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## EFFICACY AND BIASES OF COVER OBJECT SURVEY DESIGN FOR SAMPLING EASTERN RED-BACKED SALAMANDERS (*PLETHODON CINEREUS*) AT FOREST EDGE AND INTERIOR LOCATIONS

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**Abstract.**—Terrestrial salamanders are adapted to moist, cool microenvironments that facilitate cutaneous respiration and decrease risk of desiccation. Warmer, drier microenvironments may induce habitat use changes by salamanders to alleviate stressful microenvironmental conditions. Changes in salamander habitat use may bias population metrics when sampling occurs in areas with different microenvironmental conditions. The objective of this study was to determine whether *Plethodon cinereus* (Eastern Red-backed Salamander) exhibit surface cover object refugia preferences or occupancy rate differences at sampling locations with different microenvironmental conditions and with respect to sampling day of year. We assessed *P. cinereus* occupancy rates and preference of surface cover refugia using artificial and natural cover objects in two sampling locations: forests along rights-of-way (EDGEFOR) and interior forests (INTFOR). *Plethodon cinereus* showed no preference for cover object type (coverboards, logs, and rocks) in either EDGEFOR or INTFOR sampling plots. Occupancy rates were greater under cover objects in INTFOR plots than EDGEFOR plots. Occupancy rates increased with increasing cover object width and decreased with day of year (spring-late summer) irrespective of cover object type or sampling location. Our study suggests that incorporating multiple cover object types into study designs will not incur bias resulting from preference of *P. cinereus* for cover objects.

**Key Words.**—central Appalachian forests; forest edge; interior forest; plethodontid salamanders; surface cover sampling

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### INTRODUCTION

Robust and unbiased sampling methods are important for assessing wildlife populations over space and time (Yoccoz et al. 2001; Hesed 2012). Sampling methods that successfully exploit species life-history traits may improve estimates of population size and health (Vesely et al. 2006). Artificial or natural cover object surveys are a useful method to sample terrestrial salamander populations because they concentrate survey effort on optimal microhabitats during daylight hours (DeGraaf and Yamasaki 1992). This sampling approach also allows for relaxed sampling designs (as opposed to pitfall traps), allows sampling to be conducted during low-movement periods (i.e., daytime), and is a useful approach in estimating population and community metrics (Grant et al. 1992; Monti et al. 2000). Because of the ubiquitous use of cover object surveys for sampling salamander populations, studies have investigated effects of cover object type (Marsh and Goicochea 2003; Caruso 2016), cover object size (Strojny and Hunter Jr.

2009), moisture regimes (Grover 1998; Grover 2000), and prior salamander presence under objects (Mathis 1990; Gabor 1995) on salamander preference for and use of cover objects. The relationships among these factors and their effects on salamander behavior are dynamic and can intensify as resources become limited or temperature and moisture levels fluctuate during the spring-summer-autumn periods (Mathis 1990).

Behavioral coping mechanisms in terrestrial salamanders can be brought on by changes to their surrounding environment. During dry periods, terrestrial salamanders are at risk of desiccation due to their physiological constraints (e.g., thin, vascular skin; Hillman et al. 2009). Therefore, terrestrial salamanders will seek refuge in leaf litter or under coarse woody debris (CWD) where conditions are better suited to their physiology (i.e., damp and moist) and their risk of desiccation is reduced (Mathis 1990; Grover 2000). This behavior allows salamanders to avoid desiccation while remaining on the surface where they predominantly forage and reproduce (Jaeger 1980; Homayack et al.

2011). As interior forests transition to forest edges, ambient air temperatures increase and humidity and soil moisture decrease due to greater solar radiation reaching the forest floor, which creates a temperature and moisture gradient perpendicular to the forest edge (Matlack 1993; Chen et al. 1995). Furthermore, edge habitats are subject to greater daily microclimate variability than interior habitats (Hofmeister et al. 2019). Drier and warmer conditions at forest edges increase the likelihood of desiccation and decrease food resources (e.g., leaf litter arthropods; van Wilgenburg et al. 2001). These factors result in interrupted movements (Gibbs 1998; Rittenhouse and Semlitsch 2006) and reduced abundances of salamanders at forest edges (DeGraaf and Yamasaki 2002; Semlitsch et al. 2007). Salamanders inhabiting forest interiors occupy wetter, cooler habitats than those inhabiting forest edges (Didham and Lawton 1999), which likely reflects differences in temperature and moisture. Sampling strategies that account for these environmentally driven mechanisms in salamanders presumably would reduce sampling bias when sampling across heterogeneous landscapes.

We assessed whether the terrestrial salamander *Plethodon cinereus* (Eastern Red-backed Salamander) used cover object types proportional to their availability at two different sampling locations relative to forest edge: in forests along rights-of-way (forest edge) and in interior forests ( $\geq 170$  m from forest edge). We posit that *P. cinereus* will use cover objects with greater moisture retention (logs and coverboards) near forest edges while cover object preferences will be proportional to cover object availability in interior forest locations. Further, we expect that these relationships will intensify over the sampling period due to increased daily temperatures, and thus increased risk of desiccation.

#### MATERIALS AND METHODS

**Study site.**—We conducted this study at two West Virginia Division of Natural Resources (WVDNR) wildlife management areas (WMA): Beury Mountain (BMWMA; 17N 0500607, 4198629) and Lewis Wetzel (LWWMA; 17N 0529291, 4374421) in West Virginia, USA (Fig. 1). West Virginia, USA, lies within the central Appalachian region, which is characterized by rugged topography and predominantly forested land cover. Both WMAs are primarily mature forest. BMWMA lies within the transition Appalachian Oak (*Quercus* spp.)–Northern Hardwood Forest type (Küchler 1964), which consists of Mixed Hardwood and Oak-Hickory (*Carya* spp.) forests. LWWMA lies within the Appalachian Oak forest type (Küchler 1964) and is dominated by Oak-Hickory and Cove Hardwoods. Elevation at BMWMA ranges from 610–1,108 m with gentle to steep slopes, while LWWMA elevation ranges from 224–475 m



FIGURE 1. Study sites (stars), Beury Mountain Wildlife Management Area (BMWMA) and Lewis Wetzel Wildlife Management Area (LWWMA), used for assessing cover object preference of *Plethodon cinereus* (Eastern Red-backed Salamander) in West Virginia, USA.

with moderate to steep slopes. The humid continental climate of West Virginia is characterized by warm summers and cool to cold winters. Annual spring and fall temperatures at BMWMA and LWWMA average  $11^{\circ}\text{C}$  and summer temperatures average  $21^{\circ}\text{C}$ . Annual precipitation levels at BMWMA and LWWMA average 114 cm, with precipitation distributed evenly throughout the year but heaviest during June–August (<https://w2.weather.gov/climate/xmacis.php?wfo=rlx>).

**Study design.**—We conducted sampling at two locations relative to forest edge: within forests along edges of rights-of-way (e.g., underground gas pipelines and overhead utility lines; hereafter EDGEFOR) and within interior forest ( $\geq 170$  m from forest edge; hereafter INTFOR). We chose these sampling locations to investigate potential differences in surface cover object selection by *P. cinereus* in relation to forest edge proximity (edge forest and interior forest). Across the two WMAs, we placed 32 sampling plots within forests 15 m from the edge, and 35 sampling plots in INTFOR.

Each sampling plot in EDGEFOR and INTFOR locations consisted of a coverboard array (hereafter coverboard) surrounded by a 5-m radius natural cover object plot. Coverboard arrays consisted of nine individual coverboards placed in a  $3 \times 3$  arrangement with each individual coverboard spaced about 10 mm apart. Coverboards were  $200 \times 100 \times 25$  mm in size and made from hardwood species of the region: American Basswood (*Tilia americana*), American Sycamore (*Platanus occidentalis*), and Yellow Poplar (*Liriodendron tulipifera*). Within a 5-m radius surrounding coverboards at each plot center, we identified any natural cover object (i.e., logs and rocks)

that measured  $\geq 40$  mm at their narrowest footprint dimension (i.e., part of the object in direct contact with ground) and we marked each object with a stake flag for consistent sampling effort across survey visits and years. We excluded natural objects that could be turned over and surveyed but not returned to their pre-disturbed position on the ground.

We included canopy cover as a variable of interest for this study because it is a primary driver of microclimate changes at the forest floor (Brooks and Kyker-Snowman 2008) and hence a primary driver of salamander abundance/presence (deMaynadier and Hunter Jr. 1995; Russell et al. 2004). Canopy manipulation for a concurrent study occurred in 16 of the 32 EDGEFOR plots (via tree cutting) and 17 of the 35 INTFOR plots (tree death via stem injection of 6% solution of Arsenal®; main ingredient Imazapyr, 28.7%). We measured post-treatment canopy cover in each plot using a  $25 \times 25$  cm transparent plexiglass panel, divided into a  $5 \times 5$  grid held overhead (Haché et al. 2013). We used a Welch's *t*-test to test for differences in canopy cover levels between EDGEFOR and INTFOR plots.

We included topographic relative moisture index (TRMI; Parker 1982) as a variable of interest for this study because it measures solar radiation exposure based on topography, similar to Beers aspect (Beers et al. 1966) or heat load index (McCune and Keon 2002). Topographic relative moisture index (TRMI) combines slope percentage, slope aspect, slope position, and slope configuration to derive an index ranging from 0 (xeric soil) to 60 (mesic soil). We used digital elevation models (DEMs) to measure TRMI at each sampling plot. We used a Welch's *t*-test to test for differences in TRMI between EDGEFOR and INTFOR plots.

**Salamander sampling.**—At each sampling plot, we conducted three annual survey visits during 2018–2019; visit 1 was 15–30 April, visit 2 was 7–16 May, and visit 3 was 22 July to 9 August each year. During each visit, we turned all coverboards and natural cover objects, which we then returned to their original position for future sampling. After capturing a salamander, we recorded species and cover object class (coverboard or natural cover object). If captured under a natural cover object, we recorded object footprint dimensions (length and width) and type (log or rock). We clipped the tail of each captured individual to identify subsequent recaptures. We sprayed clipped tails with an antiseptic to minimize potential for infection, and sterilized clippers with 95% ethanol and an open flame to minimize potential for disease transmission.

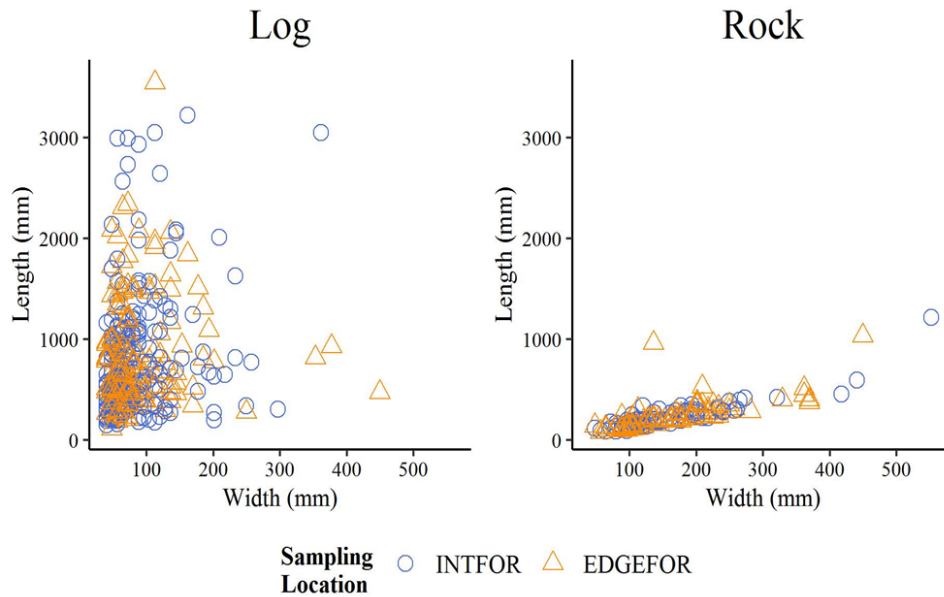
**Statistical analyses.**—For all statistical analyses, we used data across BMWMA and LWWMA study sites because *P. cinereus* was the dominant species

captured at both study sites, study sites were subjected to similar environmental conditions (i.e., temperature and precipitation regimes), and using both BMWMA and LWWMA allowed for a more balanced sampling design between EDGEFOR and INTFOR plots. Additionally, we included study site as a covariate in Generalized Linear Mixed Models (GLMMs) to account for differences between sites. We restricted analyses to *P. cinereus* captures, which comprised 81% of all salamander captures in our study. We excluded recaptured salamanders from all analyses. We assessed possible nuisance variable effects of canopy cover and TRMI on *P. cinereus* presence using Logistic Regression. Canopy cover ( $Z = 1.760$ ,  $P = 0.079$ ) and TRMI ( $Z = 3.345$ ,  $P < 0.001$ ) were significant predictors of *P. cinereus* presence at  $\alpha = 0.10$ . Therefore, we included both canopy cover and TRMI as nuisance variables in analysis where applicable (i.e., in GLMMs).

We used a Multinomial Goodness-of-Fit test to determine whether observed capture frequency followed expected capture frequency based on the distribution of cover objects (coverboards, logs, and rocks; Cressie and Read 1984). We ran analyses of EDGEFOR and INTFOR plot data separately because we were interested in whether cover object use by *P. cinereus* was proportional to availability within each sampling location. Canopy cover and TRMI were not included as nuisance variables in this analysis.

We used a GLMM to test whether naïve occupancy (hereafter occupancy) varied among cover types (coverboard, log, and rock) and sampling locations (EDGEFOR and INTFOR). The response variable, presence/absence, was specified with a binomial distribution. We included cover type and sampling location as main effect predictor variables and cover type-sampling location as an interactive predictor variable in the model. Canopy cover and TRMI were included as nuisance variables and sampling block (i.e., groupings of sampling plots based on location within BMWMA and LWWMA) was included as a random effect. Canopy cover and TRMI were standardized to have means of 0 and standard deviations of one prior to analysis.

We tested for relationships between *P. cinereus* occupancy and log and rock footprint width in EDGEFOR and INTFOR plots, separately, using a GLMM. Our response variable, presence/absence, was specified with a binomial distribution. Object width and the interaction between object width and object type-sampling location combination (log-EDGEFOR, log-INTFOR, rock-EDGEFOR, and rock-INTFOR) were our predictor variables. We included canopy cover and TRMI as nuisance variables and sampling block as a random effect. We standardized object width, canopy cover, and TRMI to have means of 0



**FIGURE 2.** Scatter-plots of untransformed dimensions (mm) of logs (left) and rocks (right) measured in edge forest (EDGEFOR) and interior forest plots (INTFOR). Both scatter plots have the same axis scales, with object width on the x-axis and object length on the y-axis. Coverboard dimensions were 200 mm (length) by 100 mm (width).

and standard deviations of one prior to analysis. We did not include coverboard data in this analysis because width of coverboards was constant. We combined data across visits and years for testing occupancy across cover object types, sampling locations, and log and rock widths.

We assessed the effect of Ordinal day of year (hereafter DOY) on occupancy rates of *P. cinereus* using a GLMM, where our response variable, presence/absence, was specified with a binomial distribution. We included DOY and the interaction between DOY and object type-sampling location as our predictor variables and canopy cover and TRMI as nuisance variables. We included study site (BMWMA and LWWMA) as a random effect because our model would not converge when sampling block (used for prior analyses) was included as a random effect. Capture data were split by year because exact DOY for survey visits varied between years. We standardized canopy cover, DOY, and TRMI to have means of 0 and standard deviations of one prior to analysis. We used data from coverboards, logs, and rocks for analysis. We ran all statistical tests using  $\alpha = 0.10$  as our threshold for significance. A less restrictive alpha is justified to minimize Type II errors, which is more important than minimizing Type I errors when examining factors associated with population viability (Duguay and Wood 2002; Wood and Williams 2013). We used the package lme4 (Bates et al. 2015) in the statistical software R (R Core Team 2019) for all GLMM analyses.

## RESULTS

We measured 371 logs (155 in EDGEFOR, 216 in INTFOR) and 160 rocks (69 in EDGEFOR, 92 in INTFOR) across 67 sampling plots (32 in EDGEFOR, 35 in INTFOR) over the study period. Log width ranged from 40–449 mm (mean  $\pm$  standard error =  $88.2 \pm 4.7$  mm) and rock width ranged from 48–449 mm (mean =  $170.7 \pm 10.2$  mm) in EDGEFOR plots. Log width ranged from 40–361 mm (mean =  $85.6 \pm 3.3$  mm) and rock width ranged from 48–552 mm (mean =  $153.7 \pm 8.5$  mm) in INTFOR plots (Fig. 2). Canopy cover in EDGEFOR plots ranged from 66–100% (mean =  $89.3 \pm 2.0\%$ ) and in INTFOR plots ranged from 74–100% (mean =  $93.0 \pm 1.3\%$ ). Canopy cover between EDGEFOR and INTFOR plots did not differ significantly ( $t = -1.595$ ,  $df = 53.3$ ,  $P = 0.117$ ). In EDGEFOR plots, TRMI ranged 9–32 (mean =  $21.2 \pm 1.3$ ) and in INTFOR plots ranged 19–28 (mean =  $23.0 \pm 0.5$ ). Topographic relative moisture index between EDGEFOR and INTFOR plots also did not differ significantly ( $t = -1.309$ ,  $df = 41.2$ ,  $P = 0.198$ ).

We captured 55 unique *P. cinereus* in EDGEFOR plots and 138 *P. cinereus* in INTFOR plots (Table 1). Observed capture frequency of coverboards, logs, and rocks in EDGEFOR or INTFOR plots did not significantly differ from expected capture frequency (EDGEFOR:  $\chi^2 = 3.658$ ,  $df = 2$ ,  $P = 0.161$ ; INTFOR:  $\chi^2 = 2.564$ ,  $df = 2$ ,  $P = 0.277$ ). Occupancy rates of *P. cinereus* were significantly higher in the INTFOR plots



**TABLE 1.** Contingency table showing number of cover objects sampled and number of *Plethodon cinereus* (Eastern Red-backed Salamander) captured for each cover object type in edge forest (EDGEFOR) and interior forest (INTFOR) sampling locations in West Virginia, USA, over six visits during 2018–2019. Values in parentheses indicate proportion of total cover objects or captures within each respective sampling location. Recaptures are not included. Abbreviations NCO = number of cover objects and NSC = number of salamander captures.

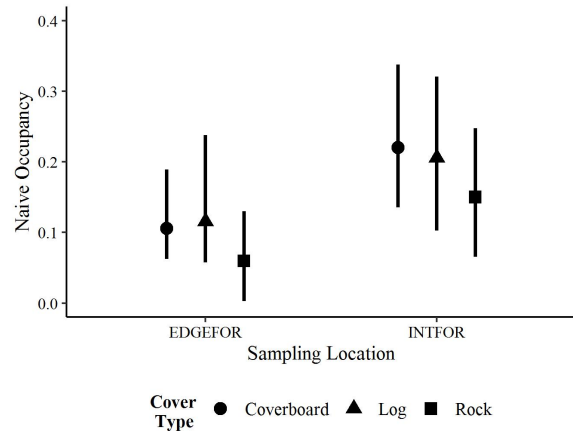
|     | INTFOR        |               |              | EDGEFOR       |               |              |
|-----|---------------|---------------|--------------|---------------|---------------|--------------|
|     | Coverboard    | Log           | Rock         | Coverboard    | Log           | Rock         |
| NCO | 315<br>(0.51) | 216<br>(0.34) | 92<br>(0.15) | 288<br>(0.56) | 155<br>(0.30) | 69<br>(0.14) |
| NSC | 75<br>(0.54)  | 49<br>(0.36)  | 14<br>(0.10) | 32<br>(0.58)  | 20<br>(0.36)  | 3<br>(0.06)  |

(mean = 0.22 ± 0.02) than EDGEFOR plots (mean = 0.11 ± 0.01;  $F_{1,1128} = 3.784, P = 0.052$ ). Occupancy rates were not significantly different between object types ( $F_{2,1128} = 1.441, P = 0.237$ ). Mean occupancy rate was highest under logs (mean = 0.19 ± 0.02), intermediate under coverboards (mean = 0.18 ± 0.02), and lowest under rocks (mean = 0.11 ± 0.02). Finally, the interaction between sampling location and object type was not significant ( $F_{2,1128} = 0.167, P = 0.846$ ; Fig. 3).

Mean occupancy of *P. cinereus* increased with increasing object width for all object type-sampling location combinations (slope coefficients: log-EDGEFOR = 0.03, log-INTFOR = 0.19, rock-EDGEFOR = 0.18, rock-INTFOR = 0.25). There was substantial variance, however, and the effect of object width was not significant ( $F_{1,523} = 1.566, P = 0.211$ ). The interaction between object width and object type within sampling location also was not significant ( $F_{3,523} = 0.059, P = 0.981$ ). Occupancy of *P. cinereus* decreased with increasing DOY for all object type-sampling location combinations (slope coefficients: coverboard-EDGEFOR = -1.18, coverboard-INTFOR = -1.53, log-EDGEFOR = -0.90, log-INTFOR = -0.64, rock-EDGEFOR = -11.34, rock-INTFOR = -0.70; Fig. 4). There was a significant DOY effect on occupancy ( $F_{5,6765} = 48.103, P < 0.001$ ) but not a significant interaction between DOY and object type within sampling location on occupancy ( $F_{5,6765} = 1.717, P = 0.127$ ).

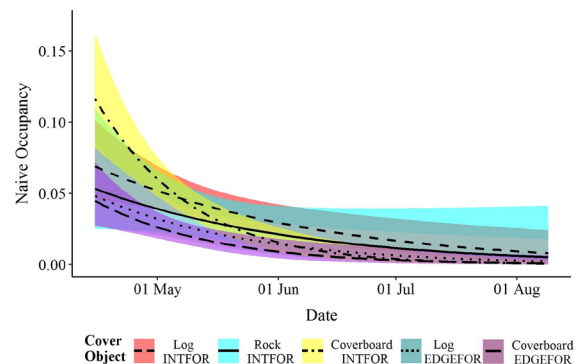
**DISCUSSION**

Type of cover object used by *P. cinereus* was proportional to availability in forest edges (EDGEFOR) and in forest interiors (INTFOR). We captured the majority of individuals under coverboards in both EDGEFOR and INTFOR (54% and 58% of total captures, respectively). However, *P. cinereus* did not show a strong preference for coverboards as observed capture frequency followed expected capture



**FIGURE 3.** Predicted mean naïve occupancy and 95% confidence intervals of *Plethodon cinereus* (Eastern Red-backed Salamander) for cover object types (coverboards, logs, and rocks) in each sampling location (edge forest [EDGEFOR] and interior forest plots [INTFOR]) over six visits during 2018–2019.

frequency. Forest interior microclimates (e.g., wetter, cooler) are generally compatible with the physiology of terrestrial salamanders (Homyack et al. 2011). Therefore, individuals in forest interiors may select surface refugia based on availability, local cues (e.g., food availability, presence of conspecifics), or other factors. We posited that *P. cinereus* would prefer either coverboards or logs in EDGEFOR plots, as these objects have greater moisture retention capacity than rocks (Harmon et al. 1986; Grover 2000) and would likely buffer salamanders more effectively than rocks from drier and warmer microclimate conditions along forest edges (Davies-Colley et al. 2000). We did not observe this, indicating moisture or humidity may not be



**FIGURE 4.** Predicted mean naïve occupancy and 95% confidence intervals of *Plethodon cinereus* (Eastern Red-backed Salamander) for cover object type (coverboards, logs, and rocks) and sampling location (edge forest [EDGEFOR] and interior forest plots [INTFOR]) combinations as a function of date over six visits during 2018–2019. Ordinal day of year (DOY) was used in analysis, but calendar date is substituted for better reference. Sampling occurred between 15 April and 9 August in both years. Lack of capture data for rocks in EDGEFOR plots resulted in limited predictive ability and is not included in graph.

an important limiting factor in EDGEFOR. Moreover, *P. cinereus* in EDGEFOR plots may select surface refugia based on similar cues as in INTFOR plots (e.g., cover object availability, food availability, presence of conspecifics). Although cover use was proportional to availability in both EDGEFOR and INTFOR plots, observed occupancy was twice as high in INTFOR as EDGEFOR plots for all cover types combined.

Although occupancy rates did not significantly increase with increasing width of logs or rocks in EDGEFOR and INTFOR sampling plots, the model did indicate positive relationships for all combinations based on slope coefficients. Intuitively, this makes sense considering greater surface area buffers against predators and adverse environmental conditions and *P. cinereus* exhibits stronger territorial behavior near larger objects relative to smaller objects (Mathis 1990). We observed, however, salamanders under objects as small as 40 mm in footprint width, the minimum used in our study. This is well below the minimum 150 mm log diameter that Strojny and Hunter Jr. (2009) detected *P. cinereus* under (they searched all logs  $\geq 100$  mm in diameter). Most studies that implement natural cover object sampling do not specify a size criterion used when sampling, preventing comparisons and inferences between studies. Of the 86 *P. cinereus* we captured under logs and rocks, 48 (56%) were under objects  $\leq 100$  mm wide. The probability of a salamander occupying an object increases with increasing cover object size, but a substantial portion of the population may go unsampled if small cover objects are not searched. This could be particularly important in areas with fewer available cover objects (Marvin 1998), where small or juvenile salamanders are possibly relegated to smaller cover items (Mathis 1990).

Occupancy rates declined from the start of sampling in mid-April to the end of sampling in early-August, but significant decreases were not specific to any object type-sampling location combination. Sampling during the spring likely increases the effectiveness of population assessments inferred from data compared to summer sampling. As wet, cool spring conditions transitioned into dry, hot summer conditions and salamanders presumably moved below the surface, occupancy rates decreased for all object types in EDGEFOR and INTFOR plots. Our reduced effectiveness of *P. cinereus* population surveys during the warmest months agrees with previous studies (Grant et al. 1992).

The results of our study indicate use of cover object types by *P. cinereus* was proportional to their availability, but use appears to be somewhat influenced by cover object size and surface availability showed strong seasonal variation. This suggests that sampling refugia (e.g., coverboards, logs, rocks) do not incur bias in relation to *P. cinereus* preferences for surface

cover across different sampling locations; however, sampling a broad size range of logs and rocks should help elucidate population size. Doing so will increase inferential ability regarding *P. cinereus* responses to environmental conditions, management activities, or other conservation priorities.

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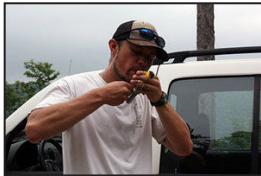
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