1-1-2013

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Heather Rena Oswald
University of South Carolina

Recommended Citation

Oswald, H. R.(2013). *The Reproductive Ecology of Plethodontid Salamanders In the South Carolina Inner Coastal Plain.* (Master's thesis). Retrieved from http://scholarcommons.sc.edu/etd/511

THE REPRODUCTIVE ECOLOGY OF PLETHODONTID SALAMANDERS IN THE SOUTH CAROLINA INNER COASTAL PLAIN

by

Heather Rena Oswald

Bachelor of Science
University of South Carolina, 2009

Submitted in Partial Fulfillment of the Requirements

For the Degree of Master of Science in

Biological Sciences

College of Arts and Sciences

University of South Carolina

2013

Accepted by:

Timothy A. Mousseau, Major Professor

Jayme L. Waldron, Committee Member

Shane M. Welch, Committee Member

Lacy Ford, Vice Provost and Dean of Graduate Studies

DEDICATION

I would like to dedicate my thesis to my husband and mother whose unwavering love, support, and patience have given me the inspiration and courage to follow my dreams.

ACKNOWLEDGEMENTS

I would like to thank Timothy Mousseau who as my advisor provided me with the opportunity to pursue my interests in ecology. He has been instrumental to my research and has provided ample resources to satisfy my project needs. His guidance and insight have been invaluable in directing my research and career goals. I would also like to thank Jayme Waldron and Shane Welch to whom I owe a debt of gratitude for their mentorship throughout my research project. They helped me develop many of the skills that were critical during my project development and have facilitated my growth as researcher, a naturalist, and a professional.

I would also like to thank several people who provided assistance and companionship during my fieldwork and professional development. Fellow graduate students, Jennifer Fill, De Anna Beasley, and Michael Martin all were tremendously helpful during the fieldwork, data analysis, and writing phases of my research. They each provided great physical and mental support for which I am tremendously grateful. I also would like to thank my field technicians Jennifer Allison, David Cann, Joseph Colbert, and Vladamir Nekrutenko who provided invaluable assistance with data collection and fieldwork.

Thanks to Steve Bennett and Andrea Bonisoli- Alquati who shared with me a great depth of knowledge and insight during my development as a researcher. Lastly, I would like to thank the NSF SC Graduate Steps to STEM program and the South Carolina DNR for providing fellowship support throughout my research project.

ABSTRACT

Nest- site selection is a critical process in the life history of amphibians, directly influencing offspring survival and parental fitness. When and where an amphibian chooses to nest is strongly influenced by a range of environmental cues. We monitored plethodontid salamander breeding behavior in seepage wetlands in the South Carolina inner Coastal Plain. Seepage wetlands are ideal for monitoring semiaquatic salamander breeding, because they provide habitat for both larval and adult salamanders while having relatively predictable, constant water temperatures and presence year round. We observed the breeding phenology of three sympatric salamanders (*Eurycea cirrigera, Desmognathus auriculatus,* and *Pseudotriton ruber)* in seepage wetlands. We determined that that these three species have temporally partitioned the year into distinct, non-overlapping breeding seasons. We believe that the partitioning of the reproductive season may serve as a mechanism for reducing competition for nest- sites. Further, we evaluated the nest- site selection behavior of the Southern Two-lined salamander, *E. cirrigera,* in relation to a range of environmental conditions over two spatial scales. *E. cirrigera* select nest- sites using environmental cues across multiple spatial scales where females actively discriminate among nesting locations based on measures of temperature, hydrology, and additional biotic factors. Because amphibians are so sensitive to environmental change and variation, understanding the factors that influence key life history processes, particularly breeding phenology and oviposition site choice, are critical to the conservation of amphibian populations and their habitats.

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CHAPTER 1

BREEDING PHENOLOGY OF PLETHODONTID SALAMANDERS IN THE SOUTH CAROLINA INNER COASTAL PLAIN

1.1 INTRODUCTION

The Plethodontidae is the largest family of salamanders reaching its greatest diversity in the southeastern United States (Petranka, 1998). All salamanders in this family are lungless and breathe through their skin and mouth lining (Petranka, 1998). This physiological constraint restricts plethodontids to habitats that maintain high levels of moisture, especially during the breeding season (Means, 2000, in press). This is important because many semiaquatic plethodontids have a biphasic life cycle, hatching as larvae in aquatic habitats and as terrestrial adults, finding shelter under moist leaf litter and coarse woody debris on land (Petranka, 1998; Semlitsch, 2000; Bruce, 2005; Crawford and Semlitsch, 2007; Niemiller and Miller, 2007; Ficetola et al., 2011). Seepage wetlands in the South Carolina inner Coastal Plain provide ideal habitat for monitoring semiaquatic plethodontid salamander breeding because they offer a heterogeneous landscape of stable terrestrial and aquatic habitats. These wetlands are unique in that they provide habitat for both larval and adult salamanders (Bruce, 2005; Means; 2000, in press; Keitzer and Goforth, 2012) and have relatively predictable, constant water temperatures and presence year round (Tufford, 2011).

The reproductive activity of amphibians is distributed non-randomly throughout the year based on distinct changes in season resulting in a distinct breeding phenology (Gottsberger and Gruber, 2004). In temperate climates, temperature and moisture are the main abiotic factors influencing temporal patterns of reproductive activity (Oseen and Wassersug, 2002; Prado et al., 2005; Lin and Kam, 2008; Arnfield, et al., 2012; Lowe, 2012). However, in seepage wetlands where abiotic factors are more predictable and stable regardless of season, temporal variation in breeding phenology may be driven by competition for other resources. Because water temperature and presence are relatively constant in seepage wetlands, plethodontid salamanders breeding sympatrically in these habitats may use temporal cues to reduce competition for oviposition sites. This would result in the temporal partitioning of breeding seasons to accommodate the different breeding schedules and nesting requirements of each species (Southerland, 1986; Pasachnik and Ruthig, 2004; Kozak et al., 2009).

The objective of this study was to use field observations of breeding plethodontids to establish breeding phenologies of three common salamanders (*Eurycea cirrigera*, *Desmognathus auriculatus,* and *Pseudotriton ruber)* nesting in the seepage wetlands of the South Carolina inner Coastal Plain. We hypothesized that these sympatric species have temporally partitioned the year into distinct, non-overlapping breeding seasons as a mechanism for reducing competition for nesting sites. We predicted that the partitioning of the reproductive season may explain how these three salamanders are able to co-occur with each other, and with other salamander species, in the same location. Further, we were interested in how the breeding phenology of each of these species is addressed in the literature and how their breeding season may change in similar salamander communities in other geographic regions.

1.2 MATERIALS AND METHODS

Study area. We monitored the breeding phenology of three plethodontid species at two headwater seepage wetlands in the South Carolina inner Coastal Plain. The Wannamaker Nature Preserve (WNP) (33° 38′ 37.05″ N, -80° 42′ 23.36″ W) and Singleton Plantation (SP) (33° 41′ 59.59″ N, -80° 43′ 11.33″ W) are located near St. Matthews in Calhoun County, South Carolina. Both study sites are headwater seepage wetlands at the base of an elevated, upland bluff dominated by a mixed-pine, hardwood forest (Tufford, 2011). The seepages at both WNP and SP are characterized by a wide, sheet flow of surface water covering a substrate of sand, gravel, and pockets of mud. These seepages provide semiaquatic salamanders with patches of both terrestrial and aquatic habitats.

Species Sampling. We sampled for *E. cirrigera*, *D. auriculatus,* and *P. ruber* adults and nests using wood coverboards (Houze, 2002), which have been documented to provide nesting habitat suitable for semi-aquatic plethodontid salamanders (S. Bennett, South Carolina Department of Natural Resources, unpub. data). We placed thirty plywood coverboards (1.2 cm x 61cm x 61 cm/ 3-Ply Rtd. Sheathing) throughout each study site using a random walk sampling design and basing direction on a random azimuth (0-350, at 10 degree intervals) and distance (5-25 meters). Coverboards were allowed to “weather” in the seeps (i.e., worn by water, sun, and rain) for approximately one year before we began monitoring them for nests.

From May 2010 through February 2012, we sampled coverboards approximately every two weeks for presence of adult salamanders and nests. During each sampling event we visually searched under each coverboard for adults and nests. Adult salamanders were collected for identification (i.e. species, gender), photographed (EOS Digital Rebel XS 1000D), measured for snout-vent length SVL (mm), total length TL (mm), and mass (g), and then returned to their nests unharmed. All nests were photographed and identified by the species of the attending female.

Breeding Phenology. Statistical analyses were performed using SAS software, version 9.2.1. We estimated breeding season using Julian dates that corresponded to reproductive events. We considered captures of dimorphic (i.e. gravid) females as breeding events because they functioned as indicators of reproductive activity. We did not uniquely identify individual females; therefore, some of our reproductive events may have been recaptures. However, the total number of gravid female captures for each species was high relative to the number of nests and did not likely influence the differences observed in our results. Further, we recorded the date corresponding to the first observation of a nest (later observations of the same nests were excluded) as a reproductive event. Because *E. cirrigera's* breeding season includes both December and January, we standardized Julian date to begin at an earlier date in the year where there were no breeding event observations from any species. “Day 1" corresponded to 17 September, thus, we added 105 days to each breeding event observations for analysis.

We used negative binomial regression (PROC GENMOD) to examine breeding season overlap using Julian date as the response and site and species as categorical predictors. We assessed goodness of fit by examining our model's deviance from negative binomial distribution. We tested for differences in Julian date to differentiate reproductive season overlap among species.

Historical Field Observations. We searched both published and unpublished literature for historical field observations of *E. cirrigera*, *E. bislineata*, *D. auriculatus*, and *P. ruber* breeding throughout the eastern United States to compare reproductive seasons. We added *E. bislineata* to this portion of the study, because in many instances the names *E. cirrigera* and *E. bislineata* were used interchangeably in the literature, especially in older sources (Jacobs, 1987). We considered each species separately during analysis, but treated them similarly for inference.

We searched Web of Science (Web of Knowledge, Philadelphia, PA) for articles on each species using the keywords: "nest," "gravid," "breeding," "breeding season," "egg," "clutch," and "mating" between the years of 1920 and 2013. We also searched the reference lists of the selected articles for additional studies that met our inclusion criteria. We also searched field guides and unpublished observations from individual researchers throughout Appalachia ("Eastern Uplands") and surrounding areas. We used approximate locations to the nearest county based on site descriptions from each source, to map breeding sites for each species using ArcGIS. Since we were only interested in breeding locations as they were related to the geographic region, we considered sources with especially vague site descriptions to be located in the center of the location described. Individual breeding locations were categorized by geographic region using a United States Department of Agriculture (USDA) Economic Research Service (ERS) Farm Resource Regions map (www.soils.usda.gov; Figure 1.2). The map divides the eastern, southeastern United States into regions with similar physiographic, soil, and climatic traits, as reflected in USDA's Land Resource Regions. We categorized the season of individual breeding events by the date or time of year that each source described having seen the breeding event. We recognize that the generic terms for “season” will vary among geographic regions; however, we used the terminology of the literature to generalize the season when the breeding event was observed. We used standard Northern Hemisphere season dates to divide the year where “winter” begins December 21, “spring” begins March 21, “summer” begins June 21, and “autumn” begins September 21. We summarized and compared the number of breeding observations for each species in each season (i.e. winter, spring, summer, and autumn) by geographic region.

1.3 RESULTS

Breeding Phenology. We counted the total number of gravid females (N=204) and the total number of nests observed (N=134) to assess the breeding season of each species. The average dates on which we observed reproductive events for *E. cirrigera* (N=178), *D. auriculatus* (N=130), and *P. ruber* (N=30) were 5 February (mean un-standardized Julian Date = 36.33 ± 27.37), 6 July (mean un-standardized Julian Date = 187.55 ± 76.43), and 22 November (mean Julian date = 326.23 ± 30.08), respectively. We failed to detect a significant site×species interaction, and thus relied on the reduced model for inference. Model deviance indicated that our data adequately fit the model (deviance = 1.06). Reproductive season differed significantly among species (χ^2 = 199.49; df = 2; $p < 0.001$; Figure 1.1). *E. cirrigera* bred primarily during winter (β = 4.97± 0.04; p < 0.001), *D. auriculatus* (β = 5.46 ± 0.04; $p< 0.001$) bred during summer, and *P. ruber* bred during the autumn (β = 4.23 ± 0.08; $p < 0.001$; Table 1.1; Figure 1.1).

We failed to detect a significant effect of site on breeding phenology ($\chi^2 = 0.89$, df = 1, $p = 0.35$).

Historical Field Observations. The historical breeding observation data from published and unpublished sources (N= 40) is summarized in Appendix A. We categorized each observation by geographic location (i.e. region) and season (i.e. winter, spring, summer, and autumn) (Figure 1.2; Table 1.2). *E. cirrigera* and *E. bislineata* in the Northern Crescent and Heartlands breed during the spring and summer and in the Eastern Uplands and Southern Seaboard during winter and spring. *P. ruber* breeding events occurred in the summer and autumn in the Southern Seaboard and autumn in the Northern Crescent and Southern Seaboard (Table 1.2). We found observations of *D. auriculatus* in both the summer and the fall in the Southern Seaboard and Fruitful Rim (Table 1.2).

1.3 DISCUSSION

Our results show a general delineation of breeding seasons between *E. cirrigera*, *D. auriculatus*, and *P. ruber* salamanders in South Carolina seepage wetlands. *E. cirrigera* breed during the winter months, starting in late November and continue through early spring. This suggests that peripherally distributed populations of *E. cirrigera* prefer nesting in cooler climates similar to the spring- like conditions of typical mountain stream habitats (Jakubanis et al., 2008). The distribution of *D. auriculatus* breeding events is the largest, encompassing most of the summer months. We observed gravid *D. auriculatus* females in every month of the year; however, we only saw nests between late April and September. This suggests that females may carry eggs for several months before laying a clutch, possibly to avoid competition for nest- sites. *Pseudotriton ruber* nest during the autumn months of October and November. Although we had observations of gravid *E. cirrigera* females in the seep at the same time that *P. ruber* was nest- guarding, we never observed an *E. cirrigera* nest and a *P. ruber* nest in the seep at the same time. This suggests that *E. cirrigera* may "wait" for *P. ruber* to finish brooding before entering the seep to nest. This is expected since large plethodontids like *P. ruber* adults are the main predator of *E. cirrigera* nests (Gustafson, 1993). *D. auriculatus* may act as a nest- site generalist in seepage habitats. Even though females carry eggs for the majority of the year, *D. auriculatus* did not lay nests in the seepage at the same time as the other species. Gravid females are likely waiting for conditions to become suitable and competition for nest- sites to become reduced before laying their nest.

Historical observations of breeding offered a broad explanation of "breeding season" for each of the three species and there was an overall ambiguity in the literature of species- specific breeding phenologies. It appears that this ambiguity is the result of seasonal variation in other geographic regions. Our results show that *E. cirrigera* and *E. bislineata* in the northern United States (i.e. the Northern Crescent and Heartlands) nest during the spring and summer. Along the Eastern Uplands and Southern Seaboard, both species seem to breed earlier in the year with observations of nesting in the winter and spring. The most southern observation of *Eurycea* breeding in the Florida Fruitful Rim provided a single observation of autumn breeding. This trend is consistent with what we expected based on our own field observations. *E. cirrigera* in South Carolina nest during the winter when temperatures are cool and mild, much like spring in more northern regions. There were few observations of *P. ruber* nesting. However, the overall breeding season is consistent with our field observations. *P. ruber* move into the seepages in late summer and nest during the autumn. In the seepages of the South Carolina inner Coastal Plain, *P. ruber* is likely to initiate the breeding season, and *E. cirrigera* breed afterward. We observed *D. auriculatus* breeding through most of the summer. Observations from the literature suggest that this trend is consistent throughout most of the *D. auriculatus* range. This supports the idea that *D. auriculatus* have flexible breeding phenologies, and nesting observations are likely most common during times of the year when competition for nest- sites is reduced (Juterbock, 1986; Hom, 1987).

In the South Carolina inner Coastal Plain, plethodontid salamanders have established discrete, non-overlapping breeding seasons in seepage wetlands. We believe that sympatric species of salamanders will temporally partition the year into distinct breeding seasons as a mechanism for reducing competition for net sites while staying within the limitations of their physiology. Therefore, this suggests that these three species of salamanders are able to coexist within the same habitat, because they have different reproductive niches (Southerland, 1986; Macarthur and Levins, 1967). Further, this trend is repeated in other geographic regions along the extent of their natural range. Plethodontids in different geographic areas apparently shift their breeding phenology to compensate for shifts in abiotic stressors related to season.

Table 1.1 Parameter Estimates (β) for fixed effects from negative binomial regression examining the effect of Julian Date on occurrence of reproductive events for three plethodontid salamanders. (SE = standard error; LCL = lower 95% confidence limit; UCL = upper 95% confidence limit)

Parameter	$\boldsymbol{\beta}$ **+/- SE**	**df**	**LCL**	**UCL**	χ^2	p
P. ruber	4.23 ± 0.08	1	4.07	4.39	2821.9	< 0.0001
D. auriculatus	5.46 ± 0.04	1	5.37	5.54	15150.5	< 0.0001
E. cirrigera	4.97 ± 0.04	1	4.90	5.05	18440.0	< 0.0001

Table 1.2 Counts of historical breeding observations (N=40) for *E. cirrigera*, *E. bislineata*, *D. auriculatus*, and *P. ruber* for each season in six United States Department of Agriculture (USDA) Economic Research Service (ERS) Farm Resource Regions. (Numbers in parentheses represent USDA Region numbering.)

Region	**Species**	**Winter**	**Spring**	**Summer**	**Autumn**
Northern Crescent (2)	*E. cirrigera*	-	-	-	-
	E. bislineata	-	2	2	-
	D. auriculatus	-	-	-	-
	P. ruber	-	-	-	1
Heartland (1)	*E. cirrigera*	-	5	-	-
	E. bislineata	-	-	-	-
	D. auriculatus	-	-	-	-
	P. ruber	-	-	-	-
Eastern Uplands (5)	*E. cirrigera*	1	4	-	-
	E. bislineata	1	5	-	-
	D. auriculatus	-	-	-	-
	P. ruber	-	-	3	2
Southern Seaboard (6)	*E. cirrigera*	2	2	-	-
	E. bislineata	1	-	-	-
	D. auriculatus	-	-	1	2
	P. ruber	-	-	-	1
Mississippi Portal (9)	*E. cirrigera*	-	1	-	-
	E. bislineata	-	-	-	-
	D. auriculatus	-	-	-	-
	P. ruber	-	-	-	-
Fruitful Rim (7)	*E. cirrigera*	2	1	-	1
	E. bislineata	-	-	-	-
	D. auriculatus	-	-	-	1
	P. ruber	-	-	-	-

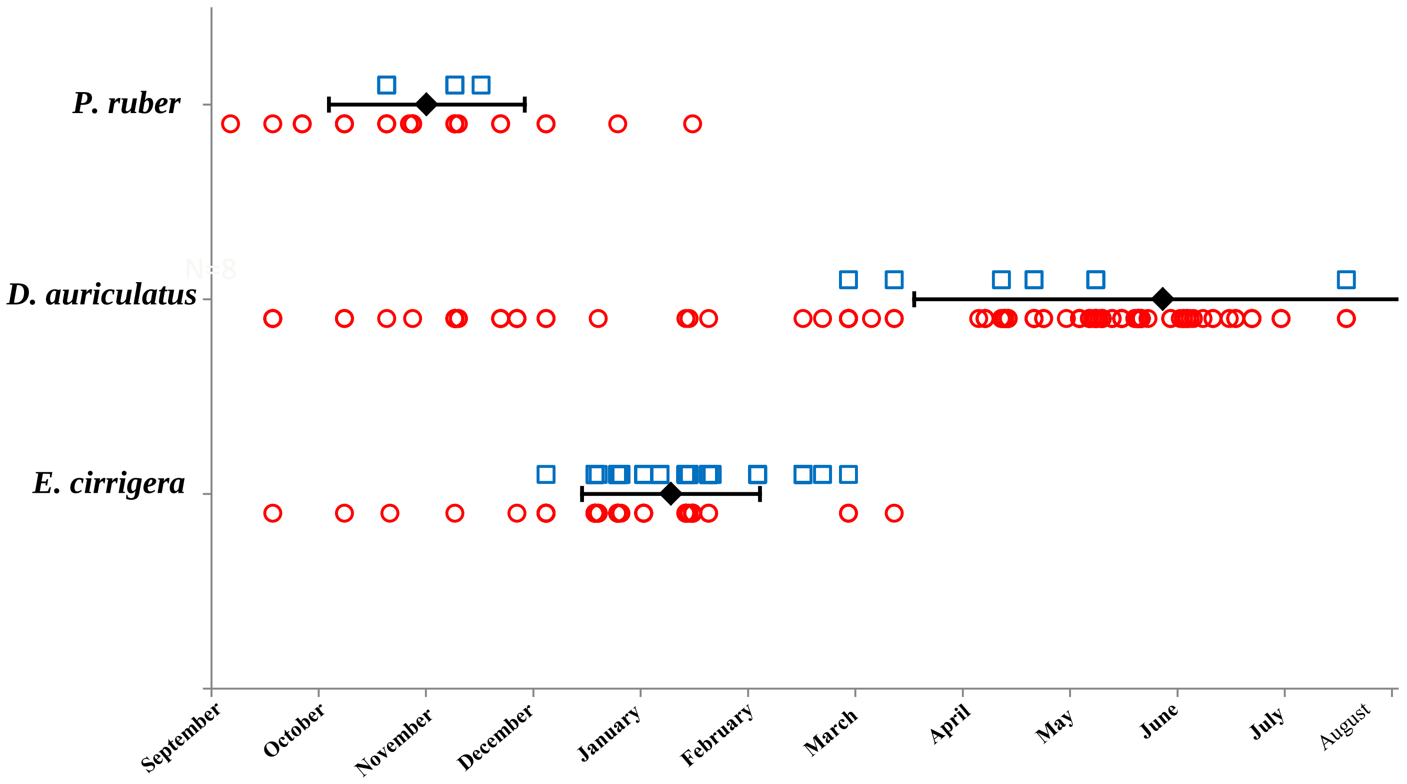

Figure 1.1. Mean Julian date of reproductive events for *E. cirrigera* (5 February) (N $_{\text{gravid}}$= 58; N $_{\text{nest}}$=120), *D. auriculatus* (6 July) (N $_{\text{gravid}}$= 122; N $_{\text{nest}}$=8), and *P. ruber* (22 November) (N $_{\text{gravid}}$= 24; N $_{\text{nest}}$=6). Error bars represent standard deviation of the mean. Red circles represent individual observations of gravid females. Blue squares represent individual observations of nests.

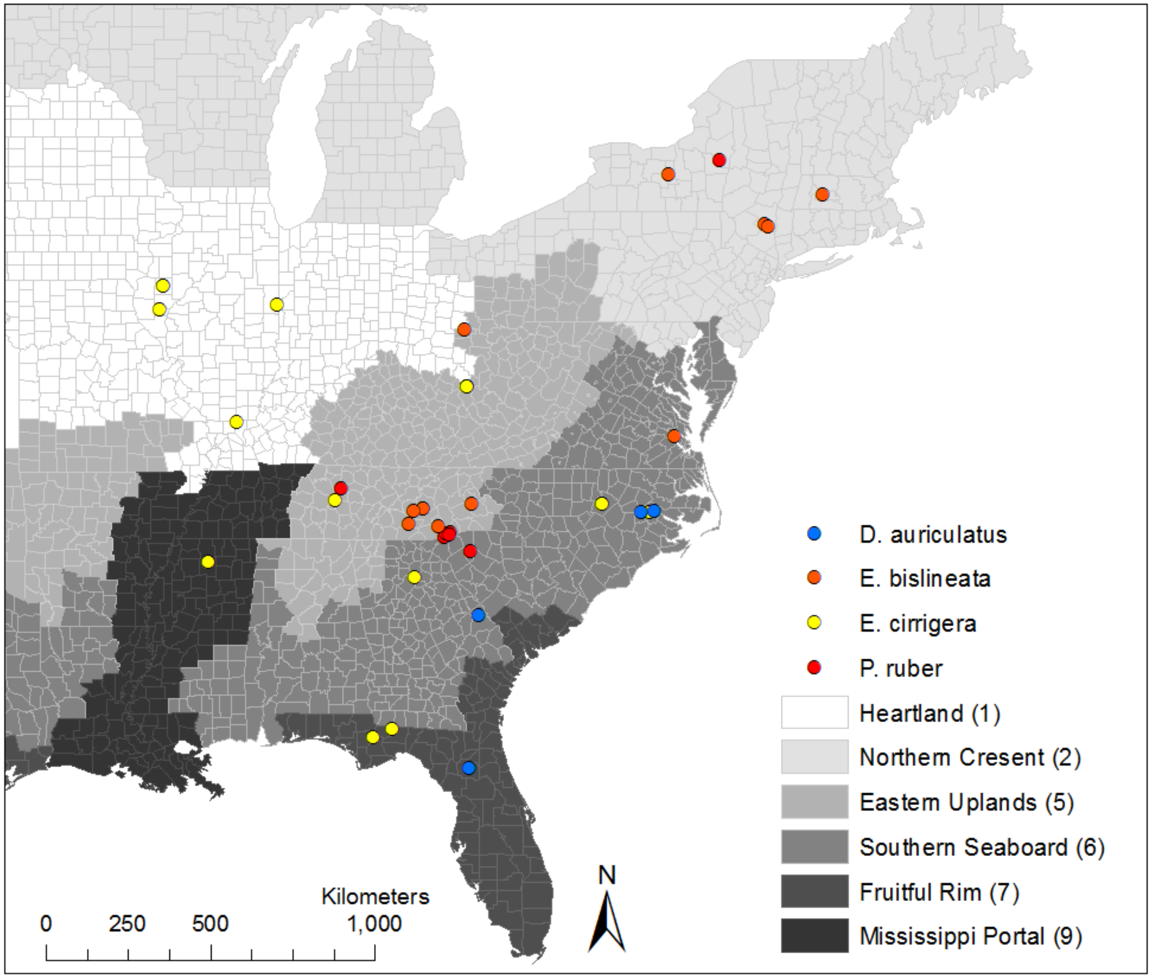

Figure 1.2 Distribution of breeding events of *E. cirrigera*, *E. bislineata*, *D. auriculatus*, and *P. ruber* (colored circles) using the United States Department of Agriculture (USDA) Economic Research Service (ERS) Farm Resource Regions.

CHAPTER 2

ENVIRONMENTAL EFFECTS ON SOUTHERN TWO-LINED SALAMANDER (*EURYCEA CIRRIGERA*) NEST- SITE SELECTION

2.1 INTRODUCTION

Nest- site selection is one of the most important and influential maternal effects by which female oviposition site choice affects offspring survival and fitness (Howard, 1978; Bernardo, 1996; Mousseau and Fox, 1998; Kolbe and Janzen, 2002). The nest-site selection process is strongly influenced by a range of environmental cues that indicate conditions for high offspring survival (Petranka and Petranka, 1981; Mousseau and Collins, 1987; Figiel and Semlitsch, 1995; Resetarits, 1996; Rudolf, 2005; Snodgrass et al., 2007). For example, some oviparous reptiles use thermal cues to assess nest- site suitability in order to optimize embryo thermoregulation (Angilletta et al. 2009; Lowenborg et al. 2010; Pike et al. 2011). Similarly, passerine birds tend to select nest-sites concealed by vegetation to reduce the risk of nest predation (Martin 1993; Liebezeit and George 2002; Davis, 2005) or environmental contaminants (Møller and Mousseau, 2007). By investigating the environmental characteristics of an organism's nest- sites, we can better understand the factors influencing how females assess nesting habitat suitability, and ultimately, increase the survival of their offspring.

For amphibians, the physiological limitations of a biphasic life cycle make scale an important consideration in characterizing nesting locations. The discrete locations of ponds or seeps in a landscape, coupled to the heterogeneity of resources within a habitat, necessitate a scale-dependent consideration of nest-site selection (Johnson 1980; Kristan et al. 2007). Distinguishing the environmental characteristics of suitable habitat at multiple spatial scales allows us to differentiate the hierarchy of factors influencing the nest- site selection process and offspring survival.

Amphibians are ideal models for examining the hierarchical effects of environmental variation on nest-site selection behavior. Because of their extreme sensitivity to environmental conditions (Blaustein et al. 1994), plethodontid salamanders exhibit physiological constraints that subject them to a narrow range of habitats (Highton, 1995). For example, many plethodontids live and breed near permanent, stream-like bodies of water because of their reliance on cutaneous respiration (Crawford and Semlitsch, 2007). As ectotherms, they have thermoregulatory constraints that necessitate discrimination among habitats based on a limited range of moisture and temperature conditions (Petranka and Petranka, 1981; Petranka, 1990; Blaustein et al., 1994). Thus, both their limited mobility and strict physiological requirements place constraints on the suitability of nest- sites.

The suitability of a nest- site, however, will be dictated by more than just physiological requirements, but also by protection from predators and minimization of competition for resources. In many cases, the presence of conspecifics, predators, and competitors serve as negative cues for reproductive success (Resetarits and Wilbur, 1989; Vredenburg, 2004). Exposure to predators and increased competition for resources becomes particularly important for female salamanders that guard egg masses, not only because of the increased metabolic costs associated with brooding, but also because of the increased risk of direct mortality (e.g., Forester 1981). Because plethodontid salamanders nest- guard throughout egg development, the role of biotic stressors become important when assessing suitability of nest- sites at the microhabitat scale.

In this study, we examined southern two-lined salamander (*Eurycea cirrigera*) nest-site selection in headwater seepage wetlands (HSWs) in the South Carolina Coastal Plain. They have a biphasic life cycle in which females deposit egg clusters under fallen trees, slabs of bark, rocks, and leaves (Petranka, 1998) and nest- guard throughout the entire egg development period and shortly after larvae hatch (Forester, 1981). *E. cirrigera* are common throughout the southeastern US and primarily breed in streams and headwater seepages. Headwater seepage wetlands are unique within the Atlantic Coastal Plain in that they mimic the mountainous, stream-like habitats typical of populations across the majority of the species' range, while providing suitable nesting habitat within an atypical landscape (i.e., seepages are generally spring-fed, quick moving, permanent bodies of water, have gravel/ sandy bottoms, and fishless) (Grenfell et al., 2005). Thus, HSWs in the South Carolina inner coastal plain are important for understanding *E. cirrigera* nesting behaviors across a broad geographic range and among peripherally distributed populations.

We observed *E. cirrigera* nest-site selection at two spatial scales using coverboard sampling. At the broadest scale, we examined nest- site selection across the landscape of the seepage. At the finest scale, we examined nest- site selection at the microhabitat scale, focusing on use versus availability of habitat beneath a coverboard. We predicted that conditional parameters with high heterogeneity at both scales (e.g., temperature, water depth, hydrologic regime, etc.) would best describe the breeding landscapes and predict *E. cirrigera* nest-site locations. We hypothesized that hydrology and temperature were the most variable and would strongly influence female nest-site selection at the landscape scale. In contrast, we expected that biotic factors would contribute more to nest-site selection at the microhabitat (coverboard) scale, where competition for resources and predator avoidance are likely to be important. This study will enable us to better understand drivers of *E. cirrigera* nesting behavior as they relate to environmental heterogeneity. This information is crucial in advancing our understanding of ecological and evolutionary drivers of nest-site selection of plethodontid salamanders at multiple spatial scales and will further the understanding of the environmental factors affecting nesting success of *E. cirrigera.*

2.2 MATERIALS AND METHODS

Study area. We monitored nesting *E. cirrigera* at two headwater seepage wetlands in the South Carolina inner Coastal Plain. The Wannamaker Nature Preserve (WNP) (33° 38′ 37.05″ N, -80° 42′ 23.36″ W) and Singleton Plantation (SP) (33° 41′ 59.59″ N, -80° 43′ 11.33″ W) are located near St. Matthews in Calhoun County, South Carolina. The WNP is owned by the National Audubon Society, and SP is located on private property. Both study sites are characterized by a headwater seepage wetland at the base of an elevated, upland bluff dominated by a mixed-pine, hardwood forest (Tufford, 2011). Seepages are the surface expression of the underground water table and serve as the primary source of stream flow for many Coastal Plain streams and rivers (Harder et al., 2007; Dai et al., 2010). The seepages at both WNP and SP are characterized by a wide, sheet flow of water covering a substrate of sand, gravel, and pockets of mud.

Coverboard Sampling. We sampled for *E. cirrigera* nests using coverboards (Houze, 2002), which have been observed to provide nesting habitat for semi-aquatic plethodontid salamanders (S. Bennett, South Carolina Department of Natural Resources, unpub. data). We placed thirty plywood coverboards (1.2 cm x 61cm x 61 cm/ 3-Ply Rtd Sheathing) throughout each study site using a random-walk sampling design and basing direction on a random azimuth (0-350, at 10 degree intervals) and distance (5-25 meters). Coverboards placed a minimum of 5 meters apart were allowed to "weather" in the seeps (e.g., worn by water, sun, and rain) for approximately one year before we began monitoring them for nests.

From May 2010 through February 2012, we sampled coverboards approximately every two weeks for *E. cirrigera* nests. We used digital photography (EOS Digital Rebel XS 1000D) to record the coverboard landscape and identify nest locations. Each coverboard was sampled by first orienting it northward to ensure that subsequent sampling of a coverboard would be recorded from the same perspective, and then flipping the coverboard to examine the area beneath for salamanders and nests (Figure 2.1). Adult salamander locations were marked with colored pegs and individuals collected for identification (photographed) were measured for snout-vent length SVL (mm), total length TL (mm), and mass (g).

Seepage Scale. We recorded daily high and low temperatures under each coverboard using a single Thermocron iButton data logger attached to the underside of coverboard. During each sampling event, we recorded coverboard temperature and water temperature using a noncontact thermometer (Raynger ST60 ProPlus). We measured average water depth (cm) by averaging the water depth of five locations (one in each corner and one in the center of the substrate). Prior to flipping each coverboard, we visually estimated the percent of the coverboard exposed to sunlight (Table 1).

All statistical analyses were performed using SAS software, version 9.2.1. In the analysis, we only included coverboards that were sampled during the *E. cirrigera* nesting season (November- April). We did not include coverboards that were lost or that where not sampled (e.g. floated away or buried in mud). All other coverboards (*N*=43) were classified as either “used” (1) or “not used” (0) for nesting during the nesting season. We averaged data collected from repeated visits to the same coverboard and then z-standardized all continuous environmental predictors (Table 1) to examine nest-site selection.

We used logistic regression to model the probability that a female nesting under a coverboard. We examined correlation coefficients for all predictors, prior to analysis (PROC CORR). If $r > 0.70$, we selected one the variables in the pair for analysis and excluded the other. We developed eight candidate models *a priori* to examine the effects of abiotic stressors on nest-site selection (Table 2). We expected that water temperature would have the greatest influence on nest location and therefore included it as a predictor in several models. We also included the coverboard and iButton temperatures, water depth, and sun exposure in separate models. We used $\mathrm{AIC_c}$ model selection (Burnham and Anderson, 2002) to rank candidate models, retaining models with $\Delta\mathrm{AIC_c} < 2.00$ for inference. We examined goodness-of- fit of the global model using the Hosmer and Lemeshow goodness-of-fit test.

Coverboard Scale. During each sampling event, we photographed each nest and the landscape of the ground beneath the coverboard, as well as the underside of the actual coverboard, for mapping using ArcGIS version 10.0. Photographs were imported to ArcGIS and georectified to a 61 cm x 61 cm template to correct for distortion in the images. We mapped locations of nests (*N*=108) in ArcGIS and paired each "used" (1) nest location with 20 "random" (0) locations under the board. We categorized the substrate beneath each nest and random point as sand, coarse woody debris (CWD), or mucky soil. Similarly, we categorized the hydrology of each used and random nest location as either saturated, standing, or flowing. We measured distances from each nest and random location to the nearest edge of board, nearest crayfish burrow, and nearest neighboring nest (Table 1).

We used logistic regression to model where a nest occurred under a coverboard. We constructed seven candidate models *a priori* to test for an effect of hydrology, substrate, and distance to edge (Table 3). We used AIC_c model selection to rank models. We retained models with a $\Delta AIC_c < 2.00$ for inference. We examined goodness of fit of the global model using the Hosmer and Lemeshow goodness-of-fit test.

2.3 RESULTS

Seepage Scale. Top ranking models for nest-site selection at the seepage scale included water temperature and water depth (Table 2.2). Hosmer and Lemeshow goodness-of-fit statistic indicated that our global model fit the data (p= 0.24). The probability of a nest occurring under a coverboard increased as the water temperature under the coverboard increased ($\beta = 1.3098 \pm 0.487$, df = 1, $\chi^2 = 7.2314$. $p = 0.0072$, 95% CI= 0.3553 to 2.2642; Figure 2.2). For every degree increase in water temperature (^{o}C), there was an increase in the probability of a nest occurring under the coverboard (odds ratio 95% CL= 3.71, 1.43-9.62). Water depth was negatively associated with the probability of nesting (β = -0.8917 ± 0.461, df = 1, χ^2 = 3.747, 95% CI=- 1.7947 to 0.0112, p = 0.0529) (Figure 2.3). For every centimeter increase in water depth, there was a decrease in the probability of a nest occurring under the coverboard (odds ratio, 95% CL= 0.41, 0.17-1.01). There was no effect of site (χ^2= 0.18, df= 1, $p<0.05$) which allowed us to pool data across locations for analysis.

Coverboard Scale. One candidate model was supported at the coverboard scale of analysis. The best model included hydrology, substrate, and distance to board edge (Table 4). The Hosmer and Lemeshow goodness-of-fit statistic suggested that the global model fit the data (p = 0.22). Nests were negatively associated with saturated hydrology (β = -0.376 ± 0.157, p <0.01) and positively associated with standing hydrology (β = 0.327 ± 0.172, p <0.05. Nest location was also positively associated with the distance to the edge of the coverboard (β=0.040 ± 0.013, p<0.01) (Figure 2.4). We failed to detect a significant effect of “flowing” hydrology or substrate on nest-site selection at the coverboard scale. These results suggest that during the nesting season (i.e. November to April) females are more likely to nest in areas of a coverboard that are slightly saturated with water and close to the interior of the coverboard. We tested for an effect of site (χ^2= 0.56) using the Chi- squared test of independence before pooling data for analysis.

2.4 DISCUSSION

Our results indicate that factors at multiple spatial scales influence nest site selection in *E. cirrigera*. At the landscape scale, the females selected areas of the seepage based on hydrologic and thermal properties. Their selection of coverboards in areas of warmer water could be due to the fact that in the coastal of plain of South Carolina, nesting occurs during the coldest months of the year (i.e. December- April). Females of peripheral populations in the coastal plain may therefore prefer warmer coverboards (analogous to the warmer, spring-like conditions of mountain streams). Warmer temperatures increase development rate, influence incubation time, and can impact offspring metabolism and survival (Bachman, 1969; Salthe and Mechum, 1974; Bradford, 1990).

Female *E. cirrigera* also preferentially nested under coverboards with relatively shallow water depth. This suggests that *E. cirrigera* prefer to nest in areas that are moist but not fully inundated. Nest location selection based on water availability is strongly driven by the pressure to reduce the risk of egg desiccation (Figiel and Semlitsch, 1995; Marco, 2001), avoid stream turbidity (Jakubanis et al, 2008), maximize oxygen exposure (Seymour and Bradford, 1987; Warkentin, 2001), and reduce exposure to aquatic predators (Barr and Babbitt, 2002; Lowe and Bolger, 2002; Petranka, 1983; Sih et al., 1992).

At the coverboard scale, female *E. cirrigera* used environmental cues to discriminate against nesting locations with the microhabitat of the coverboard. Though we were unable to detect a direct effect of biotic stressors on nest-site selection at the coverboard scale, the tendency of salamanders to nest closer to the coverboard center suggests a predator avoidance mechanism, as locations at the interior may be less exposed to predators. In addition, nest-site selection at the coverboard scale was influenced by the hydrology of the coverboard microhabitat. Females preferred to nest in areas of the coverboard that were slightly saturated with water as opposed to areas with deeper standing water or channels of flowing water. Nesting in the interior of the coverboard on moist, saturated substrate could help protect nests from being dislodged in a high flow event or from drying out during a period of drought (Guy et al., 2004).

In conclusion, nest-site selection by *E.cirrigera* is driven by environmental cues across multiple spatial scales. Female *E. cirrigera* actively select nesting locations based upon temperature, hydrology, and additional biotic factors. Fluctuations in environmental parameters, like temperature and water availability, are known to affect nesting behavior in salamanders, as well as other aquatic amphibians (Brodman, 1995; Figiel et al., 1995; Resetartis, 1996; Snodgrass et al., 2007, and Wahl et al., 2008). Because amphibians are particularly sensitive to environmental change, understanding the factors that influence key life history processes, particularly oviposition site choice, are critical to the conservation of amphibian populations and their habitats. Since female *E. cirrigera* discriminate among nesting habitats based on environmental variation, we can measure the fitness consequences associated with trade-offs between different nesting locations. Future developments in this area are critical in advancing our understanding of ecological and evolutionary drivers of nest-site selection and nesting success.

Table 2.1 Predictors and descriptions for *E. cirrigera* seepage- and coverboard-scale nest-site selection.

Scale	Predictor	Description
Seepage (landscape)	Water Temperature	Temperature of water beneath board during sampling
	Board Temperature	Temperature of water on top of board during sampling
	Water Depth	Average of five water depths taken at each corner and center of board
	iButton Daily High/ Low	Daily high and low temperature recorded from an iButton beneath board
	Sun Exposure	Visual estimation of percent of board exposed to sunlight
Coverboard	Substrate	
	Sand	Consisting of fine grains of sand and very small rocks
	Muck soil	Highly organic, dark colored mud
	CWD	Small scale decaying sticks/ wood, roots, and similar landscape structures
	Hydrology	
	Saturated	Soaked with water, but not pooling above the substrate surface
	Standing	Water is pooling on top of the substrate and is not moving
	Flowing	Water is moving over the substrate in a visible current
	Edge	Distance from nest to the nearest edge of the board
	Nest	Distance from nest to the nearest conspecific nest
	Crayfish	Distance from nest to the nearest crayfish burrow

Table 2.2 Logistic regression models, ranked according to statistical support, examining *E. cirrigera* nest-site selection at the Seep scale. Highest ranked logistic models using AICc model selection. The table shows model rank, number of estimated parameters (*k*); AICc differences (ΔAIC_c) and Akaike weights (w_i).

Rank	**Model**	***k***	**AIC_c**	**ΔAIC_c**	**w_i**
1	Water Temp and Water Depth	2	52.25	0.00	0.55
2	Water Temp	1	54.74	2.49	0.16
3	Water Temp and Board Temp	2	56.22	3.98	0.08
4	Water Temp and Sun Exposure	2	56.61	4.36	0.06
5	Board Temp	1	58.54	6.29	0.02
6	iButton Low	1	58.54	6.29	0.02
7	Water Depth	1	60.74	8.50	0.01
8	Sun Exposure	1	63.19	10.94	0.00

Table 2.3 Logistic regression models, ranked according to statistical support, used to examine *E. cirrigera* nest-site selection at the Coverboard scale. Highest ranked logistic models using AIC_c model selection. The table shows model rank, number of estimated parameters (*k*); AICc differences (ΔAIC_c) and Akaike weights (w_i).

Rank	**Model**	***k***	**AIC_c**	**ΔAIC_c**	**w_i**
1	Hydrology, Substrate, Distance	3	848.46	0.00	0.67
2	Hydrology	1	853.18	4.72	0.06
3	Substrate, Hydrology	2	855.38	6.93	0.02
4	Edge	1	857.23	8.77	0.01
5	Substrate	1	859.09	10.64	0.00
6	Crayfish	1	866.43	17.97	0.00

Figure 2.1 Image of the underside of a coverboard after flipping. White triangles mark the corners of the landscape, the white arrow designates north, and the black arrows show the colored pegs used to mark locations of salamanders upon flipping the coverboard.

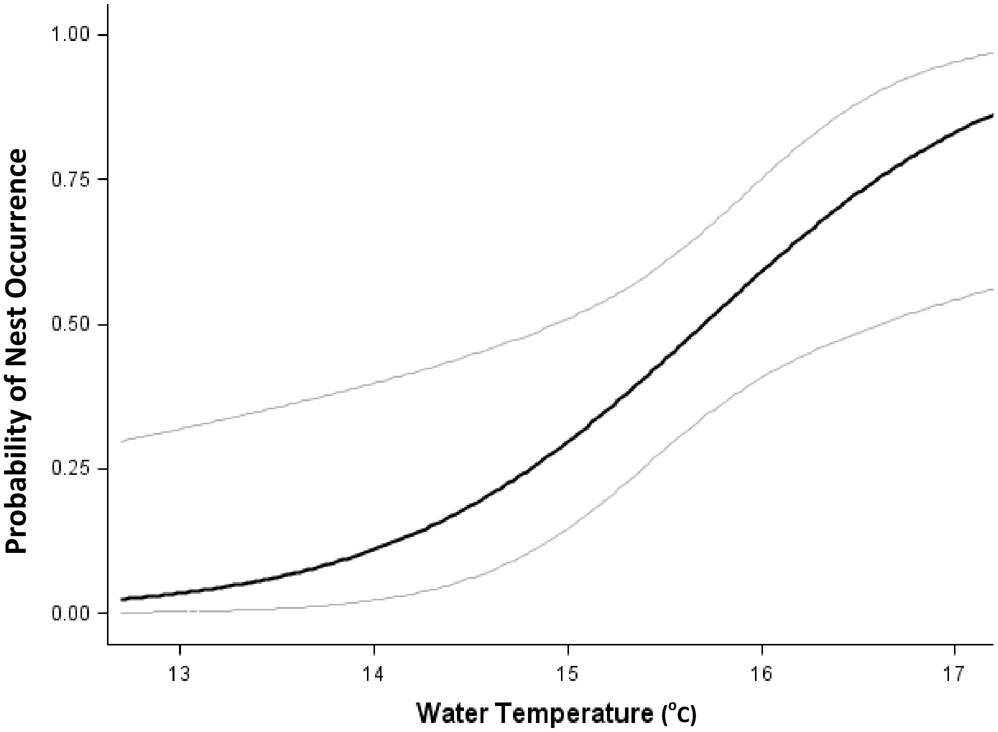

Figure 2.2 The relationship between *E. cirrigera* nesting probability and water temperature, based on output from the top supported model. As water temperature increases, the probability of nest occurrence increases. Gray lines represent 95% confidence intervals.

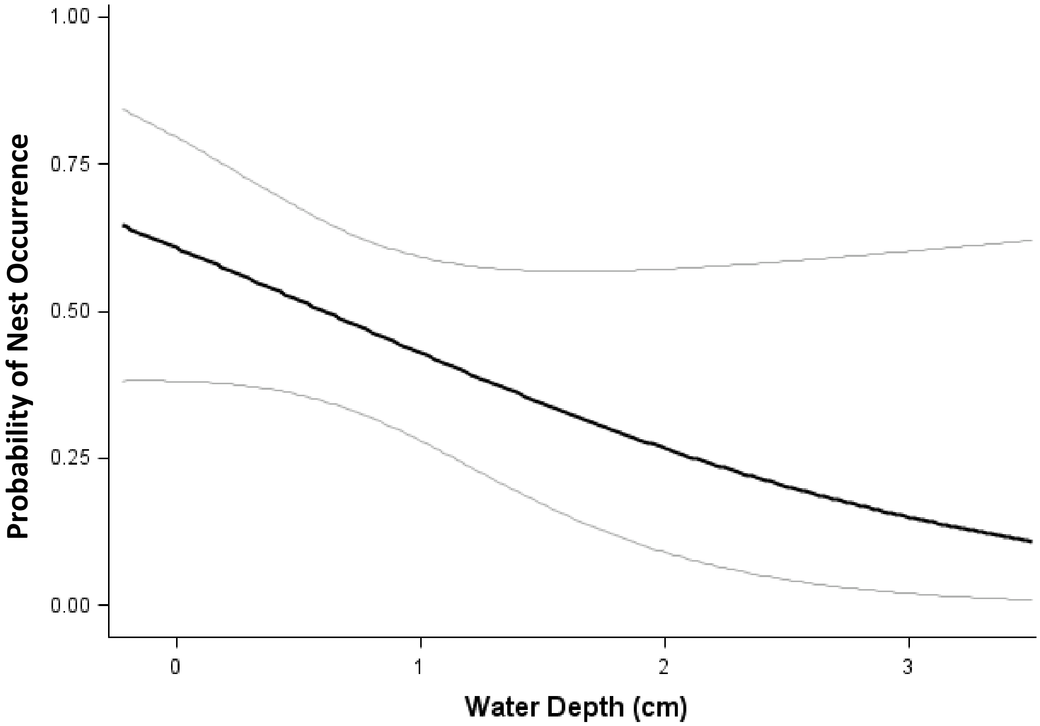

Figure 2.3 Relationship between *E. cirrigera* nesting probability and water depth (cm) underneath a coverboard, based on output from the top supported model. As water depth increases, the probability of nest occurrence decreases. Gray lines represent 95% confidence intervals.

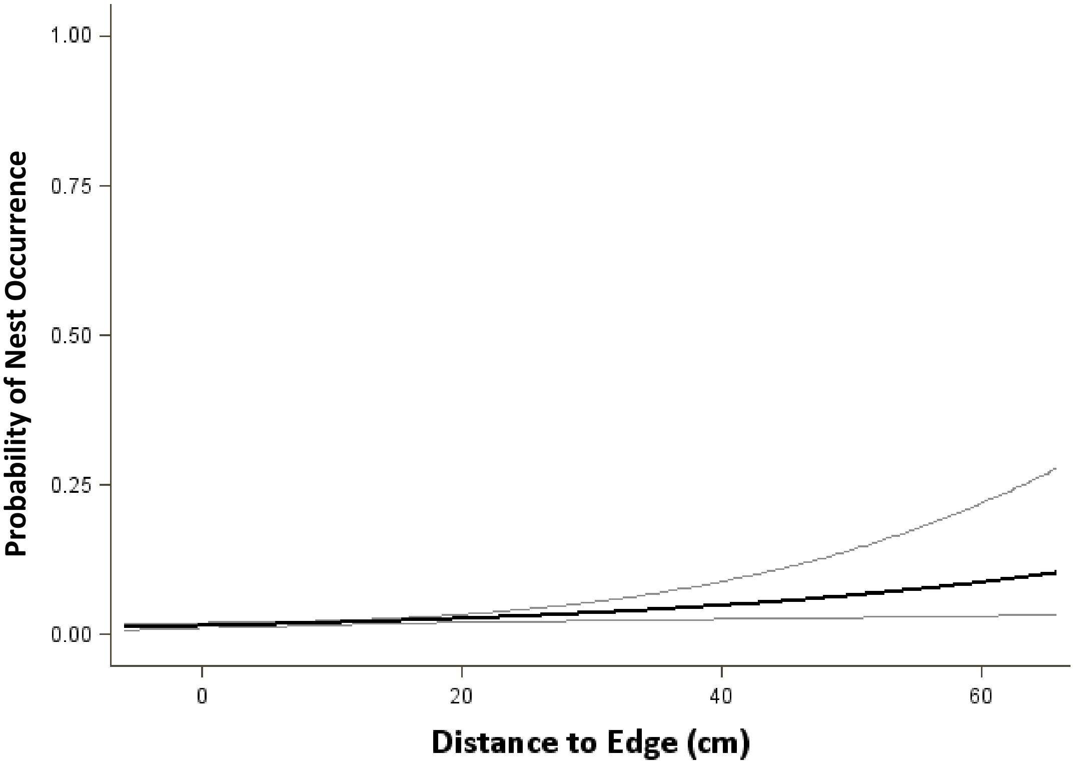

Figure 2.4 Relationship between *E. cirrigera* nesting probability and distance to the edge of a coverboard, based on output from the top supported model. As the distance to the edge of the board increases, the probability of nest occurrence increases. Dotted lines represent 95% confidence intervals.

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APPENDIX A – BREEDING EVENT OBSERVATIONS

Reference	Location	Species	Breeding Event	Time
Bahret, 1996	Lake Minnewaska, NY	E. bislineata	1. Gravid Females (8) 2. Nests (11)	1. March, April, June, July 2. Late May- Mid-July
Baumann and Huels, 1982	Pine Creek: Hocking County, OH	E. bislineata	Nests (49)	May
Beane, 2009 (Unpub.)	Wake County, NC	E. cirrigera	1. Nest 2. Nest	1. January 2. February
Bishop, 1925	"the north" (New York)	P. ruber	Nest	Fall
Brimley, 1939	Pitt County, NC	E. cirrigera	Nest	March- April
Bruce, 1974	SC Piedmont	P. ruber	Gravid Females	August- early Autumn
Bruce,1978	Jackson, Macon, Transylvania, NC and Oconee, SC	P. ruber	Gravid Females	Late Spring-Summer to Autumn
Bruce,1979	Jackson, Macon, Transylvania, NC and Oconee, SC	P. ruber	Males (11)	July 15-Sept. 19
Bruce, 1980	Jackson, Macon, Transylvania, NC and Oconee, SC	P. ruber	Breeding	Summer
Bruce, 1981	Jackson, Macon, Transylvania, NC and Oconee, SC	P. ruber	Nests	Fall
Bruce,1982	Tuckasegee River basin, Jackson County, NC	E. bislineata	Nests	Late Winter, Early Spring
Bruce, 1982	Sateetlah Creek, Graham County, NC	E. bislineata	Nests (2)	15-May-13

Carr, 1940	Liberty and Jackson County, FL	E. cirrigera	Gravid females/ Nest	November
Eaton, 1953	5 miles east of Greenville, NC	D. auriculatus	Nest	October
Enge, 1998	Havana, Gadsden county, FL	E. cirrigera	Breeding	November – mid-March
Goin, 1951	3 miles N Gainesville, Alchua County, FL	D. auriculatus	Nest (10)	October
Brophy and Pauley, 2002	Fitzpatrick's Branch, Huntington, Cabell County, WV and Beech Fork, Bowen, Wayne County, WV	E. cirrigera	1.Gravid (7), Males (30) 2. Breeding 3. Nest 4. Males	1.February – March 2. March 3. mid-March-early-April 4. February-March
Guy et al., 2004	Boscoe Creek and Lee Creek, Thompson Mill Forest, GA Piedmont	E. cirrigera	Nest (37)	April
Jakubanis et al., 2008	Vermilion River Observatory Research Area, IL	E. cirrigera	Nests (441)	Spring – Summer
King, 1939	Elkmont, Gatlinburg, TN (Little River) Great Smoky Mountains	E. bislineata	1. Gravid 2. Nests	1. March 2. April
Marshall, 1996	Poplar Cove Spring Uni. Miss. Biological Field Station Lafayette, MS	E. cirrigera	Nests (7)	April-May
McDowell, 1995	11 km N of Glendale, Pope County, IL	E. cirrigera	Gravid (33)	April
Miller and Niemiller, 2005	Caney Fork River, Dekalb County, TN	P. ruber	Nest	September
Neill and Rose, 1949	Coastal Plain side of Augusta, Richmond County, GA	D. auriculatus	Nest	June

Niemiller and Miller, 2007	SW slope of Short Mountain, near head of Mountain Creek, Camon County, TN	E. cirrigera	Nest (9)	January- February
Robertson and Tyson, 1950	Little Contentnea Creek, 10 miles west of Greenville, Pope County, NC	D. auriculatus	Nest (6)	September
Stewart, 1968	New York State, Finger Lakes Region	E. bislineata	Eggs	April- May
Wilder,1924	Northampton, Western, MA	E. bislineata	Eggs	May- June
Wood, 1949	Indian Gap, TN and Wayah Bald, Hyatt Ridge and Mt. Mitchell, NC	E. bislineata	Gravid Females (7)	April- May
Wood, 1949	Mt. Mitchell, NC	E. bislineata	Nest	May
Wood, 1953	near Williamsburg, James City County, VA	E. bislineata	Nests (4)	February
Thurow, 1997	Brown County, IN	E. cirrigera	Nests (8)	April
Thurow, 1998	Central McDough County, IN	E. cirrigera	1. Nests (1) 2. Nests (3)	1. May 2. May