Running Head: Biagas et al.—Salamander visual encounter surveys

TIME OF DAY DOES NOT AFFECT DETECTION IN VISUAL ENCOUNTER SURVEYS OF A SPRING-DWELLING SALAMANDER, *EURYCEA NAUFRAGIA*

TIFFANY D. BIAGAS, ALEXANDER S. HALL, ALEXIS L. RITZER,
AND BENJAMIN A. PIERCE*

Department of Biology, Southwestern University, Georgetown, Texas 78626, USA

* Correspondent: pierceb@southwestern.edu

Key Words: salamanders, *Eurycea, Eurycea naufragia,* visual encounter surveys, methodology, time of day

This paper is currently under review for publication in the *Southwestern Naturalist*.

ABSTRACT—Aquatic salamanders are important components of many spring and headwater stream ecosystems, and determining density and abundance of these animals is an integral part of many research and monitoring projects. Visual encounter surveys are commonly used for assessing aquatic salamanders, yet few studies have examined the influence of environmental factors on detection in this method. We studied the influence of time of sampling and other environmental variables on number of salamanders observed during daytime visual encounter surveys of the Georgetown Salamander (*Eurycea naufragia*), an aquatic species of special conservation concern. Salamander visual encounter surveys were conducted at a spring pool over a ten-week period. One morning and one afternoon survey, separated by approximately 48 h, were conducted weekly. Water temperature, dissolved oxygen concentration, specific conductivity, water depth, and percent cloud cover were recorded during each survey. No significant differences were found between the number of salamanders observed during morning and afternoon visual encounter surveys. Similarly, no differences were observed between percent of cover objects with salamanders during morning and afternoon surveys. The number of salamanders detected and the percent of cover objects with salamanders were not significantly correlated with any of the environmental variables measured. Based on our results, we suggest that visual encounter surveys conducted during daylight hours are not biased by sampling at different times of day.

RESUMEN— Las salamandras acuáticas son un componente importante de los ecosistemas de muchos manantiales y riachuelos, por lo que determinar la densidad y abundancia de estos animales es una parte integral de muchos proyectos de investigación y de supervisión. Comúnmente se utilizan encuestas sobre encuentro visual para evaluar las salamandras acuáticas, sin embargo, pocos estudios han examinado cómo los factores ambientales afectan la detección de las mismas al utilizar esta técnica. En este estudio, investigamos la influencia del tiempo de muestras y otras variables ambientales en el número de salamandras observadas en encuestas de encuentro visual durante el día de salamandras de Georgetown (*Eurycea naufragia*), una especie acuática de alto interés de conservación. Las encuestas de encuentro visual de la salamandra fueron realizadas en un manantial durante un periodo de diez semanas. Semanalmente, se realizaron encuestas matutinas y vespertinas separadas por 48 horas aproximadamente. Durante cada encuesta, se registraron la temperatura del agua, la concentración de oxígeno disuelto, la conductividad específica, la profundidad del agua y el porciento de neblina. No se encontraron diferencias significativas entre el número de salamandras observadas durante las encuestas de la mañana y de la tarde. Asimismo, no se observaron diferencias entre el porciento de objetos de cobertura junto a las salamandras en las encuestas de la mañana y de la tarde. El número de salamandras detectado y el porciento de objetos de cobertura junto a ellas no están significativamente correlacionados con ninguna de las variables ambientales medidas. Basado en los resultados, sugerimos que las encuestas de encuentro visual realizadas durante las horas diurnas no están inducidas por muestreo en diferentes momentos del día.

Salamanders are important components of many spring and head-water stream communities. Although frequently understudied, they are often the most abundant vertebrates in these habitats (Burton and Likens, 1975; Peterman et al., 2008), playing significant roles as both predators and prey. Stream and spring salamanders are also important indicators of ecosystem health (Welsh and Ollivier, 1998; but see also Kerby et al. 2010). For example, a number of studies have demonstrated that these animals are sensitive to effects of urbanization (Orser and Shure, 1972; Grant et al., 2009; Willson and Dorcas, 2003; Bowles et al., 2006; Miller et al., 2007; Barrett et al., 2010), road construction (Welsh and Ollivier, 1998; Ward et al., 2008), and timber harvest (Corn and Bury, 1989; Lowe and Bolger, 2002). Furthermore, many stream and spring salamanders are of conservation concern (Chippindale and Price, 2005). For all of these reasons, stream and spring salamanders are often included in watershed assessment programs and are frequently the focus of research and monitoring projects (e.g., Jung et al., 2000).

Different techniques have been used to assess richness and abundance of stream and spring salamanders, including dip netting (Nowakowski and Maerz, 2009), quadrat sampling (Jung et al., 2000), electroshocking (Sepulveda and Lowe, 2009), funnel trapping (Griffiths, 1985; Willson and Dorcas, 2004), sampling with leaf litter bags (Chalmers and Droege, 2002; Waldron et al., 2003), and visual encounter surveys (Crump and Scott, 1994; Jung et al., 2000). Visual encounter surveys have become widely employed because they require little equipment, have low impacts on target species, and can be used with many species and a range of habitats. In this technique, one or more researchers visually detects and records the presence of salamanders, often with active searching of potential cover objects in the stream (Barr and Babbitt, 2001; Quinn et al., 2007; Marsh, 2009).

Previous studies have examined the accuracy and precision of different visual encounter survey methods and/or compared visual encounter surveys to other survey methods (Barr and Babbitt, 2001; Jung et al., 2000; Quinn et al., 2007; Marsh, 2009; Mackey et al., 2010). A few studies investigated the effects of environmental and sampling variables, such as season (Orser and Shure, 1975) and multiple observers (Marsh, 2009) on the results of visual encounter surveys. A criticism of traditional visual encounter surveys is that they often fail to account for variation in detection probability (Mackenzie et al., 2006; Mazerolle et al., 2007). One potential source of variation in detection probability is the time of day in which the survey is conducted. Some stream salamanders are nocturnally active (Orser and Shure, 1975; Petranka, 1984), but for logistical reasons including improved visibility, visual encounter surveys are often conducted during daylight hours. If species are nocturnally active, surveys conducted during morning hours might yield more observations than surveys conducted in late afternoon. In this study, we investigated the effect of time of day and several environmental variables on the number of salamanders observed during visual encounter surveys of the Georgetown Salamander, *Eurycea naufragia*, a threatened species endemic to the San Gabriel river drainage in central Texas (Chippindale et al., 2000; Pierce et al., 2010).

MATERIALS AND METHODS—Weekly visual encounter surveys were conducted at a permanent spring on the North San Gabriel River fed by the Edwards Aquifer in Williamson County, Texas. The area sampled was always the same and consisted of the first 5 m of the spring run, a well-delineated rectangular area consisting of approximately 30 m^2 of wetted surface area. Previous research (Pierce et al., 2010) indicated that the majority of salamanders at this site occur within this segment of the spring run. The area sampled contained riffles and pools; the substrate was varied, consisting largely of silt, gravel, and large limestone rocks.

We conducted a total of ten weeks of surveys between 16 September and 20 November, 2009. One morning and one afternoon survey were conducted each survey week. The weekly morning and afternoon surveys were separated by 48 to 52 h. We randomly chose morning or afternoon for the first survey of the week and surveyed during the alternative time period for the second survey. Morning surveys were conducted between 0730 and 0930 h and afternoon surveys were conducted between 1600 and 1800 h. The survey team consisted of the four authors, who trained to conduct the surveys in a consistent manner. To avoid observer bias, two members of the team were randomly assigned to each survey, with the constraint that no person conducted both morning and afternoon surveys in the same survey week.

During each survey, the observers overturned all submerged and partially-submerged objects that could provide cover for a salamander. Potential cover objects included rocks, leaves, and woody debris. Rocks that were deeply embedded within the substrate or could not be lifted by one person were not overturned. For each survey, we recorded number of salamanders observed, number of overturned objects, percent cloud cover, water temperature (°C), specific conductivity (μS), dissolved oxygen (mg O_2 / L H_2O), and water depth (cm). Water temperature and specific conductivity were measured with a YSI Model 30-10 FT conductivity meter and dissolved oxygen was measured with a YSI Model 550A dissolved oxygen meter. Water depth was measured with a ruler during each survey at the same location within the sampled pool. Percent cloud cover was estimated visually. Each salamander observed was assigned to one of three size classes based on a visual estimate of total length from the tip of the snout to the tip of the tail: < 2.5 cm, 2.5 – 5.1 cm, or > 5.1 cm.

We used paired t-tests to compare the number of salamanders observed during morning and afternoon surveys, as well as the percent of cover objects with salamanders in the morning and afternoon surveys. To test for order effects (e.g. reduced number of salamanders on the surface due to multiple surveys in a relatively short time period), we also compared the mean number of salamanders observed and percent cover objects with salamanders in the first and second survey of the week. We used Pearson correlations to assess the influence of environmental variables (water temperature, specific conductivity, dissolved oxygen, water depth, and percent cloud cover) on the number of salamanders observed and on the percent of cover objects with salamanders. All statistical analyses were performed using SPSS statistical software (SPSS 13.0 for Windows, Release 13.0.1, SPSS Inc., Chicago, IL).

RESULTS—There was no effect of time of day on the number of salamanders observed during surveys: the mean number observed during morning surveys was exactly the same as the mean number observed during afternoon surveys (Fig. 1). In addition, the mean percent of cover objects with salamanders did not differ between during morning and afternoon surveys (t = -0.131, df = 9, P = 0.899; Fig. 2). Across all visual encounter surveys, the number of salamanders observed was strongly correlated with the percent of cover objects with salamanders (r = 0.92, $P < 0.001$).

We found no order effect of the treatments (i.e. whether the morning or afternoon survey came first). There was no significant difference between the mean number of salamanders observed in the first survey of the week and the second survey (t = -0.924, df = 9, P = 0.379) or between the mean percent of cover objects with salamanders in the first survey and second survey of the week (t = -0.971, df = 9, P = 0.357).

Measured ranges of the environmental variables during the course of the study were as follows: water temperature (21.1 – 21.3 °C), specific conductivity (550 – 662 μS), dissolved oxygen (6.44 – 7.56 mg O_2 / L H_2O), water depth (12.9 – 24.10 cm), percent cloud cover (0 – 100%). All correlations between the number of salamanders observed and these environmental variables were weak and not significant (Table 1).

DISCUSSION—Visual encounter surveys are one of the most commonly used techniques for assessing and monitoring stream and spring salamanders (Crump and Scott, 1994; Jung et al., 2000). Because these surveys are based on visual detection of salamanders, environmental factors that alter salamander behavior and/or visual acuity may influence the probability of their detection. However, few studies have examined the influence of environmental and methodological factors on detection in visual encounter surveys.

One factor that potentially affects detection in visual encounter surveys is the time of day when the survey is conducted. Studies have compared the results of surveys conducted at night with those conducted during daylight hours. For example, Orser and Shure (1975) found that mark-recapture surveys of *Desmognathus fuscus* in a spring-fed stream in Georgia yielded higher population estimates when conducted at night than when surveys were conducted during daylight hours. They found that the different population estimates were largely the result of capturing more adults at night; the densities of juveniles were similar at night and during the day. Similarly, Petranka (1984) observed that *Eurycea bislineata* larvae were located under rocks and leaf litter during the day, but emerged from cover objects at night and moved about in the open. Petranka found that larvae fed continuously during night and day, suggesting that the lack of movement during daylight was a predator-avoidance response.

These studies suggest that, at least for some species, visual encounter surveys conducted during nighttime hours may yield higher counts than those conducted during daylight hours, but we know of no study that has explicitly examined the question of whether visual encounter surveys of stream and spring salamanders conducted during daylight hours are affected by the time of day in which the survey was conducted. This question has considerable practical importance because most surveys are conducted during daylight hours and monitoring and research programs frequently combine and compare surveys conducted at different times during the day.

Because some stream and spring salamanders are more active at night (Orser and Shure, 1975; Petranka, 1984), we hypothesized that surveys conducted during morning hours might yield higher counts than surveys conducted in late afternoon. However, our results demonstrated that whether the survey is conducted during morning or afternoon had no effect on the number of *E. naufragia* observed. The average number of salamanders observed in morning and afternoon surveys was exactly the same; the percent of cover objects occupied by salamanders was also similar in morning and afternoon surveys. Although our sample size is not large (20 surveys), the degree of similarity of results for morning and afternoon surveys suggests that increasing the sample size would have little effect on the outcome. Our results suggest that outcomes of visual encounter surveys of *E. naufragia* are not biased by the time of day in which sampling occurs, allowing researchers to generalize visual encounter survey data taken during various daylight hours.

We also found no associations between the number of salamanders observed or percent of cover objects with salamanders and water temperature, dissolved oxygen, conductivity, water depth, or percent cloud cover. The site where our study was conducted is a spring-fed pool with relatively constant water temperature and chemistry. For other sites and species, where more environmental variation occurs, these parameters might play a larger role in salamander abundance. A limitation of our study was the relatively short time frame of the sampling effort (10 weeks). The absence of significant correlations between environmental variables and salamander abundance may result from the short duration of the study.

Traditional visual encounter surveys have been criticized because often they do not account for probability of detection, which may affect inferences about population size and occupancy (Mazerolle et al., 2007). Although we consistently surveyed all available habitat within the spring pool, the extent to which salamanders may move into or out of the study area and access the subterranean aquifer is not known. Our objective in this study, however, was not to draw inferences about population size, but rather to determine whether the time of day in which the sample is taken and environmental factors affect detection of salamanders in visual encounter surveys.

It is important to note that *E. naufragia* is a permanently neotenic species, and most of the salamanders we observed were adults (we rarely encounter juvenile *E. naufragia* in our visual encounter surveys at this site). Whether juveniles exhibit a similar lack of diurnal variation in detection is unknown. Orser and Shure (1975) found no differences in densities of juvenile *Desmognathus fuscus* between day and night samples. However, in his study of *E. bislineata*, Petranka (1984) found that smaller larvae were more active during predawn and afternoon hours than adults, but the effect was not strong and diurnal activity was independent of body size. Additional information about the effect of diurnal variation on larval salamanders detected in visual encounter surveys would be helpful.

Our study was conducted near the end of a severe drought in central Texas; however, major rainfall event occurred near the beginning of our study and spring flow was high through our sampling period. A limitation of our study is that it focused on a single season. Diurnal activity of salamanders might vary seasonally, although Petranka (1984) found no strong seasonal effect in his study of *E. bislineata* activity. Our conclusions are also limited to a single species. Further studies on additional species of stream and spring salamanders are warranted.

We thank the property owners who generously gave us permission to survey salamanders on their land and the Texas Parks and Wildlife Department for scientific permits. We thank Maria Cuevas for translating the abstract into Spanish. This research was supported by a Vision Grant from the 3M Foundation. The study complied with all applicable institutional animal care guidelines and was approved by the institutional animal care and use committee.

LITERATURE CITED

BARR, G. E., AND K. J. BABBITT. 2001. A comparison of 2 techniques to sample larval stream salamanders. Wildlife Society Bulletin 29:1238–1242.

BARRETT, K., B. S. HELMS, C. GUYER, AND J. E. SCHOONOVER. 2010. Linking process to pattern: causes of stream-breeding amphibian decline in urbanized watersheds. Biological Conservation 143:1998–2005.

BOWLES, B. D., M. S. SANDERS, AND R. S. HANSEN. 2006. Ecology of the Jollyville Plateau Salamander (*Eurycea tonkawae*: Plethodontidae) with an assessment of the potential effects of urbanization. Hydrobiologica 533:111–120.

BURTON., T. M., AND G. E. LIKENS. 1975. Salamander populations and biomass in the Hubbard Brook Experimental Forest, New Hampshire. Copeia 1975:541–546.

CHALMERS, R. J., AND S. DROEGE. 2002. Leaf litter bags as an index to populations of Northern Two-Lined Salamanders (*Eurycea bislineata*). Wildlife Society Bulletin 30:71–74.

CHIPPINDALE, P. T., A. H. PRICE, J. J. WIENS, AND D. M. HILLIS. 2000. Phylogenetic relationships and systematic revision of central Texas Hemidactyliine Plethodontid salamanders. Herpetological Monographs 14:1–80.

CHIPPINDALE, P. T., AND A. H. PRICE. 2005. Conservation of Texas spring and cave salamanders (*Eurycea*). *In* M. Lannoo (ed.), Amphibian Declines, pp. 193–197. University of California Press, Berkeley.

CORN, P. S., AND R. B. BURY. 1989. Logging in western Oregon: responses of headwater habitats and stream amphibians. Forest Ecology and Management 29:39–57.

CRUMP, M. L., AND N. J. SCOTT, JR. 1994. Standard techniques for inventory and monitoring: visual encounter surveys. *In* W. R. Heyer, M. A. Donnelly, R. W. McDiarmid, L. C. Hayek, and M. S. Foster (eds.), Measuring and Monitoring Biological Diversity: Standard Methods for Amphibians, pp. 84–92. Smithsonian Institution, Washington, DC.

GRANT, E. H. C., L. E. GREEN, AND W. H. LOWE. 2009. Salamander occupancy in headwater stream networks. Freshwater Biology 54:1370–1378.

GRIFFITHS, R. A. 1985. A simple funnel trap for studying newt populations and an evaluation of trap behavior in Smooth and Palmate Newts, *Triturus vulgaris* and *T. helveticus*. Herpetological Journal 1:5–10.

JUNG, R. E., S. DROEGE, J. R. SAVER, AND R. B. LANDY. 2000. Evaluation of terrestrial and streamside salamander monitoring techniques at Shenandoah National Park. Environmental Monitoring and Assessment 63:65–79.

KERBY, J. L., K. L. RICHARDS-HRDLICKA, A. STORFER, AND D. K. SKELLY. 2010. An examination of amphibian sensitivity to environmental contaminants: are amphibians poor canaries? Ecology Letters 13:60–67.

LOWE, W. H., AND D. T. BOLGER. 2002. Local and landscape-scale predictors of salamander abundance in New Hampshire headwater streams. Conservation Biology 16:183–193.

MACKEY, M. J., G. M. CONNETTE, AND R. D. SEMLITSCH. 2010. Monitoring of stream salamanders: the utility of two survey techniques and the influence of stream substrate complexity. Herpetological Review 41:163–166.

MARSH, D. 2009. Evaluating methods for sampling stream salamanders across multiple observers and habitat types. Applied Herpetology 6:211–226.

MAZEROLLE, M. J., L. L. BAILEY, W. L. KENDALL, J. A. ROYLE, S. J. CONVERSE, AND J. D. NICHOLS. 2007. Making great leaps forward: accounting for detectability in herpetological field studies. Journal of Herpetology 41:672–689.

MACKENZIE, D. I., J. D. NICHOLS, , J. A. ROYLE, K. H. POLLOCK, L. L. BAILEY, AND J. E. HINES. 2006. Occupancy Estimation and Modeling: Inferring Patterns and Dynamics of Species Occurrence. Academic Press, New York.

MILLER, J. E., G. R. HESS, AND C. E. MOORMAN. 2007. Southern two-lined salamanders in urbanizing watersheds. Urban Ecosystems 10:73–85.

NOWAKOWSKI, A. J., AND J. C. MAERZ. 2009. Estimation of larval stream salamander densities in three proximate streams in the Georgia Piedmont. Journal of Herpetology 43:503–509.

ORSER, P. N. AND D. J. SHURE. 1972. Effects of urbanization on the salamander *Desmognathus fuscus fuscus*. *Ecology* 53:1148-1154.

ORSER, P. N., AND D. J. SHURE. 1975. Population cycles and activity patterns of the Dusky Salamander, *Desmognathus fuscus fuscus*. The American Midland Naturalist 93:403–410.

PETERMAN, W. E., J. A. CRAWFORD, AND R. D. SIMLITSCH. 2008. Productivity and significance of headwater streams: population structure and biomass of the Black-Bellied Salamander (*Desmognathus quadramaculatus*). Freshwater Biology 53:347–357.

PETRANKA, J. W. 1984. Ontogeny of the diet and feeding behavior of *Eurycea bislineata* larvae. Journal of Herpetology 18:48–55.

PIERCE, B. A., J. L. CHRISTIANSEN, A. L. RITZER, AND T. A. JONES. 2010. Distribution and ecology of the Georgetown Salamander, *Eurycea naufragia*. Southwestern Naturalist 55:296–301.

QUINN, T., M. P. HAYES, D. J. DUGGER, T. L. HICKS, AND A. HOFFMANN. 2007. Comparison of two techniques for surveying headwater stream amphibians. Journal of Wildlife Management 71:282–288.

SEPULVEDA, A. J., AND W. H. LOWE. 2009. Local and landscape-scale influences on the occurrence and density of *Dicamptodon aterrimus*, the Idaho Giant Salamander. Journal of Herpetology 43:469–484.

WALDRON, J. L., C. K. DODD, JR., AND J. D. CORSER. 2003. Leaf litterbags: factors affecting capture of stream-dwelling salamanders. Applied Herpetology 1:23–36.

WARD, R. L., J. T. ANDERSON, AND J. T. PETTY. 2008. Effects of road crossings on stream and streamside salamanders. Journal of Wildlife Management 72:760–771.

WELSH, H. H., JR., AND L. M. OLLIVIER. 1998. Stream amphibians as indicators of ecosystem stress: a case study from California's redwoods. Ecological Applications 8:1118–1132.

WILLSON, J. D., AND M. E. DORCAS. 2003. Effects of habitat disturbance on stream salamanders: implications for buffer zones and watershed management. Conservation Biology 17:763–771.

WILLSON, J. D., AND M. E. DORCAS. 2004. A comparison of aquatic drift fences with traditional funnel trapping as a quantitative method for sampling amphibians. Herpetological Review 35:148–150.

TABLE 1—Correlations (*r*) and associated probabilities (*P*) between environmental variables and number of salamanders observed and percent of cover objects with salamanders.

Environmental Variable	Number of Salamanders Observed		Percent of Cover Objects Occupied	
	r	*P*	*r*	*P*
Water Temperature	-0.221	0.350	-0.208	0.379
Specific Conductivity	0.239	0.310	0.234	0.322
Dissolved Oxygen	0.227	0.351	0.118	0.630
Water Depth	0.181	0.473	0.058	0.818
Percent Cloud Cover	-0.011	0.964	-0.115	0.650

Figure Legends

FIG. 1—Mean number of *Eurycea naufragia* observed during visual encounter surveys (± SE) conducted in the morning (*N* = 10) and in the afternoon (*N* = 10) at a spring in Georgetown, Texas.

FIG. 2—Percent of cover objects with salamanders during visual encounter surveys (± SE) conducted in the morning (*N* = 10) and in the afternoon (*N* = 10) at a spring in Georgetown, Texas.

Figure 1

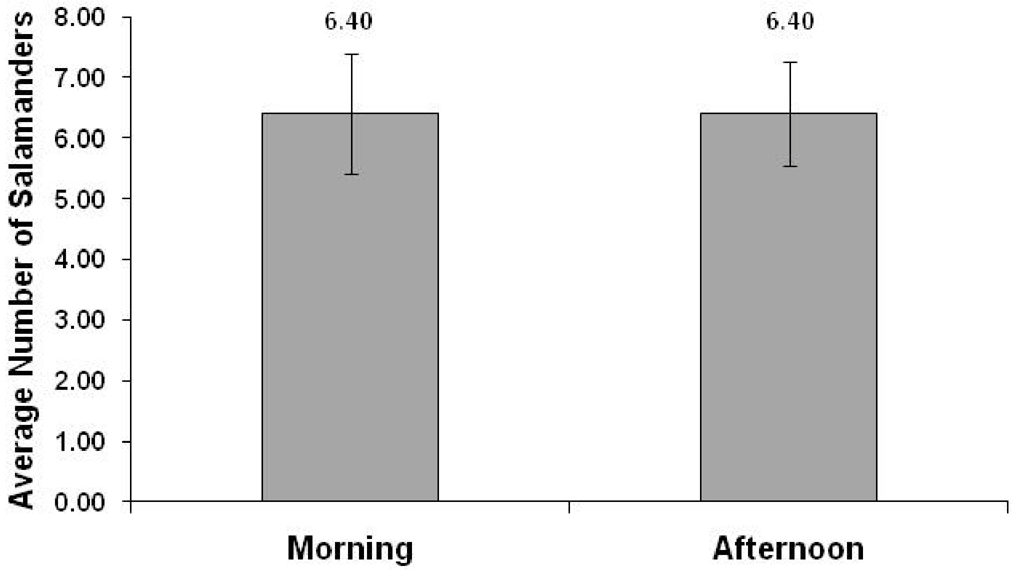
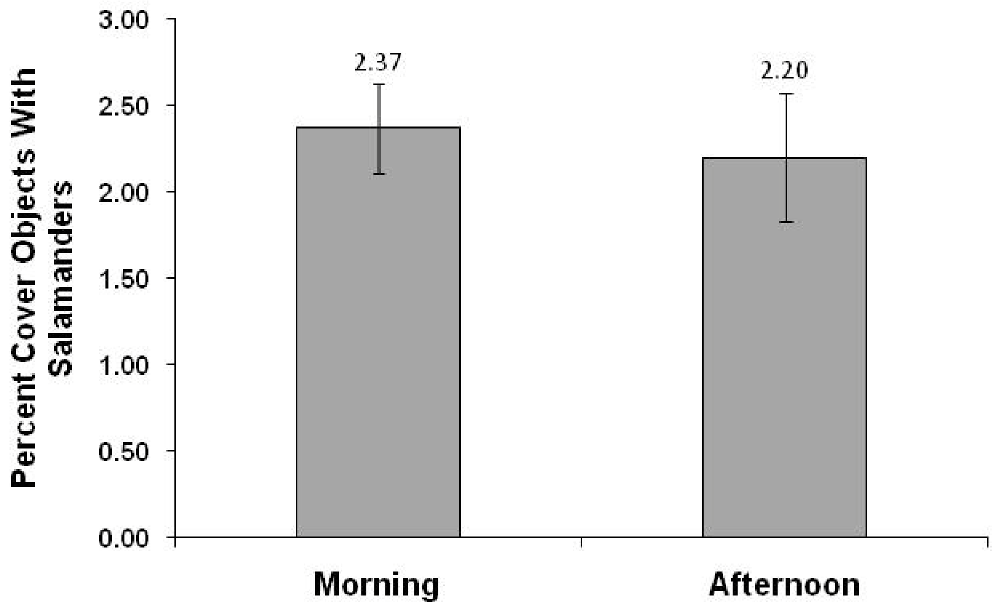

Average Number of Salamanders
8.00
7.00
6.00
5.00
4.00
3.00
2.00
1.00
0.00
6.40
6.40
Morning
Afternoon

Figure 2


Percent Cover Objects With Salamanders
3.00
2.50
2.00
1.50
1.00
0.50
0.00
2.37
2.20
Morning
Afternoon