume equal capture probability without first testing the assumption.

Ours is the first study to apply temporary emigration models to salamander populations. Additional research is needed to determine if the results presented here are consistent in other taxa and across larger geographic areas.

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TABLE 1. Reference chart for parameter variations of 12 competing models.

Model	Parameters							
	Temporary Emigration		Capture Probabilities				Surface Population Size	
	None	Random	Constant Time	Time-specific	Constant Time	Time-specific	Constant Time	Time-specific
	$\gamma(.) = 0$	$\gamma(.)$	No Trap Response $p(.) = c(.)$	No Trap response $p(i.) = c(i.)$	Trap Response $p(.), c(.)$	Trap Response $p(i.), c(i.)$	$N(.)$	$N(i)$
1		X			X		X	
2	X				X		X	
3		X				X	X	
4		X				X		X
5	X					X	X	
6	X					X		X
7		X		X			X	
8		X		X				X
9	X			X			X	
10	X			X				X
11		X	X				X	
12	X		X				X	

TABLE 2. Percentage of the site-year data sets for which the 12 different models were selected based on AIC_c criteria (n = number of site-years in each disturbance class). All models assume apparent survival rate is fixed at $\varphi(.) = 1$. Model 1 was by far the most frequently selected model.

Disturbance History	Models												n
	$p(\cdot), c(\cdot)$		$p(i), c(\cdot)$				$p(i) = c(i)$				$p(\cdot) = c(\cdot)$		
	1	2	3	4	5	6	7	8	9	10	11	12	
Undisturbed	33.3	0.0	7.4	18.5	7.4	11.1	7.4	3.7	3.7	0.0	3.7	3.7	27
Disturbed	40.0	4.0	0.0	28.0	0.0	4.0	8.0	4.0	0.0	0.0	8.0	4.0	25
Total	36.5	1.9	3.8	23.1	3.8	7.7	7.7	3.8	1.9	0.0	5.8	3.8	52

Notes: **Model 1:** $\gamma(\cdot), p(\cdot), c(\cdot), N(\cdot)$; Model 2: $\gamma(\cdot) = 0, p(\cdot), c(\cdot), N(\cdot)$;
 Model 3: $\gamma(\cdot), p(i), c(\cdot), N(\cdot)$; Model 4: $\gamma(\cdot), p(i), c(\cdot), N(i)$;
 Model 5: $\gamma(\cdot) = 0, p(i), c(\cdot), N(\cdot)$; Model 6: $\gamma(\cdot) = 0, p(i), c(\cdot), N(i)$;
 Model 7: $\gamma(\cdot), p(i) = c(i), N(\cdot)$; Model 8: $\gamma(\cdot), p(i) = c(i), N(i)$;
 Model 9: $\gamma(\cdot) = 0, p(i) = c(i), N(\cdot)$; Model 10: $\gamma = 0, p(i) = c(i), N(i)$;
 Model 11: $\gamma(\cdot), p(\cdot) = c(\cdot), N(\cdot)$; Model 12: $\gamma(\cdot) = 0, p(\cdot) = c(\cdot), N(\cdot)$;

TABLE 3. Time-specific estimated rates of conditional capture probability, $p(i)$, recapture probability, $c(i)$, and surface population size, $N(i)$, for salamanders on an disturbed site (CG009, 2001). Model 4 contains time variation and behavioral (trap-shy) effects. Model 8 contains time variation but no behavioral effects. Estimates for period 2 were imprecise with high standard errors.

Sampling Period	Model 4: $\gamma(\cdot), p(i), c(\cdot), N(i)^a$						Model 8: $\gamma(\cdot), p(i) = c(i), N(i)^b$			
	$\hat{p}(i)$	$\hat{SE} p(i)$	$\hat{c}(\cdot)$	$\hat{SE} c(i)$	$\hat{N}(i)$	$\hat{SE} N(i)$	$\hat{p}(i) = \hat{c}(i)$	$\hat{SE} (p(i) = c(i))$	$\hat{N}(i)$	$\hat{SE} N(i)$
1	0.59	0.08	0.07	0.02	38.54	1.40	0.14	0.04	81.40	20.95
2	0.08	0.10			-	-	0.02	0.02	-	-
3	0.20	0.08			33.59	10.61	0.09	0.04	65.50	29.56
4	0.66	0.10			16.00	0.00	0.09	0.05	51.84	27.47

^a $\Delta AIC_c = 0.0$

^b $\Delta AIC_c = 27.06$

TABLE 4. Estimated rates of temporary emigration parameters, $\gamma''(\cdot)$ and $\gamma'(\cdot)$, and recapture probability, $c(\cdot)$, for salamanders on one disturbed site (CG016, 2001). $p(\cdot)$ is considered conditional capture probability for Model 1 and Markovian and resembles an effective capture probability for Model 2. $N(\cdot)$ is interpreted as surface population for Model 1 and Markovian. Apparent survival rate is fixed at $\varphi(\cdot) = 1$, and all parameters are constant across primary sampling periods.

Parameter	Model 2 : $\gamma(\cdot) = 0$ ^a		Model 1: $\gamma(\cdot)$ ^b		Markovian, $\gamma'(\cdot)$ ^c	
	Estimate	SE	Estimate	SE	Estimate	SE
$\gamma(\cdot)$			0.92	0.03	0.91	0.04
$\gamma'(\cdot)$					0.94	0.03
$p(\cdot)$	0.02	0.01	0.43	0.06	0.43	0.06
$c(\cdot)$	0.05	0.02	0.05	0.02	0.05	0.02
$N(\cdot)$	217.88	70.14	31.27	1.17	31.27	1.17

^a $\Delta AIC_c = 29.37$

^b $\Delta AIC_c = 0.0$

^c $\Delta AIC_c = 1.98$

TABLE 5. Estimated rates of random temporary emigration, $\gamma(i)$, for salamanders on one undisturbed site (RG016, 2001). Model 1 has been modified to include time-specific random temporary emigration and contains behavioral (trap-shy) effects in conditional capture probability. Ad hoc estimates were calculated using equations 11 and 12 in Kendall et al (1997). Ad hoc estimates allow for either heterogeneous variation, or both heterogeneous and behavioral variation in conditional capture probabilities. Apparent survival rate for all estimators is assumed to be 1, $\phi(.) = 1$.

Parameter	Model 1 : $\gamma(i)$		Ad hoc: M_h		Ad hoc: M_{bh}	
	Estimate	SE	Estimate	SE	Estimate	SE
$\gamma(2)$	0.63	0.10	0.58	0.09	0.71	0.10
$\gamma(3)$	0.86	0.05	0.82	0.05	0.87	0.01

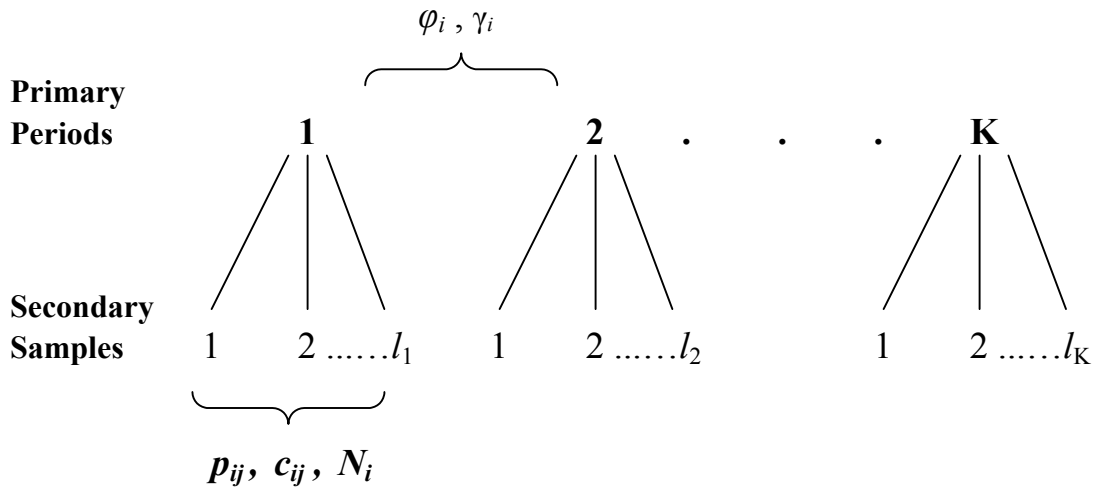


Figure 1. Pollock's robust design for a k -period study, each primary period i contains l_i closely-spaced secondary samples. Conditional capture probability, p_{ij} , recapture probability, c_{ij} , and surface population size, N_i , are estimated over secondary samples using closed-population models. Survival, φ_i , and temporary emigration rates, γ_i , are estimated between primary periods using open-population models (e.g. Jolly-Seber). Our salamander study contained capture-recapture data from 14 sites in 1999 and 19 sites in 2000 and 2001 (52 site-years). All site-years contained 4 primary periods each with 3-4 secondary samples (consecutive sampling days).

Chapter 2. Spatial and temporal variation in detection probability of *Plethodon* salamanders using the “robust” capture-recapture design

Larissa L. Bailey¹, Theodore R. Simons¹, and Kenneth H. Pollock²

¹ Cooperative Fish and Wildlife Research Unit, Department of Zoology, North Carolina State University, Campus Box 7617, Raleigh, NC 27695-7617, USA

² Department of Statistics, Biomathematics, and Zoology, North Carolina State University, Campus Box 8203, Raleigh, NC 27695-8203, USA

ABSTRACT

Recent worldwide amphibian declines have highlighted a need for long-term, large-scale monitoring programs. Scientific or management objectives, appropriate spatial sampling, and detectability all need to be considered when designing monitoring programs (Yoccoz et al. 2001). The ability to establish meaningful monitoring programs is currently compromised by a lack of information about amphibian detection probabilities. We used Pollock’s robust design and capture-recapture models that included temporary emigration to test *a priori* hypothesis about spatial and temporal variation in salamander detection probability parameters for populations found in Great Smoky Mountains National Park. We explored the effects of the 3 large-scale habitat characteristics (disturbance history, elevation, vegetation type) and found vegetation type and elevation were correlated with detection probabilities. Vegetation type was a significant covariant in estimates of temporary emigration, conditional capture

probability, and surface population size. Contrasts that isolated elevation effects were significant for all detection probability parameters, except recapture probability, despite our small elevational range (only 330m). When detection probability parameters vary over time and space, investigators should develop monitoring designs that permit the estimation of detection probabilities.

Key words: capture-recapture, detection probability, Great Smoky Mountains National Park, MARK, plethodontid salamanders, Pollock's robust design, spatial variation, temporary emigration.

INTRODUCTION

Recent worldwide amphibian declines have highlighted a need for long-term, large-scale monitoring studies to establish quantitative baseline data and document species range and status. Ideally, monitoring programs should have clear objectives (e.g. periodic assessment of population status) and their design should incorporate two important sources of variation: spatial variation and detectability (Yoccoz et al. 2001, Pollock et al. 2002). While some recent amphibian studies have attempted to estimate spatial variability (e.g. Hyde and Simons 2001), most lack necessary geographic and temporal scale to reliably detect spatial and temporal patterns in abundance estimates. Although amphibian monitoring initiatives are widespread (e.g. Amphibian Research and Monitoring Initiative, North American Amphibian Monitoring Program, Partners in Amphibian and Reptile Conservation, Declining Amphibian Populations Task Force, and US State and Federal agencies) most current monitoring programs are compromised because detection probabilities are not estimated.

Amphibian population studies often use one or more sampling methods that produce relative abundance indices (usually counts) to compare population trends over time or space. For these comparisons to be valid there must be: (1) a direct linear relationship between the count and the population size, and (2) the probability of ‘detection’ must be constant over time and space (Lancia et al. 1994). It is difficult to meet these assumptions for terrestrial salamander populations because only a small proportion of the population may be on the surface and available for capture during any given sampling interval. Surface counts are believed to comprise a small and variable proportion of the total populations, and the extent to which counts correlate to the total population may be minimal (Smith and Petranka 2000). To our knowledge no previous study has rigorously explored variations in salamander detection probabilities over time or space.

Previous studies have highlighted several ways in which detection probability might vary. The size of the surface population may be influenced by large-scale habitat characteristics such as vegetation type, elevation or previous disturbance history (Pough et al. 1987, Petranka et al. 1993, Dupuis et al. 1995, DeMaynadier and Hunter 1998, Harpole and Haas 1999, Hyde and Simons 2001) or small-scale habitat characteristics such as the type and number of cover objects (Petranka et al. 1994, Grover 1998). Surface population size at a given site is expected to change temporally due to environmental conditions (Hairston 1987, Grover 1998, Petranka and Murray 2001) or seasonal behavioral patterns (DeMaynadier and Hunter 1998, Petranka 1998). Additionally, the capture probability for salamanders near the surface (conditional capture probability) may vary spatially with habitat characteristics such as the amount of

natural cover (Grover 1998) or temporally as a result of changing moisture conditions (Heatwole 1962, Jaeger 1980). Furthermore, salamander capture probabilities likely vary among species, possibly producing heterogeneity among individual capture probabilities (Grover 2000, Petranka and Murray 2001). Therefore, the salamander community composition may influence the overall salamander detection probability at a given location.

In a companion paper, we demonstrate the usefulness of Pollock's robust design to separate and estimate population parameters important for salamander detection (Bailey et al, Chapter 1). Here we use estimates from capture-recapture models to test hypotheses about spatial and temporal differences in temporary emigration (the probability of being temporarily unavailable for capture, i.e. below the surface), conditional capture probabilities (the probability an animal is captured given it is available), and available (or surface) population sizes. We examine: (1) spatial and temporal variations in temporary emigration; and (2) variations in temporary emigration rates among species groups. Additionally, we explore whether conditional capture probabilities for salamanders near the surface differ: (1) spatially with large-scale habitat characteristics (disturbance history, vegetation type, elevation) or site-specific characteristics (soil moisture, natural cover); (2) temporally among years; and (3) among species groups. Finally we test whether estimates of average salamander surface population sizes vary with large-scale habitat characteristics or among years.

METHODS

Study area and field methods

Our study was conducted in Great Smoky Mountains National Park (GSMNP), located along the Tennessee-North Carolina border. GSMNP is the largest contiguous forest (205,665 ha) in the eastern US and is recognized for its rich temperate ecosystem and high salamander diversity (Petranka 1998).

We restricted our sites to the Roaring Fork Watershed (Mt. LeConte USGS Quadrangle) in GSMNP. Sites were stratified according to previous land use history. Land use history was determined from maps (Pyle 1985) that described 5 disturbance history classes: undisturbed, settlement areas and three types of logging: selective, light commercial, and industrial cut. All sites are now completely forested following the establishment of the Park in 1934. We combined disturbance history into 2 classes: undisturbed and disturbed (settlement and all logged classes). Forest community classifications of 90m Landsat imagery (MacKenzie 1993) were combined into 2 vegetation types: mixed deciduous (cove hardwood, mixed mesic and tulip poplar) and mixed pine (pine-oak and pine). Sites were assigned to 1 of 3 120-m elevation classes beginning at 740 m.

We captured and marked salamanders from 15 plots (15 x 15 m) in 1999 and 20 plots in 2000 and 2001. For a detailed description of plot layout and sampling methods, see Bailey (this thesis, Chapter 1). Plots were sampled according to Pollock's robust design (Pollock 1982, Bailey this thesis, Chapter 1). From 1 April to mid-June, each plot was searched during 4 primary periods each consisting of 3-4 consecutive sample days (secondary samples). Primary periods were separated by 6-10 days. Captured animals

were inspected for marks, and unmarked animals were marked with colored elastomer (Northwestern Marine Technology Inc., Shaw Island, Washington, USA) at four body locations for individual identification (Bailey this thesis, Chapter 1).

During the first day of each primary period, we collected leaf litter and soil samples at 3 locations within each plot to determine soil moisture. Samples were placed in cloth bags and sealed in plastic to prevent drying in the field. Cloth bags were weighed at the end of each day, dried at a low temperature, and re-weighed. Percent moisture was calculated as $1 - (\text{dry weight}/\text{wet weight})$.

We visually estimated the percent natural cover (logs, sticks, rocks) at 5 randomly selected 3 x 3m quadrants within each plot each year. Natural cover was categorized into 1 of 4 cover classes (<5%, 5-15%, 15-30%, >30%) for each quadrant.

Model Description and Selection

Numerous capture-recapture studies have demonstrated the advantages of Pollock's robust design over standard open-population sampling (Nichols et al. 1984, Kendall et al. 1995, Kendall et al. 1997, Schwarz and Stobo 1997, Nichols et al. 1998). A variety of models can be fit to data collected in this manner, including models that estimate temporary emigration (Kendall and Nichols 1995, Kendall et al. 1997). In previous work, we developed a series of models to test *a priori* hypotheses about the nature and importance of different salamander detection probability parameters (Bailey this thesis, Chapter 1). All competing models assumed no heterogeneity in capture probabilities and fixed apparent survival rates over primary periods, $\phi(.) = 1$. The model selected most often assumed seasonally invariant random temporary emigration, $\gamma(.)$, and

average surface population size, $N(\cdot)$, and included a trap-shy behavioral response with different conditional capture, $p(\cdot)$, and recapture, $c(\cdot)$, probabilities. We used this model to test our *a priori* hypothesis of the effects of large-scale habitat characteristics on salamander population parameters because: (1) the model was selected as the ‘best’ more often than any other competing model, (2) parameter estimation under this model was possible for nearly all sites in all years, and (3) the model allowed us to test our *a priori* hypotheses on sites with both high and low temporary emigration rates.

Summary of the Analysis

We used program MARK (White and Burnham 1999) to compute parameter estimates under our chosen model for each site, each year (site-year). We modeled parameter estimates as a function of large-scale habitat characteristics (previous disturbance history, vegetation type, and elevation class). These habitat characteristics are often confounded within GSMNP because disturbed sites are usually found at lower elevations. In addition, one of our disturbed, mixed pine sites had insufficient numbers of salamanders for parameter estimation. We eliminated it from the analysis, leaving few mixed pine sites for vegetative comparisons. Therefore we condensed the habitat characteristics into 5 different habitat ‘treatments’: disturbed/deciduous/low-elevation, disturbed/pine/low-elevation, disturbed/deciduous/mid-elevation, undisturbed/deciduous/mid-elevation, and undisturbed/deciduous/high-elevation. We modeled parameter estimates as a function of these 5 habitat ‘treatments’ using a split-plot ANOVA to handle the repeated measurement of sites over years (PROC GLM, SAS Institute 1999). Average surface population size was modeled as log (estimate). The models included

habitat treatments as the whole plot factor, and year and habitat x year as the repeated measurement factors. We tested for a habitat treatment main effect and habitat treatment x year interaction. We used contrast statements to test for: (1) vegetation effect between disturbed, low-elevation sites, (2) elevation effect between disturbed, deciduous sites, (3) elevation effect between undisturbed, deciduous sites, (4) disturbance effects between deciduous, mid-elevation sites, and (5) low-elevation, disturbed sites vs. high-elevation, undisturbed sites with deciduous vegetation type only. On rare occasions, program MARK yielded poor or nonsensical estimates for certain site-years, usually due to low numbers of recaptured animals. We eliminated parameter estimates where the standard error (estimate) > estimate. Thus we included only those site-year estimates that we felt were reliable.

Most capture-recapture salamander studies report an ‘effective capture probability’ (Kendall 1999, Bailey this thesis, Chapter 1). This probability is interpreted as the probability that an animal is captured given it is in the ‘superpopulation’, but not necessarily near the surface (Kendall 1999). ‘Superpopulation’ refers to the population of salamanders both on and beneath the surface within a sampled area. We used our estimates of temporary emigration, $\gamma(\cdot)$, and conditional capture probability, $p(\cdot)$, to calculate effective capture probability, $p^o(\cdot) = (1 - \gamma(\cdot)) p(\cdot)$ for each site-year. We ran the same split-plot, repeated measures ANOVA using this derived parameter to obtain estimates that could be compared to studies that do not estimate temporary emigration. In addition we tested for spatial and temporal differences in effective capture probability.

We averaged percent leaf litter and soil moisture, obtained at the beginning of each primary period, for each site-year. Natural cover classifications from 5 random

quadrats (3 x 3m) were used to calculate mean and standard deviations of natural cover for each site-year. We used simple linear regression to determine if either the mean or standard deviation of moisture or natural cover were associated with temporary emigration, conditional capture probability, or log (average surface population estimates).

We refer to 4 taxonomic and size groupings similar to those described by Smith and Petranka (2000). The large *Plethodon* group contains the *glutinosus* complex (including *Plethodon glutinosus* and *Plethodon oconluftee*), *Plethodon jordani* and hybrids. The small *Plethodon* group includes *Plethodon cinereus* and *Plethodon serratus*. The large *Desmognathus* group contains *Desmognathus imitator*, *Desmognathus ocoee*, and members of the *fuscus* complex including: *Desmognathus conanti*, *Desmognathus santeelah* and *Desmognathus fuscus fuscus*. The species *Desmognathus wrighti* was considered as its own group. Parameter estimation was not possible for each species group on all site-years. Each site-year had a unique composition of species, therefore we only used species-specific parameter estimates from site-years where the model yielded reliable values (i.e. standard error (estimate) < estimate). We used split-plot, repeated measures analysis of variance, with species as the whole plot factor and year and species × year as the repeated measurements factors, to test for species and year differences in temporary emigration, conditional capture probability, and recapture probability estimates.

RESULTS

The overall average estimate of temporary emigration (the probability of being absent from the study area) was high (0.87) and varied from 0.61 to 0.98 ($n = 50$ site-

years). Random temporary emigration rates varied across habitat treatments and among years and there was a habitat treatment \times year interaction effect (Table 1). The model explained a high proportion of variation in the random temporary emigration estimates ($r^2 = 0.81$, $P = 0.0044$). Salamanders on low-elevation, disturbed deciduous sites had higher temporary emigration rates (estimated probability = 0.94 ± 0.02 , $n = 10$ site-years) than those on high-elevation, undisturbed deciduous sites (estimated probability = 0.77 ± 0.02 , $n = 10$ site-years, $F_{1,14} = 10.07$, $P = 0.0068$) (Fig. 1A). Vegetation type showed a significant effect among low-elevation, disturbed sites with deciduous sites having higher temporary emigration rates than pine sites ($F_{1,14} = 9.55$, $P = 0.0080$) (Fig. 1A). There is no elevation effect between disturbed deciduous sites, but mid-elevation undisturbed deciduous sites had higher temporary emigration rates than high-elevation sites (Table 1). There was no disturbance history effect between deciduous sites within an elevation class (Table 1). Low-elevation, disturbed pine sites and high-elevation undisturbed sites had higher temporal variation in temporary emigration likely driving the significant habitat \times year interaction (Fig. 2A).

Average conditional capture probability was 0.29 ± 0.01 ($n = 50$ site-years). The model explained a high proportion of the variability in this parameter estimate ($r^2 = 0.72$, $P = 0.06$). Conditional capture probability varied both across habitat treatments and over years (Table 2, Fig. 1). There was also a habitat treatment \times year interaction effect, but no additional site effect (Table 2). Salamanders on low-elevation, disturbed deciduous sites had the highest conditional capture probability (estimated probability = 0.34 ± 0.03 , $n = 10$ site-years) while those on high-elevation, undisturbed deciduous sites had the lowest estimates (estimated probability = 0.20 ± 0.03 , $n = 10$ site-years). Conditional

capture probabilities rose steadily over the 3 years in our study (Fig. 1B). Both vegetation type and elevation appear to have an effect on conditional capture probability as contrasts involving these factors were significant (Table 2, Fig. 1A). Disturbance history did not have a strong effect among mid-elevation deciduous sites ($F_{1,14} = 0.65$, $P = 0.4352$). The pattern of temporal conditional capture probability was different for low-elevation, disturbed deciduous sites and high-elevation undisturbed sites, and was likely the basis for the habitat treatment \times year interaction effect (Fig 2B). Average recapture probability (estimated probability = 0.07 ± 0.003 , $n = 52$ site-years) showed little spatial variation, but did vary across years ($F_{2,23} = 4.74$, $P = 0.0189$, Fig 1B). The model explained approximately 75% of the variation in the recapture probability parameter ($P = 0.0146$). Elevation was the only factor that showed any influence on recapture probability, and only between mid and high-elevation undisturbed deciduous sites; high-elevation sites had higher recapture probabilities ($F_{1,14} = 4.64$, $P = 0.0492$, Fig. 1A).

Average surface population size varied among habitat treatments and sites, but not among years (Table 3, Fig. 3). The model explained a high proportion of the variation in estimated surface populations ($r^2 = 0.87$, $P = 0.0002$). Vegetation type and elevation both appeared to affect estimated surface populations and high-elevation, undisturbed sites supported higher surface populations than low-elevation disturbed sites (Table 3, Fig. 1A). Estimated surface populations did not differ among disturbed and undisturbed mid-elevation deciduous sites ($F_{1,14} = 0.34$, $P = 0.5693$). We calculated the average ‘superpopulation’ size, $N^o = N(.) / (1 - \gamma(.))$, for each site, each year. The log (superpopulation) estimates are shown in Figure 3 for comparison with log (surface population) estimates. Notice that the ratio of estimated log (surface population) to log

(superpopulation) increases over treatments and the largest ratio is among high-elevation undisturbed deciduous sites (~75%).

The average estimate of our derived effective capture probability, $p^o(.) = (1 - \gamma(.))p(.)$, was 0.03 ± 0.02 ($n = 50$ site-years). This parameter was invariant across habitat treatments and among years.

Mean soil and leaf litter moisture explained little variation in temporary emigration, conditional capture probability, or log (surface population size) (all correlation coefficients: $-0.20 < r < 0.20$). Recapture probability was negatively related to both average soil and leaf litter moisture ($r = -0.23$, $P = 0.10$; $r = -0.33$, $P = 0.02$, respectively). Additionally, standard deviation of soil moisture was negatively related with log (surface population estimates) ($r = -0.24$, $P = 0.08$). No other moisture measurements showed strong relationships to model parameters. The quantity of natural cover at a site had a negative relationship to recapture probability ($r = -0.35$, $P = 0.01$). Standard deviation of natural cover was positively related to conditional capture probability ($r = 0.36$, $P = 0.01$) and negatively associated with temporary emigration ($r = -0.33$, $P = 0.02$) and log (surface population size) ($r = -0.41$, $P = 0.003$). No other natural cover measurements showed strong relationships to model parameters.

We found some species-specific differences in salamander population parameter estimates. There was some evidence of species-specific differences among temporary emigration estimates ($F_{3,37} = 2.39$, $P = 0.0846$, Fig. 4A). Large salamanders had slightly lower temporary emigration rates than small salamanders ($F_{1,37} = 3.50$, $P = 0.0692$, Fig 4A). Conditional capture probabilities also showed some evidence of differences among species groups ($F_{3,37} = 2.46$, $P = 0.08$, Fig. 4A), with plethodontids having higher

estimates than *Desmognathus* species ($F_{1,37} = 5.93, P = 0.0199$). There was a strong species effect on estimates of recapture probabilities ($F_{3,37} = 10.28, P = <0.0001$). In general, recapture probability increased with increased species size (Fig. 4A). Temporal differences in estimates of species-specific parameters mimicked the trends detected in the species-combined analysis. Both conditional capture probability and recapture probability tended to increase over the 3-year study (Fig. 4B).

DISCUSSION

Parameters affecting estimates of salamander detection probability and abundance varied both spatially and temporally within one watershed in GSMNP. We found that temporary emigration varied spatially and was higher on low-elevation, disturbed deciduous sites than high-elevation, undisturbed deciduous sites. This result was expected as older, more mature forests often have less daily and seasonal microhabitat variability than younger forests (Harris 1984, Dupuis et al. 1995, Herbeck and Larsen 1999). However, only the standard deviation of natural cover levels was correlated with temporary emigration estimates. Neither the mean nor standard deviation of soil moisture levels explained the spatial variation in temporary emigration. There are numerous other factors (i.e. temperature, leaf litter depth, slope, aspect, soil type) that we did not explore that could explain the observed differences in temporary emigration among habitats. Temporary emigration estimates seemed to vary between species with larger salamanders having lower estimates than smaller salamanders. We might have lacked adequate sample sizes to provide statistical significance in this test, but biologically we expect smaller species to dehydrate more rapidly than larger salamanders

due to larger surface-to-volume ratios (Grover 2000). These smaller species may therefore retreat below the surface more often than larger species.

Conditional capture probabilities govern our ability to ‘detect’ salamanders at a given location. Conditional capture probabilities varied both spatially and temporally at our study sites. Salamanders on low-elevation, disturbed deciduous sites had higher conditional capture probabilities than those at high-elevation or pine dominated sites. Environmental factors and surface population sizes likely affect conditional capture probability. Conditional capture probability had a positive relationship with the variability in natural cover. It is possible that the natural cover on low-elevation, disturbed sites was more clustered which concentrated salamanders and made them easier to capture. Furthermore, high-elevation, undisturbed deciduous sites had the highest estimates of surface populations while low-elevation, disturbed deciduous sites had the lowest estimates. Surface population size was negatively related to the standard deviation of both soil moisture and natural cover. Surface population estimates were higher and conditional capture probabilities were lower on sites with relatively constant soil moisture conditions and uniformly distributed cover. Sites with variable soil moisture conditions or patchily distributed natural cover had smaller surface populations and higher conditional capture probabilities.

Temporary emigration and conditional capture and recapture probabilities increased during the three years of our study. This could indicate real differences or an increase in the efficiency of our catching and marking techniques. We had large yearly turnover in our field personnel and believe the estimated variation in capture probabilities was not the result of increased observer abilities over time. Low-elevation disturbed pine

and high-elevation undisturbed sites showed higher temporal variation in temporary emigration and conditional capture probabilities. These sites may be more sensitivity to environmental changes than sites found in other habitats.

Temporary emigration and conditional capture probability are confounded in most capture-recapture models, and the confounded variable is often referred to as an ‘effective capture probability’ (Kendall et al. 1997, Kendall 1999). We used our temporary emigration and conditional capture probability estimates to calculate an effective capture probability that could be compared to other salamander studies. Our overall estimate (0.03) is within the range of similar studies on terrestrial salamanders (Kramer et al. 1993, Jung et al. 2000, Smith and Petranka 2000). Interestingly, temporary emigration and conditional capture probabilities were often inversely related such that effective capture probabilities showed little spatial or temporal variation. This finding may encourage investigators to use counts as indices of population size, but we would discourage this practice because we have demonstrated the processes governing effective capture probabilities vary both spatially and temporally.

This is the first time temporary emigration models have been applied to salamander populations. Additional studies to determine how salamander detection probabilities vary across larger geographic areas are needed. Recent studies in the southern Appalachians support our findings, and indicate that effective capture probabilities vary spatially (Jung et al. 2000) and among species (Petranka and Murray 2001). Studies in Europe indicate that conditional capture probabilities vary spatially, temporarily, and but not among age classes (Salvidio 2001). These results suggest that

investigators should be cautious about drawing inferences based on count indices unless detection probabilities are estimated.

MANAGEMENT IMPLICATIONS

What are the implications of our findings for the development of monitoring strategies? Assume, for example, that our low-elevation, disturbed deciduous sites represent poor-quality or fringe habitat for salamanders in the southern Appalachians. Relatively high conditional capture probabilities might encourage monitoring these sites for population decline. However, the combination of high levels of temporary emigration, low recapture probabilities, and low proportions of surface/superpopulation size would make precise population estimates nearly impossible on these ‘poor-quality’ areas. Moreover, these factors would produce imprecise count indices that cannot accurately measure population change (Hyde and Simons 2001). Sampling a larger number of low-quality sites for presence-absence data and using the ‘proportion of area occupied’ as an index of population status may be more efficient (see MacKenzie et al. in press for details).

In contrast, assume that undisturbed, high-elevation deciduous sites represent high-quality habitat in our region. Relatively low levels of temporary emigration, high recapture probability, and high surface/superpopulation ratios might encourage the use of simple count indices to document population change. Variability of counts is likely lower on high-quality sites (Smith and Petranka 2000, Hyde and Simons 2001, and see Welsh and Droege 2001 for review) and high surface/superpopulation ratios on these sites suggest that changes in the superpopulation should be reflected by the count indices.

However, even on these high-quality sites, the surface population represents less than 20% of the superpopulation and conditional capture probabilities are also relatively low. Thus, superpopulations may decline considerably before declines are seen in count indices. The sudden disappearance of amphibians from pristine habitats may reflect this phenomenon (Carey 1993, Berger et al. 1998, Lips 1999). Admittedly, most of these declines have affected aquatic amphibians (Laurance et al. 1996, Lips 1998, Carey et al. 1999) and they have been attributed to synergistic effects between pathogens and environmental conditions (Carey et al. 1999, Kiesecker et al. 2001). Nevertheless, these cases represent situations in which count indices in high-quality habitats went from high to low very quickly. Capture-recapture methods are likely to yield reliable, less variable surface and superpopulation estimates, in habitats with low temporary emigration rates and high recapture probabilities. Therefore, we recommend a dual sampling design for large-scale studies (Pollock et al. 2002) when it is not feasible to estimate detection probability at every sampling location. This approach relies on a good sampling design to select a large number of sites in a variety of habitats and the use of count indices or 'proportion of area' occupied as state variables (MacKenzie et al. in press). The dual sampling design uses capture-recapture studies on a subset of reference sites to estimate detection probabilities and calibrate counts for a more extensive sampling effort. We believe this approach may provide better monitoring data than programs that are based solely on count indices.

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TABLE 1. Split-plot ANOVA results testing the effects of 5 habitat treatments (combinations of disturbance history, vegetation type, elevation, and site) on the probability of temporary emigration, $\gamma(\cdot)$ (probability of being temporary absent from the study area). Parameter estimates were obtained at each site-year using a model with seasonal invariant random temporary emigration, $\gamma(\cdot)$, conditional capture probability, $p(\cdot)$, recapture probability, $c(\cdot)$, and average surface population size, $N(\cdot)$. $n = 50$ site-years.

Source	df	Type III SS	<i>F</i>	<i>P</i>
Habitat Treatment	4	0.1327	6.18	0.0044
Site (Habitat treatment)	14	0.0752	1.79	0.1105
Year	2	0.0281	4.68	0.0209
Habitat Treatment \times Year	8	0.0834	3.48	0.0104
Error	21	0.0630		

Contrasts	df	Contrast SS	<i>F</i>	<i>P</i>
Vegetation type within Disturbed /Low-elevation	1	0.0513	9.55	0.0080
Elevation level within Disturbed/Deciduous	1	0.0041	0.77	0.3955
Elevation level within Undisturbed/Deciduous	1	0.0657	12.24	0.0035
Disturbance type within Deciduous/Mid-elevation	1	0.0004	0.08	0.7846
Low EL/Dist vs. High EL/Undist – Deciduous Only	1	0.0541	10.07	0.0068

Note: The tests of the effects of the pairwise interaction involving year is also reported

TABLE 2 Split-plot ANOVA results testing the effects of 5 habitat treatments (combinations of disturbance history, vegetation type, elevation, and site) on the conditional capture probability, $p(\cdot)$. Parameter estimates were obtained at each site-year using a model with seasonal invariant random temporary emigration, $\gamma(\cdot)$, conditional capture probability, $p(\cdot)$, recapture probability, $c(\cdot)$, and average surface population size, $N(\cdot)$. $n = 50$ site-years.

Source	df	Type III SS	<i>F</i>	<i>P</i>
Habitat Treatment	4	0.1035	4.42	0.0162
Site (Habitat treatment)	14	0.0820	0.81	0.6487
Year	2	0.0646	4.49	0.0239
Habitat Treatment \times Year	8	0.1593	2.76	0.0295
Error	21	0.1513		
Contrasts	df	Contrast SS	<i>F</i>	<i>P</i>
Vegetation type within Disturbed /Low-elevation	1	0.0358	6.12	0.0268
Elevation level within Disturbed/Deciduous	1	0.0154	2.63	0.1272
Elevation level within Undisturbed/Deciduous	1	0.0583	9.95	0.0070
Disturbance type within Deciduous/Mid-elevation	1	0.0038	0.65	0.4352
Low EL/Dist vs. High EL/Undist – Deciduous Only	1	0.0224	3.83	0.0707

Note: The tests of the effects of the pairwise interaction involving year is also reported

TABLE 3 Split-plot ANOVA results testing the effects of 5 habitat treatments (combinations of disturbance history, vegetation type, elevation, and site) log (estimated average surface population size, $N(\cdot)$). Parameter estimates were obtained at each site-year using a model with seasonal invariant random temporary emigration, $\gamma(\cdot)$, conditional capture probability, $p(\cdot)$, recapture probability, $c(\cdot)$, and average surface population size, $N(\cdot)$. $n = 50$ site-years.

Source	df	Type III SS	<i>F</i>	<i>P</i>
Habitat Treatment	4	14.6465	7.68	0.0017
Site (Habitat treatment)	14	6.6779	2.32	0.0393
Year	2	0.7772	1.89	0.1755
Habitat Treatment \times Year	8	2.0185	1.23	0.3307
Error	21	4.3116		
Contrasts	df	Contrast SS	<i>F</i>	<i>P</i>
Vegetation type within Disturbed /Low-elevation	1	4.1049	8.61	0.0109
Elevation level within Disturbed/Deciduous	1	0.5279	1.11	0.3106
Elevation level within Undisturbed/Deciduous	1	6.8883	14.44	0.0020
Disturbance type within Deciduous/Mid-elevation	1	0.1620	0.34	0.5693
Low EL/Dist vs. High EL/Undist – Deciduous Only	1	7.4321	15.58	0.0015

Note: The tests of the effects of the pairwise interaction involving year is also reported

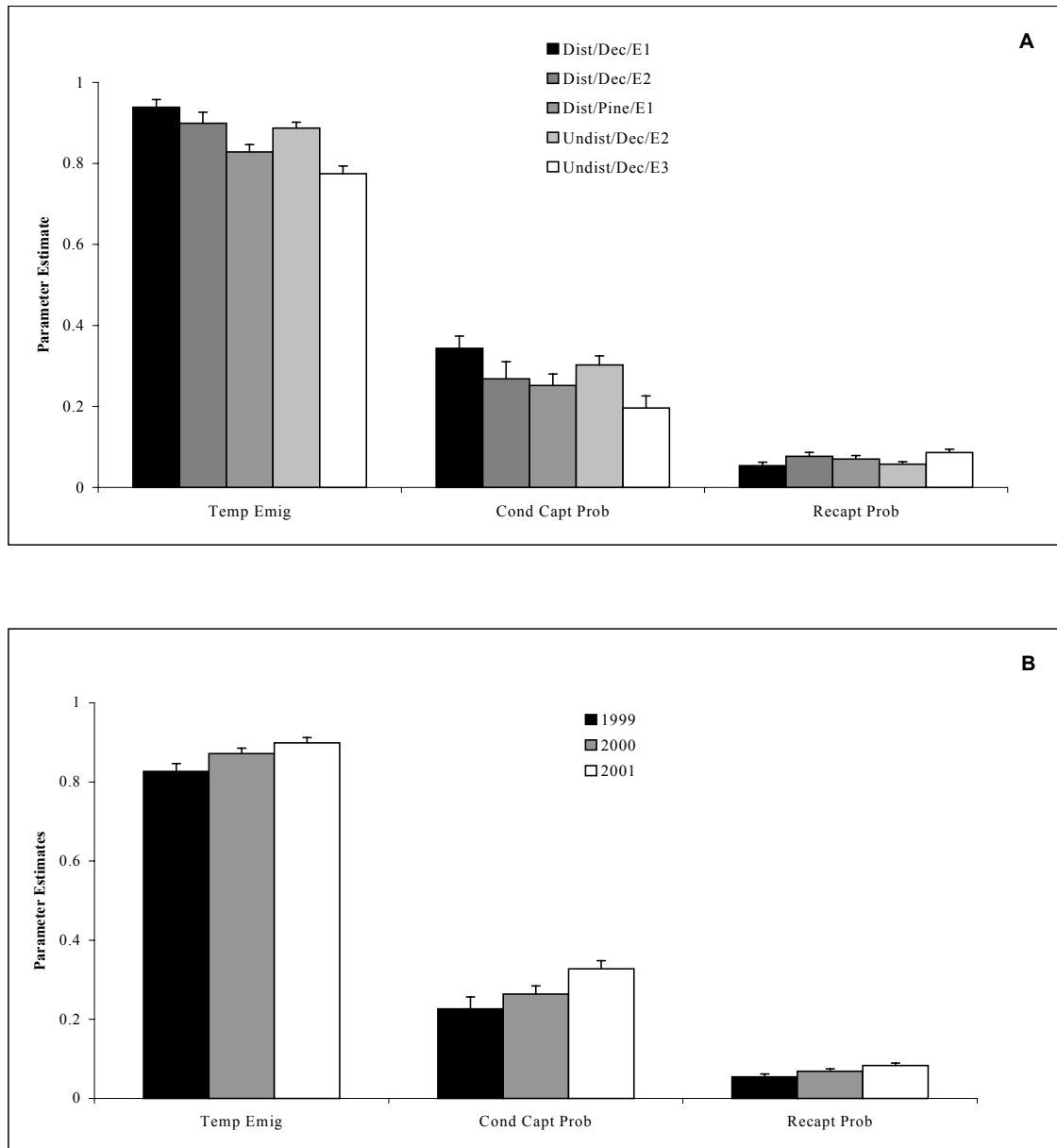


Figure 1. Parameter estimates (Mean \pm 1 SE) for 5 habitat treatments (A) and 3 years (B). See text for description of habitat treatments. Parameter estimates were obtained using a model with seasonal invariant random temporary emigration, $\gamma(\cdot)$, conditional capture probability, $p(\cdot)$, recapture probability, $c(\cdot)$, and average surface population size, $N(\cdot)$. Spatial and temporal differences in parameter estimates were tested using split-plot analysis of variance (PROC GLM; SAS Institute 1999).

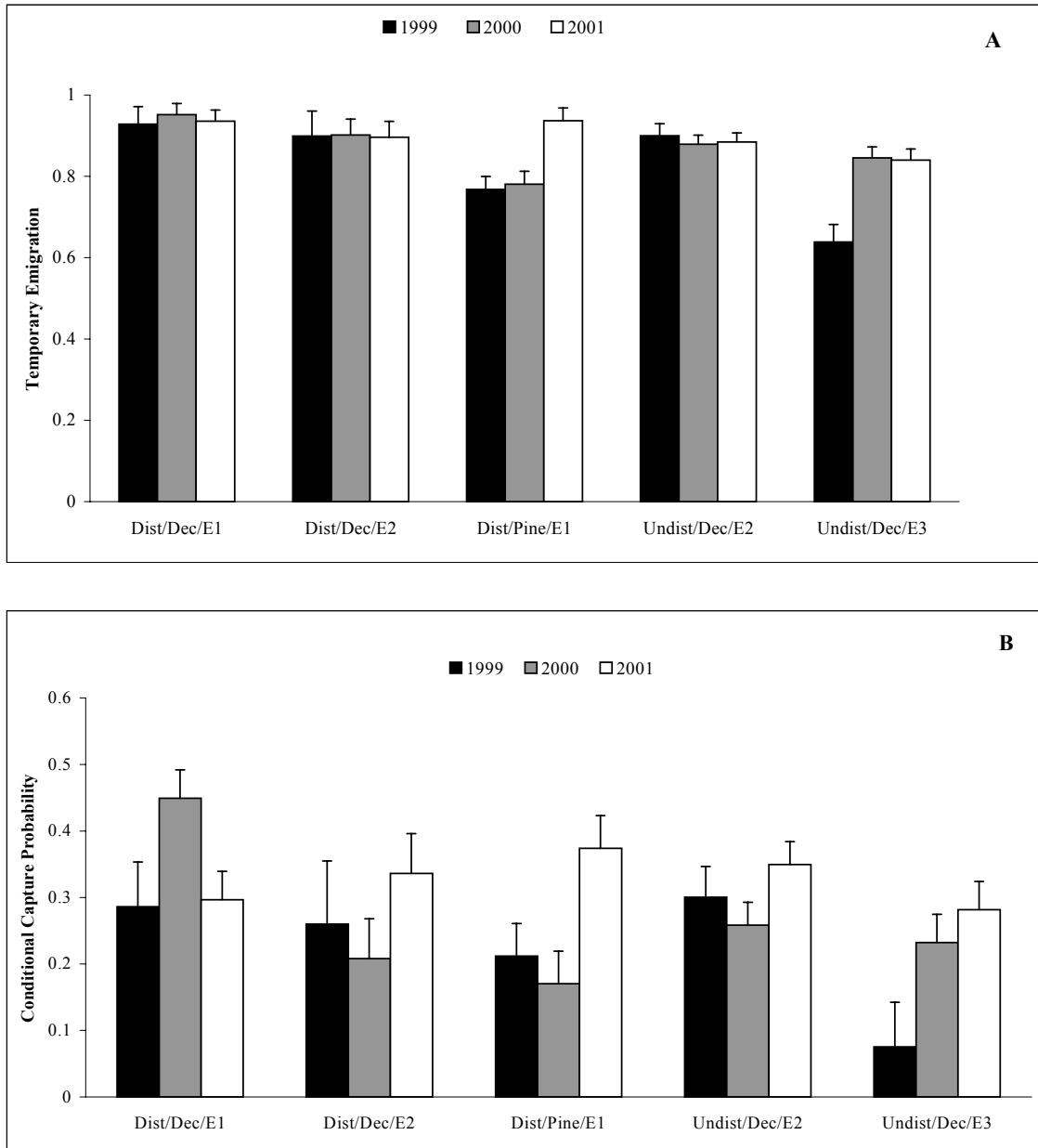


Figure 2. Habitat treatment x year interaction effects. Temporary emigration (A) and conditional capture probability estimates (B) for 5 habitat treatments and 3 years (Mean \pm 1 SE). See text for description of habitat treatments. Parameter estimates were obtained using a model with seasonal invariant random temporary emigration, $\gamma(\cdot)$, conditional capture probability, $p(\cdot)$, recapture probability, $c(\cdot)$, and average surface population size, $N(\cdot)$.

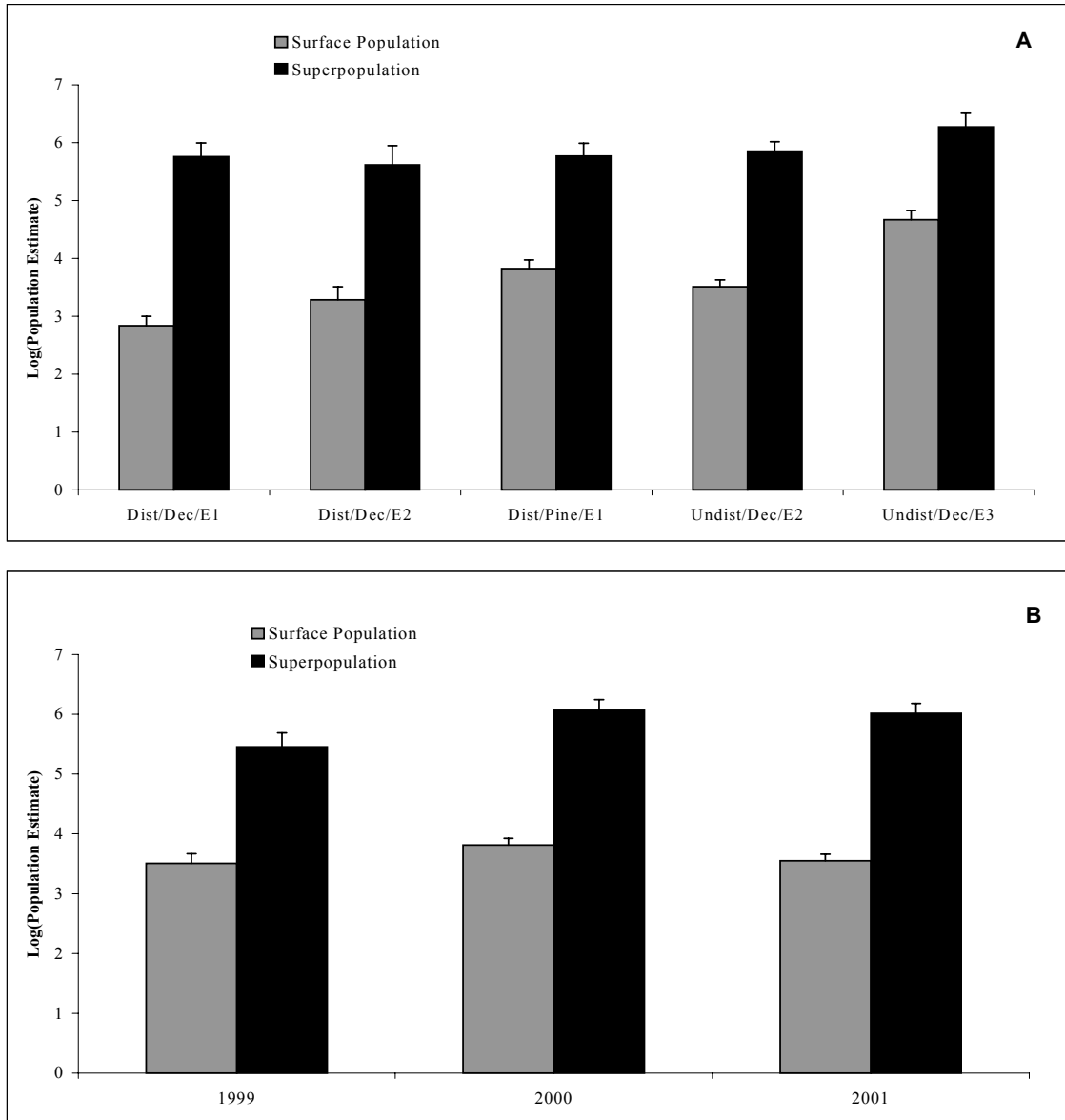


Figure 3. Log (average salamander population) estimates (± 1 SE) for 5 habitat treatments (A) and 3 years (B). Parameter estimates were obtained using a model with seasonal invariant random temporary emigration, $\gamma(\cdot)$, conditional capture probability, $p(\cdot)$, recapture probability, $c(\cdot)$, and average surface population size, $N(\cdot)$. Differences in population size were tested using split-plot analysis of variance (PROC GLM; SAS Institute 1999). Light bars represent log (average available surface populations). Dark bars represent log (average superpopulation) associated with the sample area.

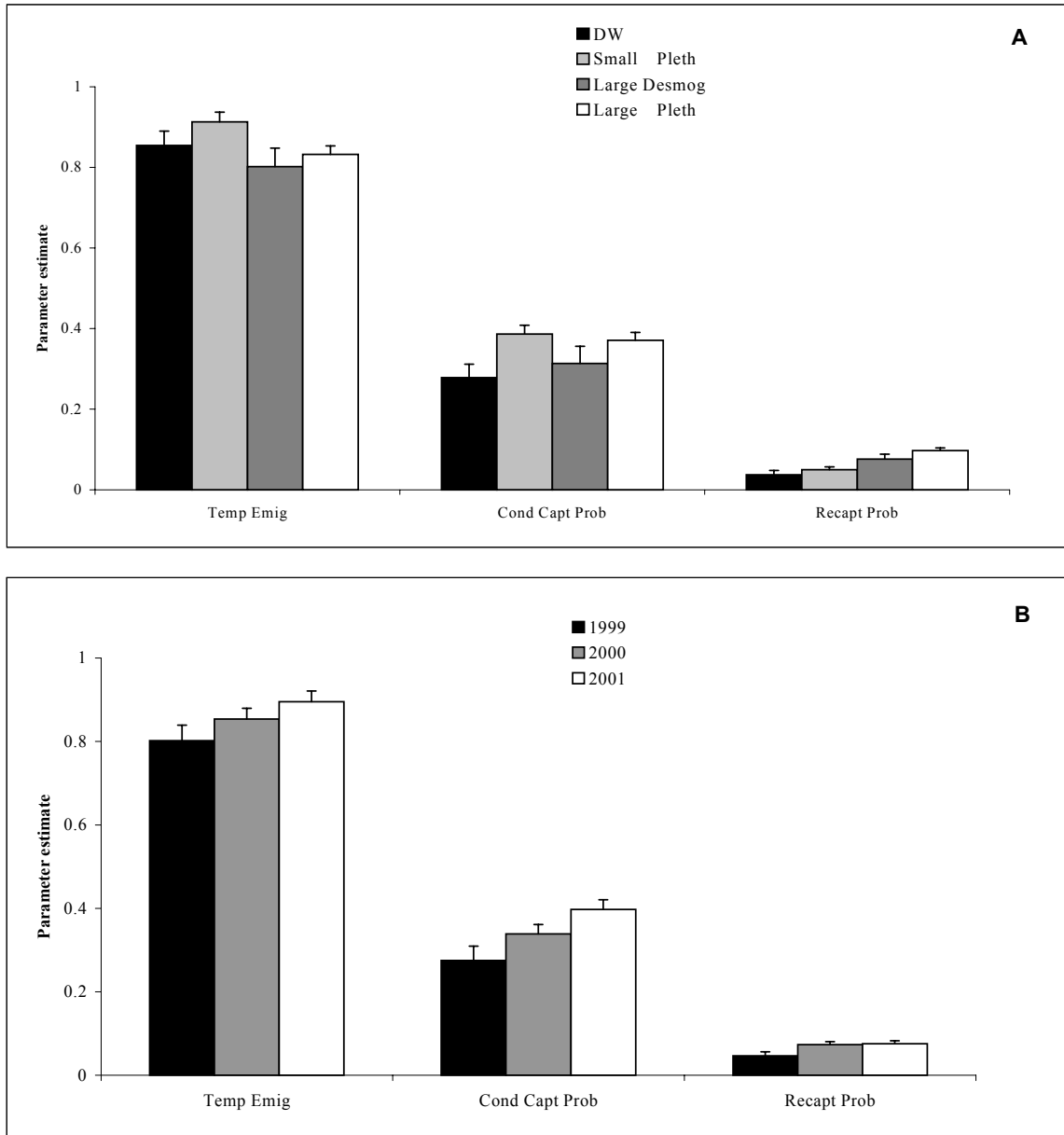


Figure 4. Parameter estimates (Mean \pm 1 SE) for 4 species groups (A) and 3 years (B).

See text for description of species groups. Parameter estimates were obtained using a model with seasonal invariant random temporary emigration, $\gamma(\cdot)$, conditional capture probability, $p(\cdot)$, recapture probability, $c(\cdot)$, and average surface population size, $N(\cdot)$.

Differences in population size were tested using analysis of variance (PROC GLM; SAS Institute 1999).

Chapter 3. Estimating site occupancy and species detection probability parameters for *Plethodon* salamanders

Larissa L. Bailey¹, Theodore R. Simons¹, and Kenneth H. Pollock²

¹ Cooperative Fish and Wildlife Research Unit, Department of Zoology, North Carolina State University, Campus Box 7617, Raleigh, NC 27695-7617, USA

² Department of Statistics, Biomathematics, and Zoology, North Carolina State University, Campus Box 8203, Raleigh, NC 27695-8203, USA

INTRODUCTION

Concern over amphibian declines has highlighted a need for more extensive and rigorous monitoring programs to document species occurrence and detect population changes. Numerous organizations are promoting long-term, large-scale studies to document, measure, and monitor amphibian populations (e.g. Amphibian Research and Monitoring Initiative, North American Amphibian Monitoring Program, Partners in Amphibian and Reptile Conservation, Declining Amphibian Populations Task Force, and US State and Federal agencies), especially species believed to be at risk. Among amphibians, plethodontid salamanders have been promoted as indicators of overall biodiversity and forest ecosystem integrity (Welsh and Droege 2001). Compared to most anurans, they are long-lived, slow to mature, and have relatively low fecundity (Petranka

1998). They also show susceptibility to a variety of natural and anthropogenic perturbations (see Welsh and Droege 2001 for a review).

General amphibian surveys have been initiated for 3 primary purposes: (1) to establish baseline data and techniques for long-term monitoring programs conducted to periodically assess community or ecosystem status (Gibbons et al. 1997; Corn 2000; Dodd et al. 2000; Hyde and Simons 2001), (2) to compare historical to current distributions (Fisher and Shaffer 1996; Shaffer et al. 1998; Corser 2001) and (3) to identify areas of high species richness and diversity for protection (Akani and Luiselli 2001). Two important sources of variation, spatial variation and detectability, constrain the inferences drawn from these types of surveys (Yoccoz et al. 2001; Pollock et al. 2002). Studies often make inferences about large areas by collecting information from sample units selected by some probability sampling technique (e.g., stratified random sample). Additionally, because not all species are detected in a sampled area, monitoring programs must incorporate methods for estimating or removing effects of variations in species detection probabilities (Pollock et al. 2002). Species detection probability is defined as the probability of detecting at least one individual of a given species during a particular sampling occasion, given that individuals of the species are present in the area (Boulinier et al. 1998; MacKenzie et al. in press). Although some salamander studies incorporate a spatial design (e.g. Hyde and Simons 2001), we know of no previous study that has estimated species detection probability. Most amphibian studies use a variety of sampling methods to document species occurrence and record the total number of individuals of each species seen at a given location. Species count (the total number of species seen) is used as an estimate of species richness (Petranka et al. 1993; Hecnar and

M'Closkey 1998; Aubry 2000). Likewise, the proportion of sites where a species was observed serves as an estimate of proportion of area occupied (Hecnar and M'Closkey 1997; Pilliod and Peterson 2001). These state variables (species richness and the proportion of area occupied) are usually compared over time or space to make inferences about temporal changes in the communities' status or the effects of environmental or anthropogenic factors (Petranka et al. 1993; Skelly et al. 1999; Pilliod and Peterson 2001).

Most studies to date assume that either (1) all species are detected or (2) the detection probability of different species is the same for the times, locations, or sampling methods being compared (Boulinier et al. 1998). The assumption of complete or equal species detection probability is unlikely to be met for terrestrial salamanders. Detection probability likely varies temporally due to seasonal behavioral patterns (Petranka 1998) and changing environmental conditions, or spatially due to site-specific habitat characteristics. Terrestrial salamander populations are believed to be largely subterranean, with only a small proportion of the population near the surface and available for capture on a given sampling occasion (Taub 1961; Heatwole 1962; Hairston 1987; Petranka and Murray 2001). Site-specific habitat characteristics influence the size of the available surface population (Bailey et al, Chapter 2). Some areas also support higher total populations of some species than others, creating clumped distributions of species throughout the landscape. Furthermore, different sampling techniques are likely to vary in their effectiveness at detecting species, making it difficult to compare results among studies with different sampling protocols (Barr and Babbitt 2001; Hyde and Simons 2001; Jenkins et al. 2002).

Two recent lines of theory accommodate situations where species detectability is <1 and varies among species or habitats. These techniques build on traditional close-population capture-recapture methods, but use either species richness or proportion of area occupied (PAO) to characterize ecosystem status. Estimation of species richness and its related community parameters are the focus of works by Burnham and Overton (1979), Boulinier et al. (1998), Nichols et al. (1998 a & b). Proportion of area (or sites) occupied is an important parameter in many herpetological studies documenting reductions in species' range over time (Shaffer et al. 1998; Tuberville et al. 2000). Estimation of this state variable is discussed in a recent publication by MacKenzie et al (in press).

In this paper, we use a variety of techniques to determine a reasonable state variable (species richness and/or PAO) for terrestrial salamander monitoring programs. Specific monitoring goals will influence the choice of appropriate state variables. However, the state variables we explore could be used for a variety of different objectives including periodic assessment of a system or to compare species distributions or richness over time or space. Estimation techniques for both species richness and PAO are relatively new; therefore, we briefly describe the assumptions and differences among methods. Specifically, we explored the effectiveness of estimating species richness using abundance information from one sample per location (limiting form of jackknife estimator, Burnham and Overton 1979) and compared it to estimates from repeated samples at the same location (Boulinier et al. 1998). We tested a series of *a priori* hypotheses about estimates of the proportion of area occupied and species detection probability for seven salamander species that occur on sites in a single watershed in Great

Smoky Mountains National Park (GSMNP). We expected both species richness and PAO estimation methods to indicate that species detection probabilities < 1 , and that detection probabilities varied among species. We tested whether the type of sampling method, the number of sites sampled, or the number of sampling occasions per site affected estimates of PAO or species detection probability. We expected detection probabilities to change with these factors but that PAO estimates would not. We explored the importance of time-specific covariates by estimating species detection probabilities among sampling occasions (within a season), and examined seasonal patterns between years. Finally, we investigated spatial variation in both PAO and species detection probabilities associated with four large-scale habitat characteristics: prior disturbance history, vegetation type, elevation, and proximity to streams.

STUDY AREA

Great Smoky Mountains National Park (GSMNP) is at the forefront of efforts to develop long-term natural resource inventory and monitoring on U.S. Department of Interior lands. Located along the Tennessee-North Carolina border, the Park's 205,665 ha of contiguous forest is internationally recognized for its rich temperate biodiversity. Geography and geology, along with steep, complex topography, create temperature and moisture gradients that produce high levels of temperate species diversity in many taxa, including salamanders. Approximately 10% of the world's salamander species are found in the southern Appalachian region (Petranka 1998). Salamanders are a high priority taxon for the Park's inventory and monitoring program due to the large number of endemic species and limited data on species distribution, abundance, and natural history.

METHODS

State Variable Estimation

All state variable estimations discussed in this paper are extensions of closed-population capture-recapture theory. Thus estimation models assume that: (1) the community of species is closed to additions (colonization), deletions (extinction), or changes in occupancy for the duration of the study period, (2) species are correctly identified, and (3) the probability of detecting a species at one site is independent of probability of detecting the species at all other sites. The fundamental differences in the methods are the state variable estimated (either species richness or PAO), and how the data are collected and compiled. Furthermore, species richness estimation allows heterogeneity, but PAO methods assume no spatial heterogeneity among sites in either probability of species detection or occupancy not attributed to specified covariates.

Species Richness Estimation.—

Several methods exist to estimate species richness from patterns of observed species occurrence (Burnham and Overton 1979; Palmer 1990; Boulinier et al. 1998). We focus on two methods that allow for variation in detection probabilities among species (heterogeneity). Investigators often record only the number of individuals seen of each species within a single sampling occasion due to logistical and financial constraints (Petranka et al. 1993; Dodd et al. 2001). This type of data can be used with a limiting form of a jackknife estimator developed by Burnham and Overton (1978, 1979) (program SPECRICH, Hines, J.E. available at: <http://www.mbr-pwrc.usgs.gov/software.html>). Boulinier et al. (1998) describe a method that utilizes detection/non-detection information

for each species rather than relative abundances, but requires multiple sampling occasions per location within a sampling season. Species detection probability may vary among sampling occasions (t), among species (h , heterogeneity) or by some ‘behavioral response’ (b), or in a combinations of factors ($M_o, M_b, M_h, M_t, M_{bh}, M_{tb}, M_{th}$) (Otis et al. 1978). Behavioral response in this case may arise when a species becomes more abundant at some point during the season (e.g. timed emergence of juvenile recruits) or if the capacity to detect a species is affected by the observer’s previous experience with a species (Boulinier et al. 1998). These differences among species detection probabilities can be treated as competing hypotheses and tested using model selection procedures in program CAPTURE (Otis et al. 1978; Rexstad and Burnham 1991). Currently, theory exists to estimate changes in species richness and community dynamics over time (Nichols et al. 1998a) or to assess community associations such as species co-occurrence or apparent species avoidance (Nichols et al. 1998b). However, an important limitation of these methods is the inability to estimate species-specific detection probabilities.

Proportion of Area Occupied.--

Recent work by MacKenzie et al. (in press) describes methods for estimating proportion of area (or sites) occupied when species detection probabilities are < 1 . Detection-non-detection information is recorded for a species at each location and sampling occasion. Sites must be visited a minimum of two times per sampling season. Estimable parameters of interest are the probability that a species is present in area i , ψ_i (PAO), and the conditional probability a species is detected in area i at time j , given the species is present, p_{ij} . PAO, ψ_i , may be expressed as a logit function of only site-

specific covariates (e.g. habitat type, elevation), but p_{ij} may be a logit function of either site-specific or time covariates (e.g. time, temperature, weather conditions) (MacKenzie et al. in press). Because species are analyzed separately, species-specific detection probabilities are possible and a computer software package, ESOR, is available for parameter estimation (MacKenzie, D. available at: <http://www.mbr-pwrc.usgs.gov/software.html>). Some methods have also been incorporated into program MARK (White and Burnham 1999). Theory is currently being developed to estimate rate of change in ψ_i and species associations similar to those explored in species richness estimation (J.D. Nichols, personal communication).

Field Methods

We extended work initiated by Hyde and Simons (2001), who sampled salamanders at 104 sites during 1999 within the Roaring Fork Watershed, GSMNP (Mt. LeConte USGS Quadrangle). These sites were located adjacent to trails and spaced approximately 250 m apart, beginning at a random point at least 250 m from each trail head. We continued to monitor a subset of 39 sites in 2000 and 2001. Sites were located in either disturbed (previously settled or logged prior to the establishment of the Park in 1934) or undisturbed areas between the elevations of 670– 1060 m. Two forest community types were considered based on 90 m Landsat imagery (MacKenzie 1993): mixed deciduous (cove hardwood, northern hardwood, mixed mesic and tulip poplar) and mixed pine (pine-oak and pine). Sites were classified as being adjacent (<50 m) to a stream, or not adjacent (>50 m) to a stream.

Two parallel relative abundance indice transects were sampled at each site: a natural cover transect (50 m long x 3 m wide) and 5 coverboard stations placed 10 m apart along a 50 m transect (see Hyde and Simons (2001) for details). Natural cover transects were sampled by 2 observers walking side by side who turned (and replaced) all nature cover (logs, sticks, and rocks) within the 50-m X 3-m area. Each coverboard station contained 3 large (26cm X 26cm) and 3 small (13cm X 26cm) boards space 1 cm apart (Hyde and Simons 2001). Ninety-one of Hyde and Simon's (2001) sites contained both natural cover and coverboard transects. The majority of these sites were in mixed deciduous forests (87%) with an average elevation of 844 ± 159 m (mean \pm 1 SD). Sixty-six percent of the sites had been previously disturbed and 36% were located near a stream. The 39 sites we continued to monitor had a slightly different breakdown of habitat characteristics: 82% deciduous, 74% disturbed, 38.5% located near a stream, and the average elevation was 816 ± 102 m (mean \pm 1 SD).

We collected data from each site during 5 sampling periods each year (1999-2001). Sampling occasions were spaced approximately 2 weeks apart, between early April and late-June. Sites were searched during the day when it was not raining. All individuals observed on each transect were identified to species. Thus, both the presence and relative abundance of each species was available for each survey method, location, and sampling occasion.

State Variable Selection

We used data from the subset of 39 sites in 1999 to assess the most appropriate state variable for our salamander system. For this analysis, we combined captures from

both natural cover and coverboard surveys. The total number of individuals observed for each species was used to build species abundance distributions at each site and single sampling occasion. We chose to use data from sampling occasion 3 and repeated the procedure using sampling occasion 5. These occasions had the highest detectability rates for most species (see results). Abundance distributions were used to estimate species richness using the limiting form of the jackknife estimator (Burnham and Overton 1979) and program SPECRICH (Hines, J.E.). This method emulates sampling designs where sites are only visited once per season.

Next, we estimated species richness at each site using detection-non detection information for recorded species using all 5 sampling occasions within a year (Boulinier et al. 1998). Model selection procedures and estimates of species richness were performed using program CAPTURE (Rexstad and Burnham 1991). Using the same data, we estimated proportion of area occupied, ψ_i , and species-specific detection rates, p_{ij} , for the seven most common species found on our sites using program ESOR (Mackenzie et al).

PAO Estimation and Covariates

We explored the effects of sampling method and intensity on PAO parameter estimates calculated using information from 4 different sampling methods: (1) detections from natural cover and coverboard transects combined for 5 sampling occasions per season (All-data), (2) detections from natural cover transects only (NCT-only), (3) detections from coverboard transects only (CBT-only), (4) detections from natural cover and coverboard transects combined from only 2 sampling occasions per season (sampling

occasions 3 and 5) (2-occs). AVOVAs were used to test for potential main effects of species, year, and method on naïve proportion of area occupied (the proportion of sites where a species was seen), estimated proportion of area occupied, $\psi(\cdot)$, and species-specific detection probabilities, $p(\cdot)$, for each year (1999-2001). To investigate how increasing the number of sites would affect PAO parameter estimates, we analyzed data from our larger set of 91 sites in 1999 and compared PAO parameter estimates to our subset of 39 sites in that same year.

We explored the importance of covariates by modeling PAO parameter estimates as a logit function of time (sampling occasion) or four large-scale habitat characteristics (previous disturbance history, vegetation type, elevation, and stream presence). *A priori*, we believed these factors might influence parameter estimates based on our previous salamander studies (Hyde and Simons 2001, Bailey this thesis, Chapter 2). Disturbance history, vegetation type, and stream proximity were treated as categorical covariates and elevation was standardized and used as a continuous variable. We tested the importance of each covariate separately for seven salamander species using variations in the basic PAO parameters: ψ_i and p_{ij} . First, we constrained the probability an area is occupied to be constant, $\psi(\cdot)$, and allowed species detection to vary with time and each covariate separately, $p(t)$ and $p(\text{Cov})$ (5 models). Next, we constrained species detection probability to be constant, $p(\cdot)$, and varied the probability an area is occupied with each covariate separately, $\psi(\text{Cov})$ (4 additional models). We used our model with no covariates, $\psi(\cdot)p(\cdot)$, as a reference model. These 10 models were ranked according to AIC values (Akaike 1973; Burnham and Anderson 1998) calculated by program ESOR. The lowest ranked $\psi(\cdot)p(\text{Cov})$ model and $\psi(\text{Cov})p(\cdot)$ model for each species were combined

in a $\psi(\text{Cov})p(\text{Cov})$ model to explore if including covariates in both parameters improved model performance. We repeated this procedure for the four different sampling methods described above to investigate if the same covariate was chosen for all sampling methods. We stress that the models were chosen *a priori* to compare several factors we felt were likely to affect PAO model parameters. The model selected ‘best’ does not necessarily represent all of the environmental or biological processes that influenced the probability of occupancy or species detection probabilities.

RESULTS

During 1999-2001 we captured a total of 2292 salamanders of 10 different ‘species’ from 39 sites. Species included *Plethodon jordani*, *Plethodon serratus*, *Desmognathus wrighti*, *Eurycea widerae*, *Desmognathus monticola*, *Gyrinophilus porphyriticus* and 3 species complexes described by Petranka (1998). The *glutinosus* complex includes *Plethodon glutinosus* and *Plethodon oconluftee*. The *fuscus* complex includes *Desmognathus conanti*, *Desmognathus santeetlah*, and *Desmognathus fuscus*. The *imitator* complex is comprised of *Desmognathus imitator* and *Desmognathus ocoee*. *Desmognathus monticola* and *Gyrinophilus porphyriticus* were only encountered on rare occasions, thus they are included in species richness estimation, but not in PAO analyses.

Appropriate State Variable

We found species richness to be an ineffective state variable for monitoring the status of our terrestrial salamander community, primarily because there are not enough different species at each site to allow meaningful comparisons across time or space.

Species richness estimates using a single sampling occasion per site were usually larger than the actual naïve species count, but data were too sparse for the models to fit well, and precision for these estimates was poor (SE (estimate)/estimate often exceeded 50%). Species richness estimates are impossible at sites where no individuals are observed during a given single sample occasion. This was the case for 1/39 sites for sample occasion 3 and 5/39 sites for sample occasion 5.

Low numbers of species also plagued species richness estimation using multiple samples per site. Model M_o (null model) was selected ‘best’ on 26 of 37 sites where model selection was possible. Heterogeneity was included in all of the other models selected ‘best’ at the remaining 11 sites. The heterogeneity model, M_h , was selected as the second most probable model on 54% of the sites. Model M_o is often the ‘default’ model for selection procedures in program CAPTURE (Menkens and Anderson 1988) and there is good biological reasons to suspect heterogeneous detection probabilities, therefore we interpreted M_o estimates cautiously. Species richness estimates under model M_o did not differ from naïve species counts tallied over the 5 sampling occasions. The average species detection probability over all 39 sites using model M_o was $0.55 \pm .025$ (mean ± 1 SE). Species richness estimates using model M_h produced estimates higher than naïve species counts on $\sim 55\%$ of the sites, but estimates were usually only 1-2 species above naïve counts. The average species detection probability over all 39 sites using model M_h was $0.46 \pm .029$ (mean ± 1 SE). Precision on the species richness estimates was poor with SE (estimate)/estimate usually over 25%.

The proportion of area occupied had several advantages over species richness as a state variable. Parameter estimates had good precision for each of the seven species (SE

(estimate)/estimate never exceeded 30 % for any species in 1999) (Fig. 1a). In addition, PAO methods can estimate species-specific detection probabilities (Fig. 1b), while species richness methods can only estimate an average species detection probability. For these reasons we concluded that PAO was a better state variable than species richness for our system, and we focus on PAO parameter estimation for the remainder of this paper.

Parameter Estimation and Covariates

Naïve PAO values (the proportion of areas where the species was actually seen), estimated PAO, $\psi(\cdot)$, and species detection probability, $p(\cdot)$, varied among all main effects: species, year, and sampling method (Fig. 1, Tables 1-3). Focusing on the sampling method containing the most information (All-data method), different species exhibit different patterns of PAO and detection probability estimates (Fig. 1).

Desmognathus wrighti had the lowest estimate of PAO ($\psi(\cdot)$ mean \pm 1 SE = 0.26 ± 0.01 , $n = 3$ years), but relatively high probability of detection ($p(\cdot)$ mean \pm 1 SE = 0.57 ± 0.06 , $n = 3$ years) (Fig. 1). *Eurycea wilderae* had relatively low probability of detection ($p(\cdot)$ mean \pm 1 SE = 0.33 ± 0.04 , $n = 3$ years), but is a wide-spread species, with high PAO estimates ($\psi(\cdot)$ mean \pm 1 SE = 0.60 ± 0.05 , $n = 3$ years). *Plethodon serratus* had relatively high estimates of both detection probability ($p(\cdot)$ mean \pm 1 SE = 0.52 ± 0.06 , $n = 3$ years) and PAO ($\psi(\cdot)$ mean \pm 1 SE = 0.82 ± 0.01 , $n = 3$ years). Notice that detection probabilities decline over years for five of the seven species (DF, DW, EW, PJ, and PS), but this does not translate to PAO, $\psi(\cdot)$, declines for those same species (Fig 1). In fact the 2 species that do show declining estimates of PAO, $\psi(\cdot)$, are the species that did not exhibit declining detection probabilities (DI and PG) (Fig 1).

The All-data method produced PAO estimates, $\psi(\cdot)$, that were similar to naïve values (mean difference ± 1 SE = 0.03 ± 0.01 , $n = 21$ species-years). However this is not the case for the other sampling methods with reduced amounts of data. The NCT-only method had good agreement between naïve and PAO, $\psi(\cdot)$ estimates (mean difference ± 1 SE = 0.05 ± 0.01 , $n = 21$ species-years), but naïve values for both CBT-only and 2-occs methods were on average 12% lower than PAO, $\psi(\cdot)$ estimates (CBT mean difference ± 1 SE = 0.12 ± 0.03 , 2occs mean difference ± 1 SE = 0.12 ± 0.03 , $n = 21$ species-years). Furthermore, precision on both PAO, $\psi(\cdot)$, and detection probability estimates is better using NCT-only methods, but declines for the CBT-only method, and is poorer still for the 2-occs method. Using the naïve estimate from the All-data method as a minimum known PAO, we investigated if this minimum PAO value was within the interval: PAO, $\psi(\cdot) \pm 1$ SE for the 3 other methods (ex. Table 4). The NCT-only sampling method did not include the minimum known PAO for *Desmognathus fuscus* complex in 2 years (1999, 2001) and *Desmognathus imitator* complex in 1999 (Table 4). The 2-occs method produced PAO estimates with large standard errors, but still failed to include the minimum known PAO for the *Plethodon glutinosus* complex in 2 years (1999, 2000) and *Plethodon serratus* in 2000. The CBT-only method performed worst, failing to include minimum known PAO for *Plethodon glutinosus* complex (1999, 2000), *Plethodon jordani* (all years), and *Plethodon serratus* (1999, 2000) (Table 4).

There was a significant species x method interaction for all the response variables (Tables 1-3), indicating an inconsistent pattern across sampling methods for the different species. The *Plethodon* pattern is consistent with increasing naïve and PAO, $\psi(\cdot)$, estimates in the following order: CBT-only < 2-occs < NCT-only < All-data (Fig 2a &

2c). This pattern is different for *Desmognathus* species and *Eurycea wilderae*. Naïve and PAO, $\psi(\cdot)$, estimates for *Desmognathus* species are more consistent across sampling methods and only slightly below estimates from the All-data method (Fig. 2b & 2d). *Eurycea wilderae* had low naïve estimates using the CBT-only method, while the NCT-only method produced the lowest naïve estimates for *Desmognathus fuscus* complex (Figure 2b). In general, species detection probabilities increase across sampling methods in the following order: CBT-only < NCT-only < All-data < 2-occs (Fig. 2e & 2f). Two species deviate from this pattern: *Plethodon serratus* (CBT-only < NCT-only < 2-occs < All-data, Fig. 2e) and *Desmognathus fuscus* complex (NCT-only < CBT-only < 2-occs < All-data, Fig. 2f).

Including more sites (91 total) in the 1999 analysis improved parameter precision, but showed similar trends to those observed in our subset of 39 sites (Table 4). Using all 5 sampling occasions, PAO, $\psi(\cdot)$, estimates differed little from naïve PAO values. Species detection probabilities did not change from those obtained with only the 39 sites, but the estimates of PAO, $\psi(\cdot)$, were slightly lower for 3 species (DF, PG, PS) (Table 4). This likely reflects differences in the proportion of important habitat characteristics between the 39-site and 91-site data sets (see discussion).

Species-specific detection probabilities were consistent over our 3-year study (no species x year interaction, Table 3), but patterns of detection probability varied among species. By including a time-specific covariate (sampling occasion) in our estimates of detection probability, $p(t)$, we were able to examine the patterns of species-specific detection probability within the sampling season (Fig 3). *Desmognathus* species and *Plethodon jordani* had low detection probabilities for the first sampling occasion and

higher probabilities in the middle and late sampling rounds (Fig. 3). *Eurycea wilderae* and *Plethodon glutinosus* complex had relatively consistent detection probabilities throughout the sampling season except for an inexplicable low detection probability during the second sampling occasion for *Eurycea wilderae* (Fig 3). *Plethodon serratus* was the only species to show a consistent decline in detection probability over the sampling season.

We explored the importance of both time-specific and site-specific covariates in species-specific PAO parameter estimates by comparing AIC values for 10 competing models. Disturbance history was an important covariate for the *Desmognathus imitator* complex, *Plethodon glutinosus* complex, and *Plethodon serratus* (Fig 4). Occupancy probability, $\psi(\text{dist})$, and detection probability, $p(\text{dist})$, were higher on disturbed sites for *Plethodon glutinosus* complex, and *Plethodon serratus*, but lower for the *Desmognathus imitator* complex (Fig 4). Stream proximity increased both PAO, $\psi(\text{stream})$, and detection probability, $p(\text{stream})$, for *Desmognathus fuscus* complex and *Eurycea wilderae* (Fig 4). The probability of occurrence and detection probability for *Plethodon jordani* increased with increasing elevation.

While different covariates were important among species, all sampling methods were capable and consistent at identifying these covariates. For example, the PAO model for *Plethodon jordani* that included elevation, $\psi(\text{elevation})p(\cdot)$, had the lowest AIC values among $\psi(\text{Cov})p(\cdot)$ models for all 4 sampling methods (Table 5). Two notable exceptions were: (1) the analysis involving CBT-only data favored models that included stream proximity more often than analyses using data from other sampling methods, and (2) disturbance history and elevation covariates were occasionally interchanged, because

these habitat characteristics are often correlated on our study sites with disturbed areas usually found at lower elevations. The species-specific covariate included in the ‘best’ $\psi(\text{Cov})p(\cdot)$ model usually yielded the lowest AIC value among $\psi(\cdot)p(\text{Cov})$ models (Fig 4, Table 5). *Plethodon serratus* was the only exception, as disturbance history was the most important covariate among $\psi(\text{Cov})p(\cdot)$ models, but a time covariant, $\psi(\cdot)p(t)$, was most important among $\psi(\cdot)p(\text{Cov})$ models (Fig 4). This is not surprising given the within season detection probability pattern for this species (Fig 3). In fact modeling detection probability as a linear function of time (sampling occasion) would likely fit the data even better. Among competing $\psi(\text{Cov})p(\cdot)$ and $\psi(\cdot)p(\text{Cov})$ models, usually one covariate fit the data much better than any other covariate; rarely were there multiple competing models with Δ AIC values < 2.0 (ex. Table 5). The only exceptions again involved situations where models that included disturbance history or elevational covariates fit the data equally well. On occasion, *Plethodon glutinosus* complex data was fit well by all competing models, thus favoring no distinct covariate. The inclusion of more sites in 1999 (91 sites) showed similar covariate results to those found using only 39 sites. Combining the best $\psi(\text{Cov})p(\cdot)$ and $\psi(\cdot)p(\text{Cov})$ models, in a $\psi(\text{Cov})p(\text{Cov})$ model, often improved model fit (i.e., resulted in lower AIC values) emphasizing the importance of including covariates in both PAO parameter estimates (ex Table 5). However, fitting the more complex model, $\psi(\text{Cov})p(\text{Cov})$, was difficult for the CBT-only and 2-occs methods which had reduced amounts of data.

DISCUSSION

The choice of an appropriate state variable for a monitoring program depends on the program's objectives, scale, and resources. The objectives of many large-scale monitoring programs often lack the clarity required to determine appropriate state variables (Olsen et al. 1999; Yoccoz et al. 2001). Large-scale amphibian monitoring programs generally include objectives such as: “to determine the status and trends of amphibian populations on Department of Interior lands” (ARMI, Dodd 2000); “to determine the geography and extent of amphibian declines; to determine the causes of those amphibian declines” (DAFTP). These objectives may have multiple interpretations that lead to different state variable choices (i.e. population size, species richness, proportion of area occupied). Monitoring population abundance usually involves capture-recapture methods that are costly in terms of time and effort. In this paper, we explored two alternative state variables that may be useful in large-scale monitoring programs whose management objectives are to determine the status of a system and periodically assess changes in the system over time. Assuming these general objectives, we found that proportion of area occupied was a better state variable than species richness for terrestrial salamanders in GSMNP. Species richness estimates were imprecise regardless of the estimation method and often did not differ from naïve species counts. However, lack of fit in species richness estimation is not an invitation to use only species count data, because model-based estimates will still exhibit less bias than simple count methods (Nichols et al. 1998a). PAO has good potential as a state variable for large-scale amphibian monitoring programs, but our results emphasize the importance of estimating PAO parameters rather than use naïve site occupancy information.

Naïve proportion area occupied based on only 1-2 sampling occasions is likely to grossly underestimate true PAO values because species detection probabilities are not 1, nor are they constant across species, time, space, or sampling method. There are multiple factors that could affect detection probability for a given species including: local density, seasonal or behavioral patterns, size of the species, weather and environmental variations, or sampling effort in terms of number of sampling occasions or amount of area surveyed. Our findings suggest that several of these factors are important in the detection of different terrestrial salamander species. Body size was the only factor that did not appear to affect our ability to detect different species. Our smallest species, *Desmognathus wrighti*, had detection probabilities comparable to our largest species, *Plethodon glutinosus* complex.

Local density is likely to affect the detection probability of most species because as the density of a given species increases, so does the probability of detecting a single individual of that species. For example, *Desmognathus wrighti* is locally abundant when it occurs in our study area, often exceeding over 100 individuals in a 15 X 15 m plot (Bailey personal observation). In contrast, *Eurycea wilderae* is a widespread species, but is never found in high abundances (>10 individuals) on the same terrestrial plots (Bailey personal observation). We cannot directly explore the relationship between the probability of detection and population sizes because we did not estimate abundances on our sites. However, recent simulation studies show that detection of (patch) occupancy is a good index of abundance in metapopulation studies (Lopez and Pfister 2001).

Estimated salamander detection probabilities exhibited seasonal behavioral patterns that are consistent with current natural history information. Many salamander

species including *Desmognathus wrighti*, members of the *Desmognathus fuscus* complex, and *Plethodon jordani* are known to disperse from winter retreats in early spring (Organ 1961; Petranka 1998) and then remain active on the surface during the warmer months of the year (Petranka 1998). Seasonal behavior was most prominent in *Plethodon serratus* whose detection probabilities consistently declined throughout the sampling season for all years and all sampling methods. *Plethodon serratus* adult surface activity is known to decline throughout April and juveniles often disappear beneath the ground by mid-May (Petranka 1998). This pattern explains why PAO estimates using 2-occs data for this species had higher standard errors than other *Plethodon* species, whose detection probabilities for sampling occasions 3 and 5 are relatively high. Seasonal behavioral patterns vary throughout a species range and with elevation (Tilley 1977; Petranka 1998), emphasizing the need to estimate species detection probabilities for studies at different geographic locations rather than simply standardize protocols. It is important to note that detection probability is conditional, thus higher species detection probabilities do not always translate to increased precision or accuracy in PAO estimates. For example, species detection probabilities are often highest for the 2-occs sampling method, but both detection probabilities and PAO estimates via this method lack precision and in some cases PAO estimates are inaccurate (not near minimum known PAO values).

Time-specific weather conditions may account for some of the variability that we see in detection probabilities, but these factors are likely less influential when monitoring PAO as a state variable compared to population size or relative abundance. Even during unfavorable environmental conditions a few individuals of a species are likely detectable at occupied sites. However, persistent drought may result in lower species detection rates

and this may be why we see yearly declines in detection probabilities for 5 of our 7 species. Total precipitation in April-June declined over the 3 years of our study and in 2001 was approximately 20 cm below rainfall levels achieved in the four preceding years (Mt. LeConte National Weather Station). Another possibility for detection probability declines is a disturbance effect incurred by sampling transects 5 times per year. There are few studies that rigorously explore cumulative sampling effects, but investigators have cautioned against using natural cover searches because of perceived disturbance effects (Heyer et al. 1994). If disturbance were an issue, we would expect detection probabilities to decline throughout the sampling season, but only *Plethodon serratus* showed such a decline and it is likely the result of seasonal activity patterns. Still investigators should strive to minimize disturbance effects and may choose to randomly vary the exact location of natural cover searches within an area to avoid repeatedly sampling the same cover objects.

Both the size of the area sampled and sampling intensity affected species detection probability. The amount of area surveyed was greater for NCT as compared to CBT sampling methods and thus had higher detection probabilities for most species. The only exception, *Desmognathus fuscus* complex, likely resulted from a flaw in our sampling design rather than a true affinity of *Desmognathus fuscus* complex to CBT sampling. On sites near streams, coverboard stations were always placed stream-side of NCT. This non-random placement of transects explains the higher CBT detection probability for *Desmognathus fuscus* complex, as members of the complex are generally considered a stream-side species and are unlikely to be found on the forest floor (Petranka 1998). This is confirmed by low *Desmognathus fuscus* complex PAO

parameter estimates calculated from NCT-only data. Additionally, transect placement also explains the tendency for stream proximity to be an important covariate for analysis using CBT-only data.

Species-specific detectability alone does not explain differences in PAO, $\psi(\cdot)$, estimates among methods. If variations in species detection probability were the only factor responsible for differences in naïve values among methods, then PAO, $\psi(\cdot)$, methods should incorporate the differences in detection probabilities and produce consistent species-specific PAO, $\psi(\cdot)$, estimates across methods; there should be no significant interaction terms in our ANOVA for PAO, $\psi(\cdot)$, estimates. Incorporating species-specific detection probabilities in PAO, $\psi(\cdot)$, estimates does remove the species x year and method x year interactions, but not the species x method interaction (Table 2). PAO, $\psi(\cdot)$, estimates are more consistent across sampling methods than naïve estimates for all 7 species (Fig 2), but there are still unaccountable differences among methods. NCT and CBT are located several meters apart and may be sampling slightly different areas which could account for the unexplainable differences among PAO, $\psi(\cdot)$, estimates among methods.

PAO estimates were capable of revealing differences in species' distribution types (clumped or widespread) as well as potentially important species-specific habitat characteristics. Our findings suggest that *Desmognathus wrighti* has the most restricted distribution of salamanders species in our watershed while *Plethodon serratus*, *Plethodon glutinosus* complex, and *Eurycea wilderae* are the most wide-spread. Analysis of data from all sampling methods consistently identified important covariates. If determining habitat associations were the main objectives of a monitoring study, our results suggest

NCT and CBT represent overlapping effort and only one of these sampling methods would be necessary. Investigators should consider stratifying sample units according to species-specific habitat characteristics important for either PAO or detection probability estimation. Including more sites in our 1999 analysis decreased PAO estimates for 3 species: *Desmognathus fuscus* complex, *Plethodon glutinosus* complex, and *Plethodon serratus*. All three species, especially *Plethodon glutinosus* complex and *Plethodon serratus*, have higher occupancy and detection probabilities on previously disturbed sites. Seventy-four percent of our 39-site subset was previously disturbed compared to only 66% of the 91-sites. This finding emphasizes the importance of having a good spatial sampling design to insure that the chosen sample sites are representative of the entire area from which inferences are to be drawn.

Although PAO may be a useful state variable for a variety of monitoring studies, there are three caveats we would like to mention. First, PAO methods only allow spatial heterogeneity attributed to specified covariates; current theory does not allow unexplained heterogeneity among sites, but more general models could be developed (K. Pollock, personal communication). Second, models that include covariates only in the detection probability parameter, $\psi(\cdot) p(\text{Cov})$ models, often yield unreasonable PAO estimates when detection probabilities were <0.15 . MacKenzie et al (in press) also encountered this problem in their simulation study, concluding that the model has difficulty distinguishing between sites with low detection probability and sites where the species is truly absent (MacKenzie et al. in press). Furthermore, PAO estimation may not be useful for some types of at-risk species, depending on their detection probabilities. If a species has low detection probability and low occurrence then PAO methods do not

perform well, for of the reason discussed above. However, if the species has adequate detection probability ($p_{ij} > 0.15$), but low occurrence (ex. *Desmognathus wrighti* species) then PAO methods should perform well. PAO methods may be extremely important in estimating the distribution for species like *Eurycea wilderae* that have relatively low detection probabilities (provided $p_{ij} > 0.15$) but are widely distributed. Naïve PAO estimates for this type of species are inclined to have strong negative bias. *Eurycea wilderae* is a Management Indicator Species for the southern region of the National Forest Service and as such, populations of *Eurycea wilderae* will be monitored to assess the effects of forest management actions. PAO may be one way to effectively monitor *Eurycea wilderae* populations in this region.

There are obvious trade-offs between sampling a large number of sites 1-2 times per season vs. multiple visits to fewer sites. Remote sites may be difficult and costly to sample multiple times within a season. We would encourage further work using PAO methods directed at optimizing allocation of sampling effort (see Pollock et al. 2002). It may be beneficial to implement a double sampling design (Pollock et al. 2002) where occupancy data are collected a large number of sites, but multiple visits are made to a subset of these sites within a single sampling season.

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TABLE 1. ANOVA results testing the effects of species, year, and sampling method on naïve estimates using model, $\psi(\cdot) p(\cdot)$.

Source	df	Type III SS	<i>F</i>	<i>P</i>
Species	6	1.7983	249.09	<0.001
Year	2	0.1621	67.36	<0.001
Method	3	0.3606	99.91	<0.001
Species × Year	12	0.0713	4.93	<0.001
Year × Method	6	0.0191	2.65	0.0314
Species × Method	18	0.2783	12.85	<0.001
Error	36	0.0433		

Note: The tests of the effects of pairwise interactions are also reported

TABLE 2. ANOVA results testing the effects of species, year, and sampling method on proportion of area occupied estimates using model, $\psi(\cdot) p(\cdot)$.

Source	df	Type III SS	<i>F</i>	<i>P</i>
Species	6	2.5518	75.80	<0.001
Year	2	0.0650	2.80	0.0066
Method	3	0.0795	4.72	0.0070
Species × Year	12	0.0958	1.42	0.2008
Year × Method	6	0.06937	2.06	0.0826
Species × Method	18	0.2214	2.19	0.0222
Error	36	0.2020		

Note: The tests of the effects of pairwise interactions are also reported

TABLE 3. ANOVA results testing the effects of species, year, and sampling method on species detectability estimates using model, $\psi(\cdot) p(\cdot)$.

Source	df	Type III SS	<i>F</i>	<i>P</i>
Species	6	0.6949	16.99	<0.001
Year	2	0.3159	23.17	<0.001
Method	3	0.8547	41.81	<0.001
Species × Year	12	0.0979	1.20	0.3225
Year × Method	6	0.1204	2.94	0.0192
Species × Method	18	0.3401	2.77	0.0045
Error	36	0.2453		

Note: The tests of the effects of pairwise interactions are also reported

TABLE 4. 1999 example of PAO parameter estimates using model, $\psi(\cdot) p(\cdot)$, from 3 different sampling methods on 39 sites: natural cover transect data from 5 sampling occasions (NCT), coverboard transect data from 5 sampling occasions (CBT), and combined natural cover and coverboard transect data with 2 sampling occasions (2-occs). Results are given for seven salamander species: *Desmognathus fuscus* complex (DF), *Desmognathus imitator* complex (DI), *Desmognathus wrighti* (DW), *Eurycea wilderae* (EW), *Plethodon glutinosus* complex (PG), *Plethodon jordani* (PJ), and *Plethodon serratus* (PS). Min is the naïve proportion of area occupied using combined data from natural cover and coverboard transects over 5 sampling occasions from 39 sites. It represents the minimum known proportion of area occupied for the NCT, CBT, and 2-occs methods. Bold entries indicate instances where the Min value was not within the interval: PAO, $\psi(\cdot) \pm 1$ SE (i.e. poor estimates). Results from an expanded data set of natural cover and coverboard transects on 91 sites (5 sampling occasions) are also listed, but they are not directly comparable because the proportions of important habitat covariates different between the 39-site and 91-site data sets (i.e. the proportion of disturbed sites is lower for the 91-site data set).

Species	Min	NCT			CBT			2-occs			91 Sites		
		Naïve	$\psi(\cdot)$ [SE $\psi(\cdot)$]	$p(\cdot)$ [SE $p(\cdot)$]	Naïve	$\psi(\cdot)$ [SE $\psi(\cdot)$]	$p(\cdot)$ [SE $p(\cdot)$]	Naïve	$\psi(\cdot)$ [SE $\psi(\cdot)$]	$p(\cdot)$ [SE $p(\cdot)$]	Naïve	$\psi(\cdot)$ [SE $\psi(\cdot)$]	$p(\cdot)$ [SE $p(\cdot)$]
DF	0.41	0.13	0.14 (0.06)	0.36 (0.11)	0.33	0.37 (0.09)	0.40 (0.07)	0.29	0.37 (0.11)	0.53 (0.16)	0.26	0.27 (0.05)	0.48 (0.05)
DI	0.38	0.26	0.27 (0.07)	0.46 (0.08)	0.26	0.35 (0.11)	0.24 (0.08)	0.28	0.30 (0.08)	0.78 (0.11)	0.40	0.41 (0.05)	0.47 (0.04)
DW	0.26	0.26	0.26 (0.07)	0.57 (0.07)	0.26	0.31 (0.12)	0.20 (0.08)	0.23	0.23 (0.07)	0.87 (0.09)	0.31	0.31 (0.05)	0.63 (0.04)
EW	0.64	0.54	0.63 (0.10)	0.32 (0.05)	0.38	0.84 (0.33)	0.12 (0.05)	0.49	0.58 (0.12)	0.59 (0.11)	0.56	0.62 (0.06)	0.37 (0.03)
PG	0.77	0.69	0.73 (0.08)	0.45 (0.05)	0.51	0.60 (0.09)	0.41 (0.05)	0.54	0.58 (0.09)	0.73 (0.09)	0.53	0.54 (0.05)	0.52 (0.03)
PJ	0.46	0.44	0.44 (0.08)	0.67 (0.05)	0.31	0.33 (0.08)	0.46 (0.07)	0.44	0.46 (0.09)	0.79 (0.09)	0.46	0.46 (0.05)	0.73 (0.03)
PS	0.82	0.74	0.76 (0.07)	0.52 (0.04)	0.54	0.60 (0.09)	0.41(0.05)	0.67	0.87 (0.15)	0.51 (0.10)	0.66	0.68 (0.05)	0.51 (0.03)

TABLE 5. 1999 example of AIC values for competing $\psi(\cdot)p(\text{Cov})$ and $\psi(\text{Cov})p(\cdot)$ models using *Plethodon jordani* data from 39 sites. Results from 4 different sampling methods are given: combined natural cover and coverboard transect data from 5 sampling occasions (All-data), natural cover transect data from 5 sampling occasions (NCT), coverboard transect data from 5 sampling occasions (CBT), and combined natural cover and coverboard transect data with 2 sampling occasions (2-occs). Lowest AIC values are highlighted for each set of competing models. Combining consistently low-ranked $\psi(\cdot)p(\text{Cov})$ and $\psi(\text{Cov})p(\cdot)$ models in a $\psi(\text{Cov})p(\text{Cov})$ model often improved model performance.

Covariate	All-data Models			CBT Models			NCT Models			2-occs Models		
	$\psi(\text{Cov})$ $p(\cdot)$	$\psi(\cdot)$ $p(\text{Cov})$	$\psi(\text{Cov})$ $p(\text{Cov})$	$\psi(\text{Cov})$ $p(\cdot)$	$\psi(\cdot)$ $p(\text{Cov})$	$\psi(\text{Cov})$ $p(\text{Cov})$	$\psi(\text{Cov})$ $p(\cdot)$	$\psi(\cdot)$ $p(\text{Cov})$	$\psi(\text{Cov})$ $p(\text{Cov})$	$\psi(\text{Cov})$ $p(\cdot)$	$\psi(\cdot)$ $p(\text{Cov})$	$\psi(\text{Cov})$ $p(\text{Cov})$
None	163.47	163.47	-	132.26	132.26	-	162.80	162.80	-	87.81	87.81	-
Time	-	168.47	-	-	138.24	-	-	167.28	-	-	89.14	-
Disturbance	145.83	160.00	-	119.83	134.17	-	143.50	159.05	-	na	72.33	-
Vegetation	161.61	160.75	-	132.93	129.81	-	161.48	161.11	-	86.50	84.45	-
Stream	165.09	165.42	-	130.94	124.15	-	164.67	164.14	-	89.69	88.50	-
Elevation	142.51	134.35	125.16	111.26	127.03	100.85	141.62	136.28	128.42	63.33	51.64	53.64

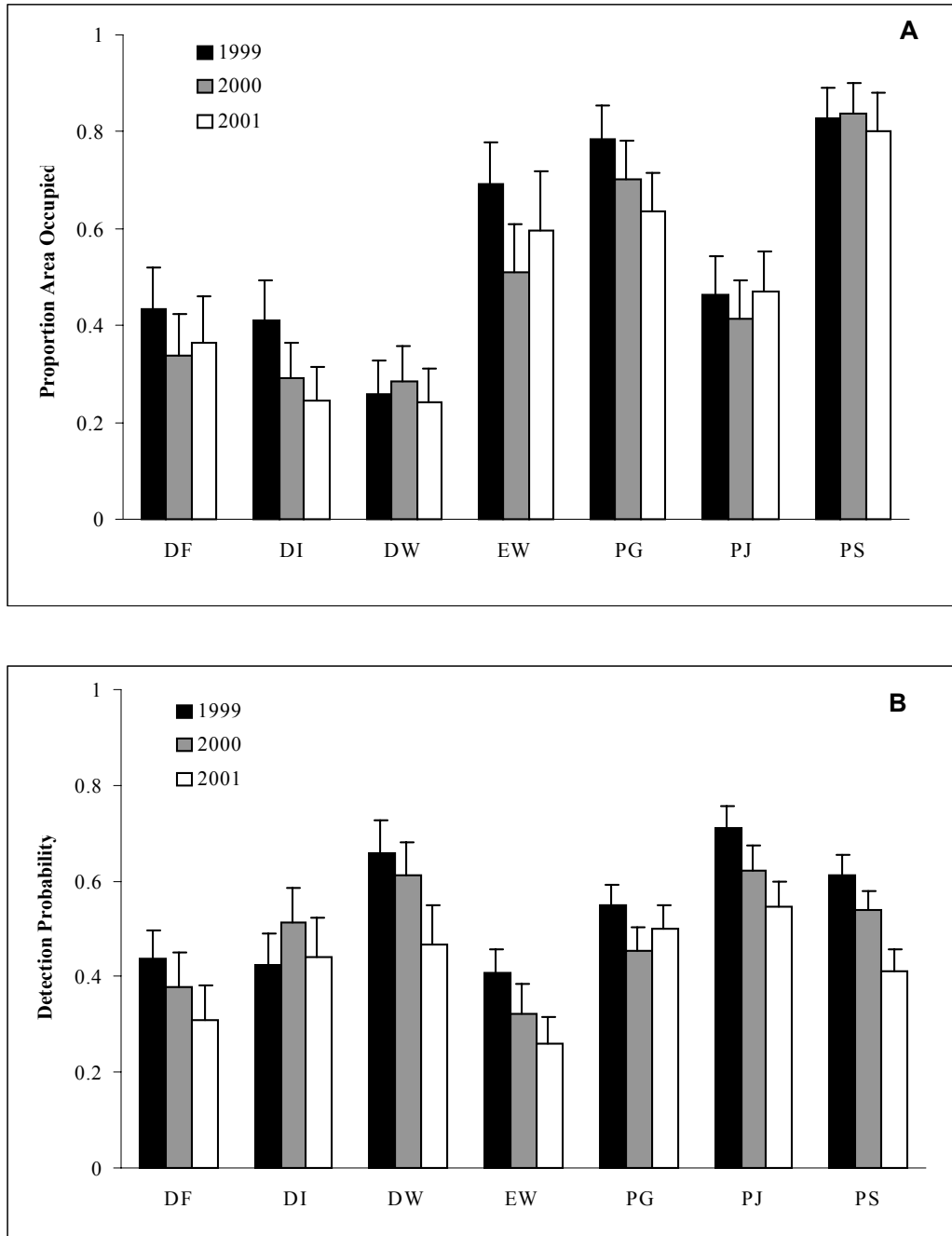


Figure 1. Parameter estimates (Mean \pm 1 SE, $n = 3$ years) for proportion of area occupied, $\psi(\cdot)$, (A) and detection probability, $p(\cdot)$, (B) for seven salamander species: *Desmognathus fuscus* complex (DF), *Desmognathus imitator* complex (DI), *Desmognathus wrighti* (DW), *Eurycea wilderae* (EW), *Plethodon glutinosus* complex (PG), *Plethodon jordani* (PJ), and *Plethodon serratus* (PS).

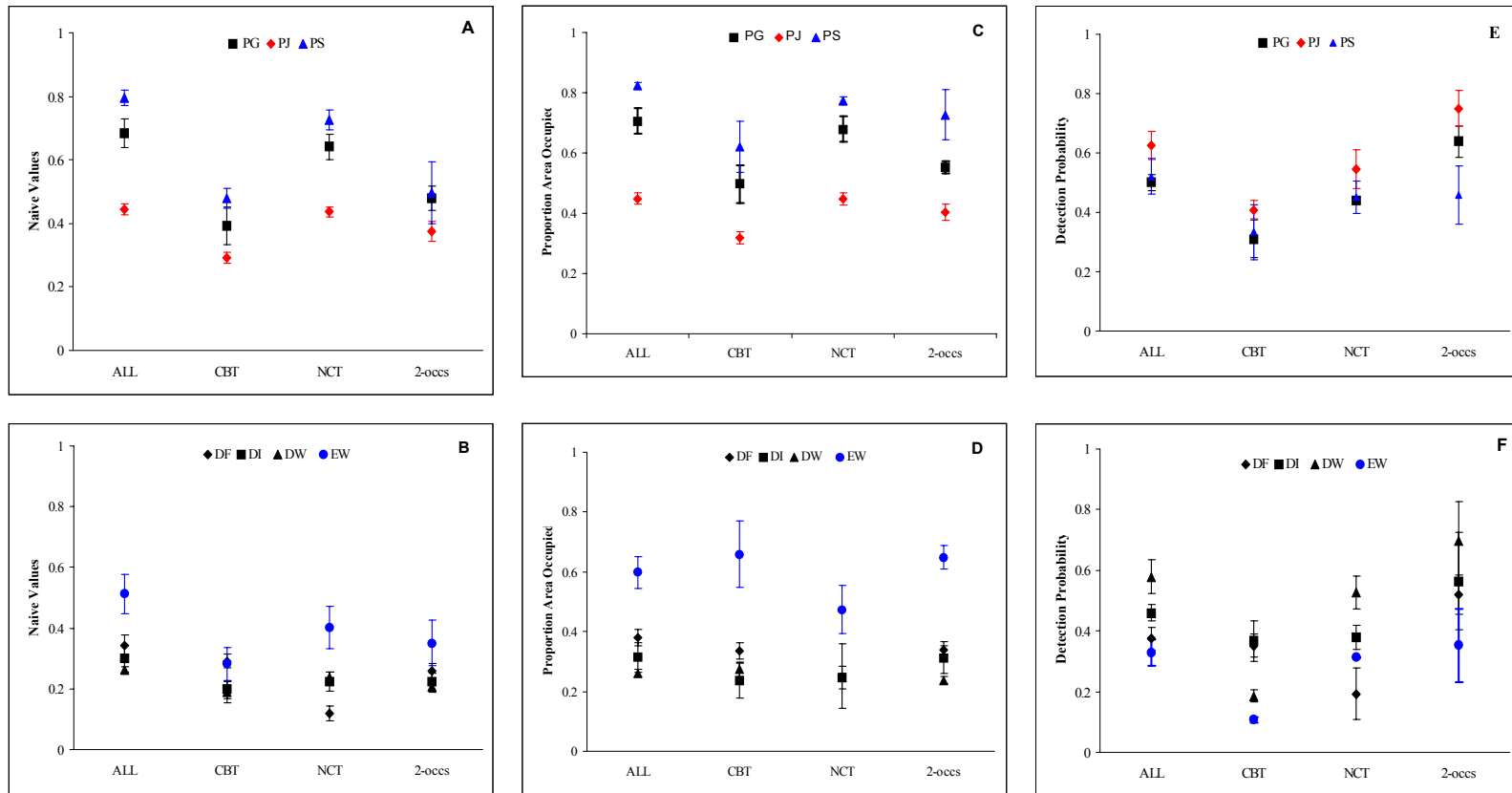


Figure 2. Species x method interactions are significant for all response parameters. Parameter values (Mean \pm 1 SE, $n = 3$ years) for naïve proportion of area occupied (A,B), estimated proportion of area occupied, $\psi(\cdot)$, (C,D) and detection probability, $p(\cdot)$, (E,F) are separated according to four different sampling methods: 5 sampling occasions per season for natural cover and coverboard transect data combined (ALL), coverboard transect data only (CBT), natural cover transect data only (NCT), and natural cover and coverboard transect data combined for 2 sampling occasions (2-occs). Species-specific estimates are given for seven salamander species: *Desmognathus fuscus* complex (DF), *Desmognathus imitator* complex (DI), *Desmognathus wrighti* (DW), *Eurycea wilderae* (EW), *Plethodon glutinosus* complex (PG), *Plethodon jordani* (PJ), and *Plethodon serratus* (PS).

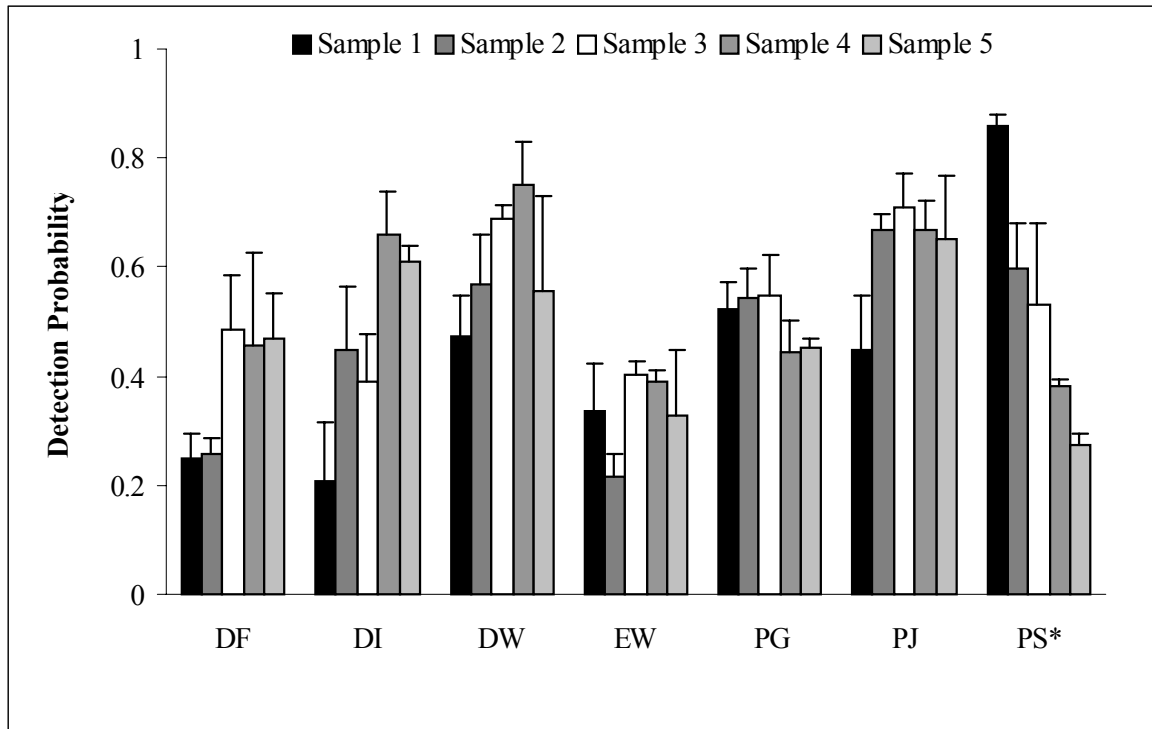


Figure 3. Time-specific species detection probability estimates, $p(t)$, ($t = 5$ sampling occasions, Mean \pm 1 SE, $n = 3$ years) for seven salamander species: *Desmognathus fuscus* complex (DF), *Desmognathus imitator* complex (DI), *Desmognathus wrighti* (DW), *Eurycea wilderae* (EW), *Plethodon glutinosus* complex (PG), *Plethodon jordani* (PJ), and *Plethodon serratus* (PS). An asterisk denotes species for which the time covariate was chosen 'best' (see text for details).

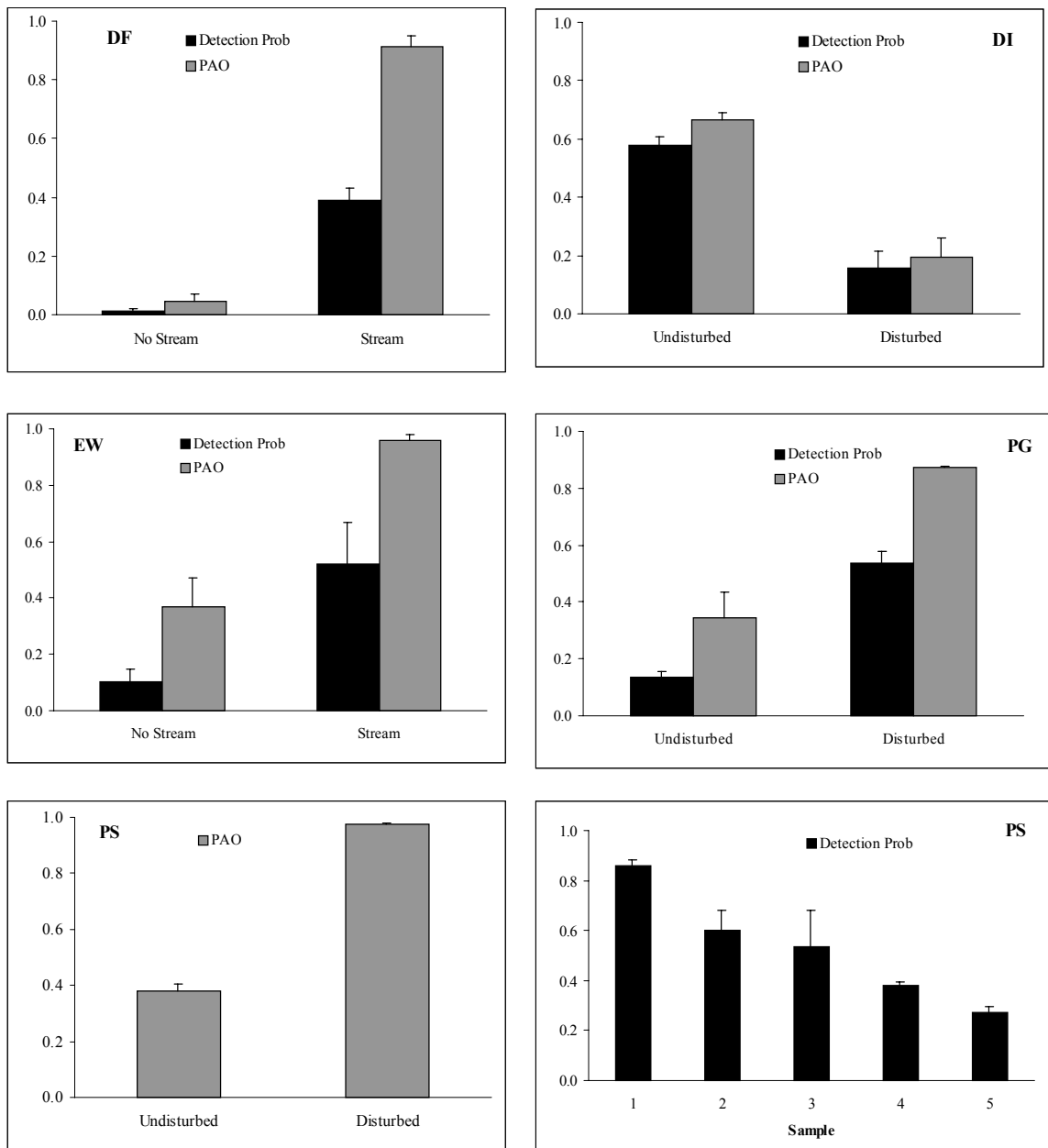


Figure 4. PAO parameter estimates with species-specific habitat covariates (Mean \pm 1 SE, $n = 3$ years) using $\psi(\text{Cov})p(\cdot)$ and $\psi(\cdot)p(\text{Cov})$ models with lowest AIC values. The most important covariate among competing $\psi(\text{Cov})p(\cdot)$ models was usually the most important covariate among competing $\psi(\cdot)p(\text{Cov})$ models; the only exception is *Plethodon serratus* (PS). All sampling methods were capable and consistent at identifying important habitat covariates and results using natural cover and coverboard transect data combined (ALL) is presented here. Species-specific estimates are given for *Desmognathus fuscus* complex (DF), *Desmognathus imitator* complex (DI), *Eurycea wilderae* (EW), *Plethodon glutinosus* complex (PG), and *Plethodon serratus* (PS).

Chapter 4. Evaluating elastomer marking and photo identification methods for terrestrial salamanders: marking effects and observer bias

Larissa L. Bailey¹

¹ Cooperative Fish and Wildlife Research Unit, Department of Zoology, North Carolina State University, Campus Box 7617, Raleigh, NC 27695-7617, USA

ABSTRACT

Capture-recapture methods are used on a wide variety of animals to estimate parameters such as population size, apparent survival rates, and recruitment rates. Two key assumptions of all capture-recapture models are that marks are not lost or overlooked by the observer and that there is no mark-induced mortality. I tested the validity of these assumptions for 2 marking methods, the injectable Visible Implant Elastomer (VIE) and photo-identification, using the Blue Ridge two-lined salamander, *Eurycea bislineata wilderae*. There was no difference in mortality, growth, or weight gain between marked and unmarked salamanders. There was 100% VIE-mark retention over 11 months with little mark migration. Mark recognition by observers was high for all identification methods (>85%) despite minimal technical training. Color misidentification was the most common mistake among misidentified VIE-marks. Giving observers more practice would likely alleviate this problem and thus eliminate the majority of VIE-mistakes. My results suggest that both VIE-marking and photo-identification methods meet capture-recapture model assumptions.

INTRODUCTION

Capture-recapture methods are commonly used in ecology and conservation biology to obtain demographic and life-history information on a wide variety of animals. These methods require individual identification to estimate parameters such as population size, apparent survival rates, and recruitment rates. Two chief assumptions of all capture-recapture models are that marks are not lost or overlooked by the observer and that there is no mark-induced mortality.

Recent global amphibian declines have prompted studies to estimate and monitor amphibian populations throughout the world. Amphibians are difficult to mark because of their sensitive skin, small size, and potential for limb regeneration (Murray and Fuller 2000). Nevertheless, a wide variety of marking methods are available including toe-clipping, tattooing, branding, tagging, skin dyeing or dusting, and subcutaneous dye injection (Donnelly 1994). Less-invasive methods such as “pattern mapping” or photo identification have also been used (Gill 1978; Tilley 1980). Few amphibian studies have evaluated marking effects on behavior and survival rates, or tested the additional assumptions of capture-recapture methods (no mark loss or observer bias). Many amphibian marking studies lack control animals, adequate sample sizes, or sufficient study duration to rigorously assess these marking issues (Donnelly 1994; Murray and Fuller 2000). Estimates of observer bias in mark recognition are almost non-existent (but see Muths et al. 2000).

The choice of an appropriate identification method depends upon the animal’s size and behavior, the necessary study duration, and cost. I studied terrestrial salamander populations at 20 capture-recapture sites in Great Smoky Mountains National Park

(GSMNP). From 1999-2001, I regularly encountered 7 different salamander species ranging in size from the pigmy salamander, *Desmognathus wrighti* (adult total length = 3.7-5.1 cm) to *Plethodon oconoluftee* (adult total length = 12-21 cm) (Petranka 1998). I needed an identification method that could be used on all species and produce recognizable marks for several years. I selected the injectable Visible Implant Elastomer (VIE) developed by Northwest Technology Inc (Shaw Island, Washington). Originally developed for fish, VIE has been recently used in studies on larval and adult amphibians (Anholt et al. 1998; Davis and Ovaska 2001; Marold 2001; Nauwelaerts et al. 2000), but it has not been tested on any of the salamander species found on my study sites. While most studies report favorable results, Close (2000) found that some marks gradually became undetectable over time in trout fingerlings. To address this concern, Northwest Technology Inc. has recently replaced their original blue-filtered viewing light with an improved deep blue LED light.

I evaluated the effectiveness of VIE marks using the Blue Ridge two-lined salamander, *Eurycea bislineata wilderae*. This species is relatively small (total length = 6.5-12 cm) (Petranka 1998) and has dual life history phases with the larval period lasting 1-2 years in the southern Appalachians (Bruce 1988). During the non-winter months, it can be found in the forest floor far from running water (Petranka 1998). It has the broadest range of any salamander species on our study sites, occurring in low densities on 90% of our capture-recapture plots. It is 1 of 4 salamander species designated as a Management Indicator Species (MIS) by the United States Forest Service for the southern region. As such, populations of *E. b. wilderae* will be monitored to assess the effects of forest management actions.

The dorsum of *E. b. wilderae* contains numerous black spots and blotches, suggesting that photo-identification may also be used for individual identification. Spot or pattern recognition is used extensively in mammals (see Kelly (2001) for review) and has been promoted as a non-invasive identification technique for several species of amphibians (Tilley 1980; Reaser 1995). Unfortunately, it may be prone to bias due to observer variation or because the spots of some species can change over time (Reaser 1995). Digital photography has reduced the cost and increased the quality of images, and improved the ease of maintaining large photographic libraries. Furthermore, computer programs continue to be developed to evaluate digital imagery (Hiby and Lovell 2001; Kelly 2001).

I evaluated the effectiveness of VIE and photo-identification techniques to individually identify *E. b. wilderae* salamanders in a laboratory study. I documented VIE-mark retention and the survival and growth rates of marked vs. unmarked salamanders. I also tested observers' ability to correctly identify individuals using: (1) digital photographs of dorsal spot patterns or (2) VIE marks viewed with either a blue-filtered or deep blue LED light.

MATERIALS AND METHODS

Marking

In June 2001, 53 *E. b. wilderae* (22 – 42 mm snout-vent length, SVL) were collected from Sevier County, Tennessee outside of Great Smoky Mountains National Park. Salamanders were temporarily stored in refrigerators at 4-5 degrees Celsius and then transported in coolers to a temperature controlled research lab at the North Carolina

Museum of Natural Sciences. I recorded the initial weight, length, age (sub-adult or adult), sex, and any abnormalities for each individual. Salamanders were housed individually in .71-liter plastic containers on a substratum of damp paper towels. Crumpled damp paper towels were provided as refuge. Each salamander was fed 6-8 small crickets per week and the substratum was also replaced weekly. Fluorescent lights maintained a 12:12 L:D photoperiod and the temperature was maintained at 18° Celsius.

Salamanders were allowed to acclimate for 2 weeks and on July 10, 2001, 47 salamanders were randomly assigned one of two treatments: unmarked but digitally photographed (control, $N = 23$) or marked via VIE ($N = 24$). Each VIE-marked salamander was sub-cutaneously injected at 2 of 4 possible locations (anterior to either hind leg or posterior to either front leg) using 1 of 3 colors (red, orange, or yellow). Marks were administered with 0.3-cc insulin syringe and needles were cleaned with 95% ethanol between injections.

Every 6 weeks from June 2001 - May 2002 salamanders were measured and weighed to the nearest 0.01 g using an electronic scale. VIE-marked individuals were examined for mark migration or mark loss using polarized glasses and both types of lights.

Observer testing

From mid-October to mid-December (approximately 3-5 months after marking), 15 observers were used to assess observer variation for each identification technique. Observers were primarily graduate students with no formal experience with either photographic or VIE marking techniques and were given a short 5-minute instruction on

the VIE-mark identification. I purposely chose observers that were unfamiliar with salamanders and gave them minimal VIE training only. Therefore, results should represent lower limits of proficiency expected from professional biologists and technicians using VIE-marking techniques in field settings. Observers were allowed to have a reference syringe of each color for comparison during the identification process. They were not informed of differences between blue-filtered or deep blue LED lights, nor were they told how many marks would appear on each salamander. To mimic field conditions, salamanders were placed in individual plastic bags and viewed with polarized glasses under a rain poncho. We use the poncho in the field to block out ambient light. Each observer viewed 10 randomly chosen marked individuals with one light (randomly assigned), then lights were exchanged, and the 10 animals were re-randomized and presented to the observer again. Observers were unaware that they were viewing the same individuals with both lights. Data recorded included the marks observed and the time required to identify the marks. The same 15 observers were used in the photo-identification experiment. Color printouts of the digital, dorsal -view photographs were given to observers (4-photos per page). The SVL for each individual was written below its picture to provide a size reference. Salamanders were placed in plastic bags and presented to observers who were asked to match the individual with the correct photograph. The time necessary to identify each individual was recorded for each observer.

Data analysis

Repeated measures analysis of variance was used to compare weight gain and growth among marked and unmarked animals. I tested for the main effects of marking,

age (sub-adult or adult), sex, and time as well as the interactions of these factors (PROC GLM with repeated statement, SAS Institute 1999).

I used a paired t-test to compare the proportion of correctly recognized VIE marks among the two different viewing lights, and a standard t-test to compare the proportion of correctly recognized salamanders between VIE and photo-identification methods.

Because all photographed salamanders were presented to each observer, I constructed a capture history matrix for each photographed individual. Correct identification was denoted with a “1” and incorrect identification was given a “0”. In this case, observer results are analagous to sampling occasions in traditional capture-recapture situations. Close-population capture-recapture models were fit using program CAPTURE (Otis et al. 1978, Rexstad and Burnham 1991) and model selection procedures tested the following competing models: (1) no effects model, M_o (equal identification among salamanders and observers), (2) individual heterogeneity, M_h , (variation among salamanders, but not observers), (3) observer effect, M_t , (equal identification among salamanders and variation among observers), and (4) variation among both salamanders and observers, M_{th} .

RESULTS

Six salamanders, 3 sub-adults (<30mm SVL) and 3 adults (>30mm SVL) died during the 2-week acclimation period (Figure 1). Following marking, 5 marked and 5 unmarked individuals died and 1 marked animal escaped. Fourteen of the 16 recorded mortalities occurred within the first 6 weeks of captivity, and only 3 of 8 sub-adults survived to the end of the study (Figure 1). None of the VIE-marks were lost and marks

on surviving salamanders could be identified with either the blue-filtered or deep blue LED lights.

Marked and control salamanders did not differ in their pre-marking weight ($t_{45} = -0.85$, $P = 0.39$) or length ($t_{45} = -1.12$, $P = 0.27$). Marking had no effect on either weight gain ($F_{1,26} = 0.00$, $P = 0.97$) or growth ($F_{1,27} = 0.29$, $P = 0.59$) and none of the interactions involving marking effect were significant. Both weight and length changed over time ($P < 0.05$ using either univariate and multivariate tests) and there was a significant time x sex interaction for the weight response variable (Wilk's lambda = 0.42, $F_{8,19} = 3.27$, $P = 0.0161$, univariate $F_{8,208} = 4.46$, G-G adjusted $P = 0.0122$, Figure 2). Females continued to gain weight throughout the study, but males stopped gaining weight after January 2002 (Figure 2).

Mark recognition by observers was high for all identification methods, especially photo-identification (Figure 3). The type of light, blue-filtered or deep blue LED, did not affect an observer's ability to correctly identify VIE-marked individuals ($t_{15} = -0.69$, $P = 0.50$), nor was there a difference between photo-identification and the currently marketed deep blue LED light ($t_{15} = -1.8$, $P = 0.08$). Observers misidentified VIE-marked individuals on 39 of 300 identification opportunities (10 salamanders x 2 lights x 15 observers). Observers had difficulty finding marks on 3 marked salamanders resulting in 15 misidentifications. One salamander accounted for 10 of 15 missed marks, because one of its marks was quite small. The remaining mistakes consisted of color misidentification (21 occasions) and incorrect mark locations (3 occasions). Observer performance was even better using photo-identification where salamanders were misidentified on only 16 of 270 possible occasions (18 salamanders x 15 observers). All

observers correctly identified 7 salamanders, 7 salamanders were misidentified once, 3 were misidentified twice, and 1 was misidentified 3 times. Model selection procedure in program CAPTURE suggested that there was variation among observers and individuals (models M_t and M_{th} were equally likely). The proportion of salamanders correctly identified by observers ranged between .67 – 1.00 ($\bar{x} = 0.94 \pm 0.02$, $N=15$).

Observers were able to identify individuals using VIE-marks more quickly than by matching individuals to photographs (VIE mean (sec) $\bar{x} = 18.0 \pm 0.75$, $N=298$; Photo mean (sec) $\bar{x} = 55.0 \pm 3.16$, $N=270$). Some observers spent over 5 minutes matching individual salamanders to photographs.

DISCUSSION

Both VIE-marking and photo-identification methods showed good potential for individual identification of *E. b. wilderae* salamanders. There was 100% VIE-mark retention over 11 months with little mark migration or mark reduction. Only 1 of 18 marked salamanders had a small mark that was consistently missed by observers. VIE marking had no effect on *E. b. wilderae* growth or weight gain, adding to similar findings from studies on western red-backed salamanders, *Plethodon vehiculum* (Davis and Ovaska 2001), and larvae of *Rana* species (Anholt et al. 1998). Marking did not inhibit reproductive development; most of our female salamanders developed eggs during the spring months. Presumably the significant time x sex interaction in the weight response variable was due to egg development. Mortality early in the study may have resulted from stress associated with handling or feeding prey that were too large for the smaller individuals. I am currently investigating VIE-marking effects on the smallest salamander

species found on our sites, *Desmognathus wright*. I adjusted prey size for these individuals and have not experienced early die-offs.

There were no differences in observer's ability to detect specific VIE-colors, but observers needed practice to correctly identify the colors. Observers commonly commented that the polarized glasses and viewing lights were necessary to see the VIE-marks, but that the colors look different when viewed with the lights: red VIE looks orange, orange VIE looks yellow, and yellow VIE looks green. Despite having reference syringes, color misidentification was the most common mistake among VIE-marked individuals ($21/39 = 54\%$ VIE misidentifications). When we mark salamanders in the field, the marks are always checked before the salamanders are released, thus new workers are exposed to hundreds of known color marks before they are required to read an unknown VIE-marked individual. This study contained minimal observer preparation, but these results show that observers with little training can still correctly identify $\sim 85\%$ of marked salamanders, and it is likely that field recognition rates with trained observers are much higher.

Photo-identification may be an underutilized identification technique in salamander studies. This technique was used in early salamander studies (Forester 1977; Gill 1978; Tilley 1980), but more recently it has only been used to verify other marking methods (Davis and Ovaska 2001). Advances in camera and computer technologies combined with good observer performance encourage the use of photo-identification as a primary identification method for species with distinctive marking patterns. Photo-identification would presumably be much more difficult and less accurate with larger numbers of individuals without computer aided matching techniques. Furthermore,

photograph quality has been shown to affect matching likelihood (Kelly 2001 and Whitehead 1990). Observers in my study commented that picture clarity was good, but glare from the camera's flash and salamander positioning sometimes inhibited identification. It is important to note that observers in my study were not given unmarked individuals (non-photographed individuals). It is likely that correct photo-identification rates would have been reduced if unphotographed individuals were also presented to observers. Finally, future studies that explore potential changes in spot patterns over time are still needed (Reaser 1995).

This study adds to the growing body of literature that supports using VIE-marking methods in terrestrial amphibian and reptile species. The technique appears to have good long-term retention, minimal marking effects, and meets the assumptions of capture-recapture studies. Photo-identification also shows good potential as a non-invasive identification technique and I would encourage investigators to use this method as a primary identification technique or in combination with VIE-marking methods.

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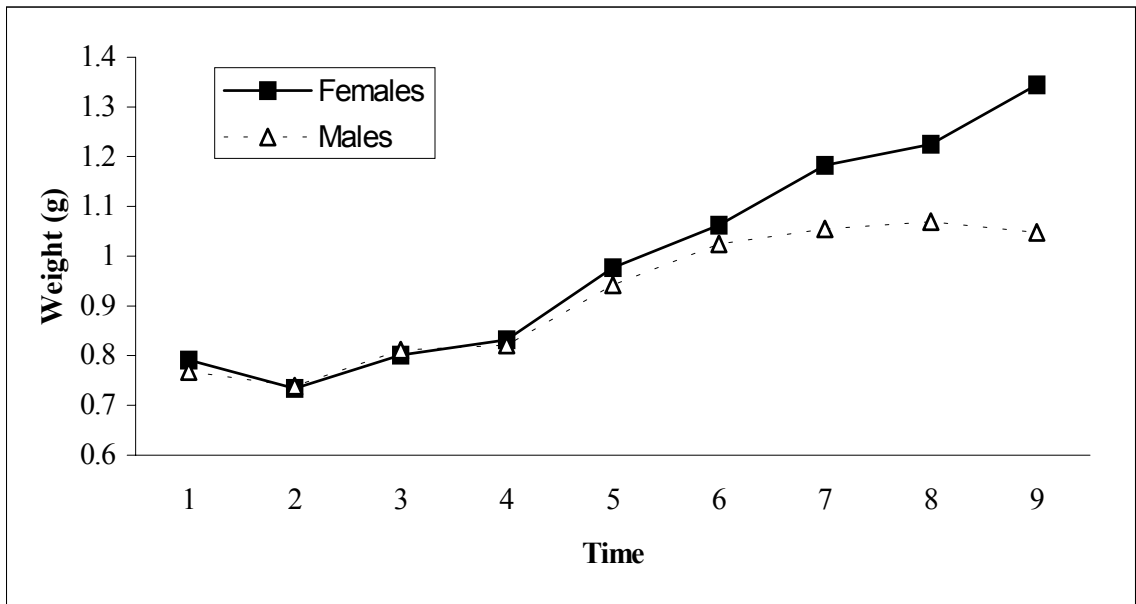


Figure 2. Average weight (g) of male and female salamanders from July 2001 – May 2002.

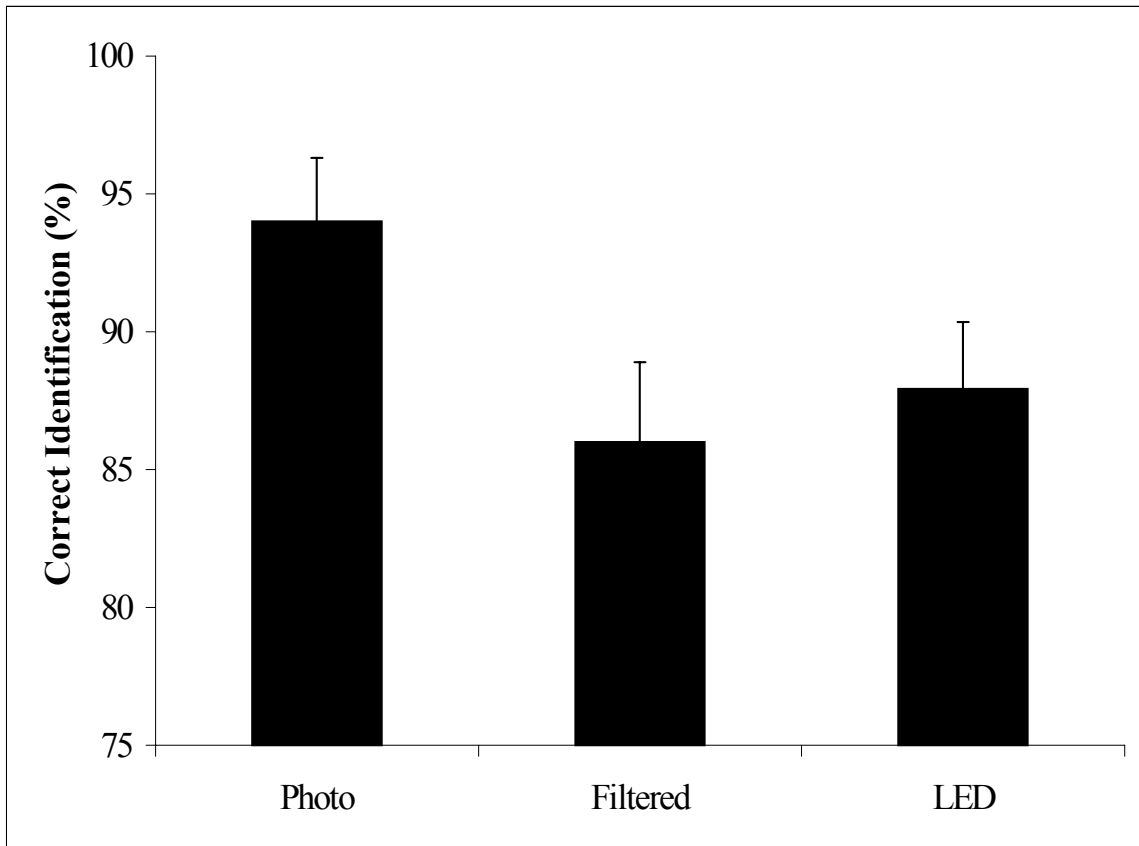


Figure 3. Average percent of salamanders correctly identified by observers using 3 different methods: photo-identification, VIE-marks viewed with blue filtered light, and VIE-marks viewed with new deep blue LED light. $N = 15$ observers.

Chapter 5. Comparing population size estimators for *Plethodon* salamanders

Larissa L. Bailey¹, Theodore R. Simons¹, and Kenneth H. Pollock²

¹ Cooperative Fish and Wildlife Research Unit, Department of Zoology, North Carolina State University, Campus Box 7617, Raleigh, NC 27695-7617, USA

² Department of Statistics, Biomathematics, and Zoology, North Carolina State University, Campus Box 8203, Raleigh, NC 27695-8203, USA

ABSTRACT

Despite growing concern over amphibian population declines, few studies actually estimate absolute abundances due to logistic and economic constraints and previously poor estimator performance. Two estimation approaches recommended for amphibian studies are mark-recapture and depletion (or removal) sampling (Heyer et al. 1994). We compared abundance estimation via various mark-recapture and depletion methods, using data from a 3-year study of terrestrial salamanders in Great Smoky Mountains Nation Park. Our results indicate that short-term closed-population, robust design, and depletion methods estimate the surface population of salamanders (i.e. those near the surface and available for capture during a given sampling occasion). In longer duration studies, temporary emigration violates assumptions of both open and closed-population mark-recapture estimation models. However, if the temporary emigration is completely random, these models should yield unbiased estimates of the total population ('superpopulation') of salamanders in the sampled area. We highly recommend using

Pollock's robust design in mark-recapture studies because of its flexibility to incorporate variations in capture probabilities, and estimate temporary emigration rates.

INTRODUCTION

Despite growing concern over amphibian population declines, few amphibian studies actually estimate population abundances due to the logistical cost of obtaining precise parameter estimates (Welsh and Lind 1992, Jung et al. 2000). Most investigators have abandoned estimation techniques in favor of relative abundance indices based on count data. Such indices assume that there is a constant, linear relationship between the index and population size and that all individuals of a particular species have the same detection probability (equal catchability) (Lancia et al. 1994). These assumptions are unlikely in most wildlife populations including the terrestrial salamander populations we studied in the southern Appalachians (Bailey this thesis, Chapters 1 and 2).

Many methods exist to estimate population size, including change-in-ratio methods, catch-per-unit-effort, removal and depletion methods, and mark-recapture methods (see Seber 1982 for reviews). Two approaches recommended for amphibian studies are mark-recapture and depletion (or removal) sampling (Heyer et al. 1994). As these methods are labor intensive, they have been recommended only when researchers require detailed knowledge of the target population; otherwise relative abundance indices are preferred (Heyer et al. 1994).

In this paper we compare abundance estimation via various mark-recapture models and depletion models, using data from a 3-year study of terrestrial salamanders in Great Smoky Mountains National Park (GSMNP). We compare population estimates from

depletion models and categories of mark-recapture models including closed-population, open-population, and robust-design models to illustrate how different model assumptions can result in wide variations in population estimates. We also consider and clarify which methods estimate surface population vs. superpopulation (total population) size.

POPULATION ESTIMATION METHODS

Mark-recapture estimators can be separated into 2 classes: closed-population and open-population models. Closed-population models make three general assumptions:

- (1) The population is closed to births, deaths, immigration, and emigration during the sampling interval (demographic closure)
- (2) All animals are equally likely to be captured in each sample (equal catchability) and no animal has probability of capture equal to zero, $p(.) \neq 0$.
- (3) Marks are not lost or overlooked by the observer

The equal catchability assumption may be relaxed by allowing capture probabilities to vary as a result of time (t), behavioral (or trap) response (b), and heterogeneity (h).

Well-known Lincoln-Peterson (for 2 samples), Schnabel, and Schumacher-Eschmeyer estimators (Seber 1982) allow time variation in capture probabilities. These methods only require batch marks. However, if animals have been individually marked, the use of program CAPTURE (Otis et al. 1978, Rexstad and Burnham 1991) to calculate maximum likelihood estimators is recommended (Pollock et al. 1990). Depletion or removal models (Zippen 1956, Seber 1982) are similar to the behavioral model, M_b , in program

CAPTURE, because only information from an animal's first capture contributes to population estimates (Pollock et al. 1990). Studies that remove individuals from the population assume demographic closure, equal sampling effort, and constant capture probabilities over time (White et al. 1982). The behavioral model, M_b , assumes that marked and unmarked individuals have different capture probabilities, but no temporal variation in capture probabilities (Otis et al. 1978, Pollock et al. 1990). Finally, capture probabilities may vary among individuals (heterogeneity, h) as a result of factors such as species type, age, size or sex. Estimators for heterogeneous populations included Burnham's "jackknife" technique (Burnham and Overton 1978, Burnham and Overton 1979), Chao's M_h estimator (Chao 1988), and finite mixture models (Norris and Pollock 1996, Pledger 2000). Program CAPTURE contains estimators for seven of the eight closed population models proposed by Otis et al. (1978; M_o , M_b , M_h , M_t , M_{bh} , M_{tb} , M_{th}). CAPTURE also contains a test for demographic closure and a model selection procedure, together with a series of goodness-of-fit tests to aid investigators in model choice. The test for demographic closure and the model selection procedure both have limitations and biologists should interpret model results carefully (Otis et al. 1978, Menkens and Anderson 1988, Pollock et al. 1990, Stanley and Burnham 1999, Pledger 2000).

In studies where the demographic closure assumption cannot be met, open-population models can estimate population size at each sampling period, and survival and recruitment rates between sampling periods (Pollock et al. 1990). The most common model, the Jolly-Seber (JS) model (Jolly 1965, Seber 1965) has three main assumptions (Pollock et al. 1990):

- (1) All animals present in the population at time i ($i = 1, 2, \dots, k$) are equally likely to be captured (equal catchability).
- (2) Every marked animal present in the population at time i has the same probability of surviving from i to $i+1$.
- (3) Marks are not lost or overlooked by the observer

An additional, implicit assumption is that all emigration from the population is permanent; temporary emigration in and out of the population violates assumptions of the Jolly-Seber (JS) model. Seber (1982) and Pollock et al. (1990) present several variations of the JS model and many are included in programs JOLLY (Pollock et al. 1990) and MARK (White and Burnham 1999). Despite these additions, the JS model is still limited in that some parameters cannot be estimated (e.g. population size in the first and last periods) and the population size estimator is not robust to variations in capture probabilities (other than time variations) (Kendall and Pollock 1992).

To address these concerns, Pollock developed the “robust-design” model (Pollock 1982). Under this design, primary sampling periods, i ($i = 1, 2, \dots, k$) contain l_i secondary sampling periods that are separated by a time interval that is short enough to assume demographic closure (i.e. no births, deaths, immigration, or emigration) (Fig. 1). Primary periods are separated by longer time intervals during which population additions (immigration and births) and deletions (emigration and deaths) can occur (Fig. 1). Data from secondary samples within each primary period can be analyzed using closed-population models that allow for unequal capture probability (White et al. 1982, Otis et

al. 1978). The closed-population models estimate conditional capture probabilities, recapture probabilities, and population size for each primary period. Data within each primary period are pooled to estimate survival rates and temporary emigration rates between primary periods (Fig. 1) (Kendall and Nichols 1995, Kendall et al. 1997, Kendall 1999). This design is especially useful for estimating temporary emigration rates (Kendall and Nichols 1995, Kendall et al. 1997, Kendall and Hines 1999, Kendall 1999).

Previous salamander studies have utilized a wide variety of closed and open-population estimation methods. Closed-populations methods include: Lincoln-Peterson for 2 samples (Welsh and Lind 1992); and for more than 2 samples: Schabel's (Stewart and Bellis 1970, Howard 1987, Smith and Petranka 2000), Schumacher-Eschmeyer (Semlitsch 1980, Howard 1987), and depletion methods (Bruce 1995, Petranka and Murray 2001, Salvidio 2001). We know of only one study that has used the multiple closed-population models discussed in Otis et al. (1978) (Jung et al. 2000). This is important because none of the other methods allow for heterogeneity of capture probabilities between different animals. JS open-population models have been utilized in salamander studies by Tilley (1980), Welsh and Lind (1992), and Marvin (1996). Most studies were conducted on 1-4 sites (but see Jung et al. 2000, Smith and Petranka 2000), and they frequently report low effective capture probabilities ($<.15$) which resulted in imprecise population estimates (Howard 1987, Welsh and Lind 1992, Jung et al. 2000). This thesis (Chapters 1 and 2) represents the most extensive terrestrial salamander population study to date and is the only study to use the robust design.

STUDY AREA

Great Smoky Mountains National Park, located along the Tennessee-North Carolina border, is internationally recognized for its rich temperate forest biodiversity. The Park's unique geology, along with steep, complex topography, creates temperature and moisture gradients across 205,665 ha of contiguous forest. These gradients produce high levels of temperate species diversity in many taxa, including salamanders. Approximately 10% of the world's salamander species are found in the southern Appalachian region (Petranka 1998), with 30 species occurring inside the Park's boundaries (Dodd et al. 2000). Salamanders are a high priority taxon for the Park's inventory and monitoring program due to their high diversity, large number of endemic species, and the limited amount of data on the distribution, abundance, and natural history of most species.

METHODS

Field Methods

In 1999 we initiated a 3-year mark-recapture study on 15 plots (15 x 15 m) in the Roaring Fork Watershed (Mt. LeConte USGS Quadrangle); five additional plots were added in 2000. Each plot was enclosed with a silt fence to inhibit salamander movement. The bottom edge of the silt fence was buried 10-15 cm into the soil and the remainder of the fence was raised and stapled to 60-cm tall wooden stakes. The top edge of the fence was draped toward the inside of the plot creating a lip that made it difficult for salamanders to escape. Three parallel 15-m transects were established within each plot including a natural cover transect, a transect of 5 coverboard arrays, and a set of 5 leaf

litter search stations (see Bailey this thesis, Chapter 1 for details). Plots were sampled according to Pollock's robust-design with 4 primary periods, each consisting of 3-4 consecutive sample days (Pollock 1982). Primary periods were separated by 6-8 days in 1999 and 12 days in 2000 and 2001. Sampling was conducted from April 1 to mid-June each year. During each sampling occasion, the 3 transects were sampled first, then the remaining natural cover was turned, and the inside edge of the fence was searched. This procedure ensured that all available animals within the plot had a non-zero capture probability during the given primary period. We recorded the following information for each captured salamander: species type, presence of previous marks, snout-vent length (SVL), and age and sex (if possible). Abnormalities and injuries were also noted. All unmarked salamanders over 18-mm snout-vent length (SVL) were individually marked using fluorescent elastomer (see Bailey this thesis, Chapter 1 for details).

Following the 30-40 day mark-recapture study, a depletion study was initiated on each plot. Plots were sampled every other day for 8 days, resulting in 4 depletion samples per plot. Captured animals were removed from plots and stored in a refrigerator at 4-5 degrees Celsius. Salamanders can be retained in this manner with minimal care for several weeks (Stewart and Bellis 1970, DeNardo 1995, Salvidio 1998)

In 1999, a complete removal study was conducted on a subset of 3 plots. All natural cover and leaf litter was removed from these plots using the method presented in Heatwole and Sexton (1966). The material was thoroughly searched before removing it was removed from the plot. Leaf litter was collected and stored in plastic garbage bags outside the plot. All captured salamanders were stored in refrigerators. Following excavation, plots were visited on consecutive nights to remove all available salamanders.

Sampling was terminated when no new salamanders were found on nightly surveys, usually after 4-5 nights. It is thought that removing cover forces burrowing species to the surface and can thus yield a complete count of salamanders in an area. After complete removal sampling was halted, the bagged leaf litter and natural cover were replaced, carefully reconstructing the plot as closely to its original design as possible. Captured salamanders were marked and released at their original capture locations.

Population Estimation

We used several methods to estimate salamander abundances at each site in each year (site-year). We used all 15-16 sampling occasions (4 primary periods x 3-4 secondary samples each) to construct capture history data sets for each site-year and used program CAPTURE to test demographic closure, perform model selection, and estimate population size under a number of closed-population models (Otis et al. 1978, White et al. 1982, Rexstad and Burnham 1991). This analysis was the first exploratory step to determine the types of variation in capture probabilities (heterogeneity, behavioral or time) that characterized each data set. Data from the 4-sample depletion study were analyzed using the behavioral model, M_b , and the 'generalized removal method', M_{bh} (program CAPTURE, Otis et al. 1978). The generalized removal method allows heterogeneity of capture probabilities.

A third closed-population estimate was obtained using the Lincoln-Peterson method. Because the Lincoln-Peterson estimator uses only 2 sampling occasions, all individuals caught in 30-40 day mark-recapture study were pooled into a single sampling period, n_1 , and the individuals obtained in the 8-day depletion study were pooled into a

single recapture period, n_2 . The number of previously marked animals in the depletion sample becomes m_2 , and an unbiased estimate of population size can be obtained using:

$$\hat{N} = \left[\frac{(n_1 + 1)(n_2 + 1)}{(m_2 + 1)} \right] - 1 \quad (\text{Chapman 1951})$$

Pollock et al. (1990) and Seber (1982) present detailed exposition on this model and its standard error (not shown here). Closed-population estimates were compared to one another and to the complete removal counts from 3 plots in 1999 (Tables 4-6).

Open-population estimates were obtained for each data set by pooling secondary samples within each primary period. We considered 3 standard JS models including the full time-specific model (Model A), the Constant Survival Model (Model B), and the model with Constant Survival and Capture Model (Model D) (Pollock et al. 1990). Analysis was performed using program JOLLY (Pollock et al. 1990).

We analyzed the mark-recapture data using the robust-design option in program MARK (White and Burnham 1999). In previous work, we fit a series of models that represented possible salamander responses (time and behavioral) with and without temporary emigration (Bailey this thesis, Chapter 1). Models that contained random temporary emigration were chosen ‘best’ for 80% of our data sets (using AIC criteria; Akaike 1973, Burnham and Anderson 1998). In this study we used a subset of robust design models, each containing constant random temporary emigration, $\gamma(\cdot)$, time-invariant average surface population size, $N(\cdot)$, and either constant, behavioral, or time-specific capture probabilities (see Bailey this thesis, Chapter 1 for model details). In order to compare robust model results to JS open-population estimates, we did not fix apparent survival rate over primary periods ($\phi(\cdot) \neq 1$; Bailey this thesis, Chapter 1), but

allowed the model to estimate a constant survival parameter, $\phi(\cdot)$. Finally, we analyzed the secondary samples within each primary period using closed-population models M_b and M_h in program CAPTURE.

RESULTS

A total of 6560 salamanders (>18 mm SVL) were captured and marked during our 3 year study (Table 1). Of these, 206 salamanders were removed from 3 plots during the complete removal study in 1999. The average number of salamanders caught per site (excluding complete removals) varied little between years: 1999 mean \pm 1 SE = 128 ± 19.89 , $n = 14$ sites; 2000 mean \pm 1 SE = 130 ± 28.02 , $n = 19$ sites; 2001 mean \pm 1 SE = 112 ± 20.32 , $n = 19$ sites.

Despite the use of a silt fence as a barrier to salamander movement, the test for closure was rejected ($p < 0.05$) for 30 of the 52 data sets (program CAPTURE). Closed-population models containing time and behavioral variation in capture probabilities fit our data sets better than other competing models according to model selection procedures in program CAPTURE (Table 2). Time variation (t) was included in 73.1% of selected models, while behavioral effects (b) and heterogeneity (h) were included in 44.2% and 42.3% respectively; constant capture probabilities were suggested for 13.5% of our data sets. Unfortunately, models with both time and behavioral effects, M_{tb} , are often difficult to fit with sparse data, and there is currently no estimator available for model M_{tbh} . Because model selection procedures in program CAPTURE often perform poorly with sparse data (Menkens and Anderson 1988), we analyzed the same data sets using the closed-captures procedure in program MARK (White and Burnham 1999) where AIC

values could be used to evaluate relative model performance. Models containing heterogeneous capture probabilities are excluded in this assessment, but model selection procedures in program CAPTURE indicated that heterogeneity was the least influential of the 3 possible capture probability effects (Table 2). A subset of 4 closed-population models (M_o , M_b , M_t , M_{tb}) were tested and all models with $\Delta AIC < 2.0$ were considered appropriate for a given data set (Burnham and Anderson 1998). Model selection results using AIC were compared to the ‘best’ subset model from program CAPTURE (i.e. the subset model with the value closest to 1.0) (Table 3). Neither model selection procedure yielded a dominant capture probability effect, but suggested that either time or behavioral or both effects influenced salamander capture probabilities.

Capture probabilities were low regardless of model choice; the highest average capture probability estimate for any data set was $\hat{p}(\cdot) = 0.06$ using the null model, M_o , and $\hat{p}(\cdot) = 0.04$ using the heterogeneity model, M_h ($n = 52$ data sets). Nearly all data sets suggested a ‘trap shy’ behavioral response, where the highest estimates of initial capture probability and recapture probability were $\hat{p}(\cdot) = 0.27$ and $\hat{c}(\cdot) = 0.07$ ($n = 42$ data sets).

Open-population estimation was difficult because pooling secondary samples reduced the number of recaptures. For example, if an animal was recaptured multiple times within a primary period, but not recaptured in other primary periods, that animal appeared to be captured only once in the open-population analysis. Pooling resulted in a 32% reduction in the number of recaptured animals and thus open-population analyses were possible for only 35 of 52 data sets. All 3 JS models and the full compliment of goodness-of-fit tests could be calculated for only 10 data sets. The goodness-of-fit test comparing JS Model D vs. Model A was possible for 31 data sets and the more restricted

Model D was favored for 81% of these data sets. Population estimates were possible for 47 depletion data sets using model M_b and 43 data sets using the ‘generalized removal method’, M_{bh} . Lincoln-Peterson population estimates were possible for 50 of 52 data sets.

We use estimates from the 3 complete removal sites in 1999 to illustrate the variation among population size estimates obtained from different types of models (closed-population, open-population, depletion models)(Tables 4-6). Population estimates obtained from different closed-population models vary widely, while estimates from open-population JS models and depletion models are more consistent (Tables 4-6). Despite these differences, a distinct pattern emerged that we believe represents 2 different populations. Depletion models yielded the lowest population estimates, but had good agreement with robust-design estimates that included temporary emigration and closed-population estimates from secondary sampling periods (Table 4-6). We feel these models estimated the population of salamanders near the surface and available for capture at a given sampling occasion (i.e. surface population). Table 6 represents one of the few exceptions to this generality, but in 1999 the RG012 site suffered from exceptionally low number of recaptured individuals (only 7 of 111 individuals were recaptured during the mark-recapture study) resulting in poor estimates and precision.

Closed-population models M_o and M_t , JS open-population models, and Lincoln-Peterson estimators all yielded much higher population estimates than depletion or robust design estimates. Population estimates from depletion model M_b were usually 5-20% of Lincoln-Peterson estimates for the same data sets (range: 4.39 - 40.42%, $n = 47$ data sets). The robust-design models listed in each table (4-6) make the same capture probability assumptions as the corresponding closed-population models, but their average

population size estimates were much less. We believe the Lincoln-Peterson model, closed-population models M_o and M_t , and JS open-population models estimated both surface and subterranean individuals (termed ‘superpopulation’, Kendall 1999). Using the robust design, random temporary emigration rates were incorporated to estimate the ‘superpopulation’ at each site and these estimates resembled those from closed-population models M_o and M_t , JS open-population models, and Lincoln-Peterson methods (Tables 4-6).

DISCUSSION

Population size estimation is an important aspect of many ecological studies and wildlife management programs. There are numerous estimation methods available and a method’s performance depends on its underlying assumptions. This is especially important when mark-recapture data are sparse. An estimator’s relative bias and coefficient of variation is expected to increase as population size or individual capture probability decrease (Menkens and Anderson 1988). Two estimation methods recommended for amphibian populations are mark-recapture and depletion methods (Heyer et al. 1994). Population size estimates derived from these methods varied widely for populations of terrestrial salamanders on our sites in GSMNP. An examination of the assumptions of each estimation method provides some insight into the sources of variability that can influence abundance estimates.

All closed-population estimators assume demographic closure and no mark loss or observer bias. Marking studies indicate that elastomer mark loss and misidentification is rare (Davis and Ovaska 2001, Bailey Chapter 4), thus it is likely that all model

assumptions involving mark retention and identification are met. The assumption of demographic closure, which is of fundamental importance (Stanley and Burnham 1999), was rejected for 58% of our data sets, despite the fact that the closure test in program CAPTURE is not particularly powerful (Stanley and Burnham 1999). Survival rates are not well known for terrestrial salamanders, but several studies suggest annual rates are above 45% (Organ 1961, Tilley 1980, Hairston 1983). Thus, survival rates during our 6-8 week sampling period should be near 1. Likewise, growth rates are sufficiently low (Petranka 1998) that we would not expect large numbers of recruits into the population (>18 mm SVL salamanders) during our study. For these reasons, we concluded that movement (immigration and emigration) was the likely cause of the closure violation. Although we constrained horizontal surface movement with silt fencing, we could not control the vertical movement of salamanders in the soil. We believe this vertical movement, surface to subsurface, explains the lack of closure.

In addition to the closure assumption, our results indicate that the equal capture probability assumption was also violated. Model selection procedures in programs CAPTURE and MARK indicate that capture probabilities varied temporally and had a 'trap-shy' behavioral response (higher initial capture probabilities than recapture probabilities). Previous salamander studies have used estimators that allow temporal variation in capture probabilities (i.e. Lincoln-Peterson, Schnabel and Schumacher-Eschmeyer estimators), but few have incorporated a behavioral response (Stewart and Bellis 1970, Semlitsch 1980, Howard 1987, Smith and Petranka 2000). Trap-shy behavioral effects result in a positive bias in population estimates.

Although other studies have shown that capture probabilities may vary with sex (Tilley 1980) or species (Smith and Petranka 2000, Petranka and Murray 2001, Bailey this thesis, Chapter 2), our results indicate that heterogeneity is the least influential of the 3 possible capture probability effects. We do not know if this is real or due to the low capture probabilities realized in our study (see Bailey this thesis Chapters 1 and 2 for capture probability estimates). Failure to account for heterogeneous capture probabilities in closed and open-population estimators will result in negatively biased estimates of abundance (Pollock et al. 1990).

Our most dramatic result was the large discrepancy between the depletion and robust design estimators and the open and closed-population estimators. We believe that the estimators actually apply to 2 different populations: surface and ‘superpopulations’. Depletion estimators utilize data collected during a short period of time where the closure assumption is likely valid, thus depletion population estimates should represent the available surface population of salamanders during the 8-day sampling period. ‘Superpopulation’ refers to the population of salamanders both near the surface and available for capture, and those beneath the surface and unavailable for capture during a given primary sampling period. Temporary emigration violates the closure assumption for the longer mark-recapture study, but if the temporary emigration is completely random, closed-population and open-population models should yield unbiased estimates of the ‘superpopulation’ in the sampled area. Using robust-design models, we were able to incorporate and estimate temporary emigration (Bailey this thesis, Chapters 1 and 2) and confirmed that random temporary emigration rates between primary periods were high (average temporary emigration estimate = 0.87 ± 0.01) (Bailey this thesis, Chapter 1).

Surface population estimates calculated using the robust-design models were similar to depletion model estimates and closed-population estimates from secondary sample periods; ‘superpopulation’ estimates from robust-design models were similar to estimates from closed-population models M_o and M_t , JS open-population models, and the Lincoln-Peterson method.

Finally, complete removal techniques have been highly successful at providing absolute abundances for a wide variety of herpetofaunal species (Heatwole and Sexton 1966, Rhodda et al. 2001, H. Heatwole personal communication) but the technique has not been validated for fossorial or subterranean species (G. Rodda, personal communication). Our results indicate that complete removal procedures described in this paper do not produce good estimates of the ‘superpopulation’ of terrestrial salamanders.

CONCLUSIONS

The choice of an appropriate population abundance estimator is study-specific and investigators must weigh logistical, economic, and biological factors when selecting a sample design. Study objectives and model assumptions must be considered when developing sampling protocols for estimating abundance. We highly recommend the use of the robust design because of its flexibility to incorporate variations in capture probabilities, and estimate temporary emigration rates. The method’s assumptions fit the biological processes occurring in most terrestrial salamander populations (see Bailey Chapter 1 and 2). The method is costly in terms of labor and time, but it may be possible to apply the method on a subset of sample sites using the dual sampling design described by Pollock et al. (2002). If intensive sampling is not possible at any sites, depletion

methods show good potential to estimate available population size over short sample periods (Bruce 1995, Salvidio 2001), while closed-population estimators over somewhat longer periods may be unbiased for superpopulation sizes.

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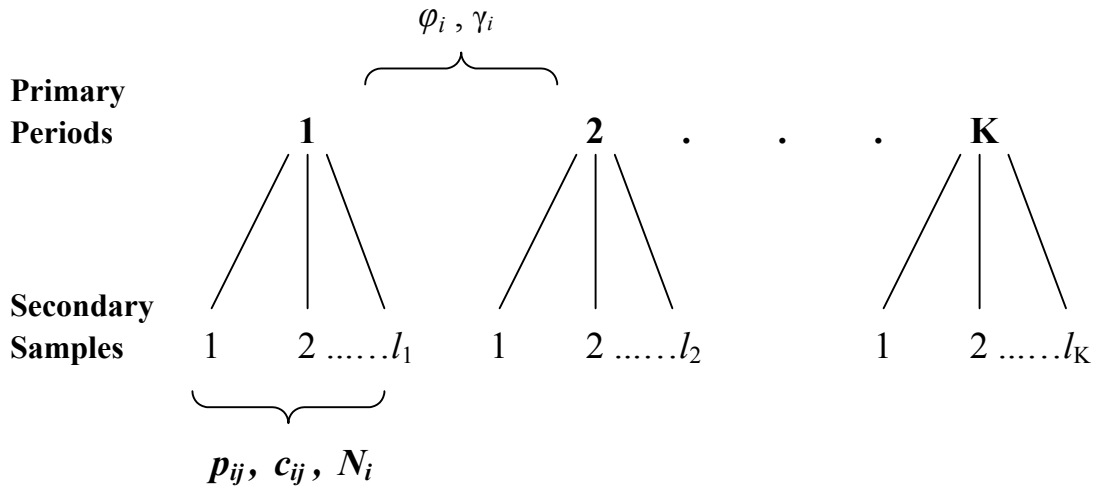


Figure 1. Pollock's robust design for a k -period study, each primary period i contains l_i closely-spaced secondary samples. Conditional capture probability, p_{ij} , recapture probability, c_{ij} , and surface population size, N_i , are estimated over secondary samples using closed-population models. Survival, φ_i , and temporary emigration rates, γ_i , are estimated between primary periods using open-population models (e.g. Jolly-Seber). Our salamander study contained capture-recapture data from 14 sites in 1999 and 19 sites in 2000 and 2001 (52 site-years). All site-years contained 4 primary periods each with 3-4 secondary samples (consecutive sampling days).

Table 1. Breakdown of the number of individual salamanders captured (>18-mm SVL) on 15 x 15-m plots in Great Smoky Mountains National Park. n plots were sampled 14 - 16 times per year.

Year	n	Mark-Recapture	Depletion		Complete Removal ^a		Total
		Caught	Unmarked	Marked	Unmarked	Marked	Caught
1999	14	1417	375	122	172	34	1964
2000	19	2137	334	189	-	-	2471
2001	19	1779	346	194	-	-	2125

^a Complete removals were only performed at 3 plots in 1999.

TABLE 2. Percentage of the data sets for which different closed-population models were selected by program CAPTURE. n = number of data sets (sites).

Year	n	Models							
		M_o	M_h	M_b	M_{bh}	M_t	M_{th}	M_{tb}	M_{tth}
1999	14	28.6	14.3	7.1	0.0	0.0	7.1	7.1	35.7
2000	19	10.5	5.3	5.3	0.0	26.3	5.3	26.3	21.0
2001	19	5.3	0.0	5.3	5.3	5.3	15.8	42.1	21.0
Total	52	13.5	5.8	5.8	1.9	11.5	9.6	26.9	25.0

TABLE 3. Percentage of the data sets for which different closed-population models were considered appropriate by AIC_c values (calculated in program MARK) and by model selection procedures in program CAPTURE. n = number of models considered appropriate for data sets in each year.

Year	MARK model selection					CAPTURE model selection				
	n	M_o	M_b	M_t	M_{tb}	n	M_o	M_b	M_t	M_{tb}
1999	23	17.4	34.8	39.1	8.7	14	71.4	7.14	0.0	21.4
2000	28	10.7	25.0	32.1	32.1	19	26.3	5.3	36.8	31.6
2001	26	15.4	30.8	26.9	26.9	19	26.3	5.3	15.8	52.6
Total	77	14.3	29.9	32.5	23.4	52	38.5	5.8	19.2	36.5

TABLE 4. Example of population size estimates for one site, CG008, in 1999. Closed-population model M_{tbb} was the 'best' model selected by program CAPTURE. Super-population estimates include both surface and subterranean salamander populations. Robust and closed-population models on the same row make the same capture probability assumptions. Values in parentheses are 'superpopulation' estimates using corresponding robust-design models (see Bailey this thesis, Chapter 1 for details). See text for model details. This site is a disturbed, mixed deciduous site.

Model	Surface Population		Model	Super Population	
	\hat{N}	SE \hat{N}		\hat{N}	SE \hat{N}
<u>Robust</u>			<u>Closed</u>		
$\varphi(\cdot), p(\cdot)=c(\cdot), N(\cdot)$	264 (264)	60.05	M_o	341	44.20
$\varphi(\cdot), p(\cdot), c(\cdot), N(\cdot)^*$	65 (260)	8.07	M_b	183	16.48
$\varphi(\cdot), p(i.)=c(i.), N(\cdot)$	264 (264)	60.25	M_t	337	43.22
<u>Depletion</u>			<u>Open</u>		
M_b	41	6.92	B	245	159.25
M_{bh}	33	4.73	D	261	128.83
<u>4 Secondary Periods</u>			<u>Lincoln-Peterson</u>	291	65.60
	- : -	- : -			
$M_b : M_h$	59 : 130	4.96 : 3.96	Day Removal ^b	27	
	46 : 86	7.39 : 11.25	Complete Removal ^c	123	
	70 : 83	41.09 : 10.94	Total Captured ^d	237	

* Robust design model with the lowest AIC values

^a For these models the data were too sparse and yielded nonsensical estimates

^b Number of individuals captured while removing all leaf litter and cover from the plot

^c Total number of individuals captured depletion and complete removal studies

^d Total number of individuals captured during mark-recapture, depletion, and complete removal studies

TABLE 5. Example of population size estimates for one site, CG009, in 1999. Closed-population model M_h was the 'best' model selected by program CAPTURE. Super-population estimates include both surface and subterranean salamander populations. Robust and closed-population models on the same row make the same capture probability assumptions. Values in parentheses are 'superpopulation' estimates using corresponding robust-design models (see Bailey this thesis, Chapter 1 for details). See text for model details. This site is a disturbed, mixed pine site.

Model	<u>Surface Population</u>		Model	<u>Super Population</u>	
	\hat{N}	SE \hat{N}		\hat{N}	SE \hat{N}
<u>Robust</u>			<u>Closed</u>		
$\varphi(\cdot), p(\cdot)=c(\cdot), N(\cdot)$	92 (188)	20.59	M_o	228	25.35
$\varphi(\cdot), p(\cdot), c(\cdot), N(\cdot)$	43 (157)	11.97	M_b	^a not est.	^a not est.
$\varphi(\cdot), p(i)=c(i), N(\cdot)^*$	112 (214)	26.15	M_t	225	24.70
<u>Depletion</u>			<u>Open</u>		
M_b	57	10.39	B	262	175.19
M_{bh}	56	33.83	D	280	196.82
<u>4 Secondary Periods</u>			<u>Lincoln-Peterson</u>	419	121.54
	- : -	- : -			
$M_b : M_h$	106 : 101	4.96 : 3.96	Day Removal ^b	11	
	40 : 81	7.39 : 11.25	Complete Removal ^c	77	
	- : 104	41.09 : 10.94	Total Captured ^d	179	

* Robust design model with the lowest AIC values

^a For these models the data were too sparse and yielded nonsensical estimates

^b Number of individuals captured while removing all leaf litter and cover from the plot

^c Total number of individuals captured depletion and complete removal studies

^d Total number of individuals captured during mark-recapture, depletion, and complete removal studies

TABLE 6. Example of population size estimates for one site, RG012, in 1999. Closed-population model M_o was the 'best' model selected by program CAPTURE. Super-population estimates include both surface and subterranean salamander populations. Robust and closed-population models on the same row make the same capture probability assumptions. Values in parentheses are 'superpopulation' estimates using corresponding robust-design models (see Bailey this thesis, Chapter 1 for details). See text for model details. This site is an undisturbed, mixed deciduous site.

Model	<u>Surface Population</u>		Model	<u>Super Population</u>	
	\hat{N}	SE \hat{N}		\hat{N}	SE \hat{N}
<u>Robust</u>			<u>Closed</u>		
$\varphi(\cdot), p(\cdot)=c(\cdot), N(\cdot)^*$	412 (839)	226.64	M_o	787	262.66
$\varphi(\cdot), p(\cdot), c(\cdot), N(\cdot)$	353 (842)	1180.05	M_b	281	145.08
$\varphi(\cdot), p(i.)=c(i.), N(\cdot)$	2435(769)	240.76	M_t	780	258.52
<u>Depletion</u>			<u>Open</u>		
M_b	31	3.46	B	729	1326.44
M_{bh}	29	1.70	D	763	1057.13
<u>4 Secondary Periods</u>			<u>Lincoln-Peterson</u>	463	150.40
	153 : 89	299.80:11.45			
$M_b : M_h$	- : -	- : -	Day Removal ^b	31	
	109 : 73	189.49:10.41	Complete Removal ^c	93	
	- : -	- : -	Total Captured ^d	192	

* Robust design model with the lowest AIC values

^a For these models the data were too sparse and yielded nonsensical estimates

^b Number of individuals captured while removing all leaf litter and cover from the plot

^c Total number of individuals captured depletion and complete removal studies

^d Total number of individuals captured during mark-recapture, depletion, and complete removal studies