Marbled salamanders (*Ambystoma opacum*) choose low elevation nest sites when cover availability is controlled

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**Abstract.** Nest site selection is an important part of adult reproductive behavior because growth and survival of young are often affected by the local environment. In terrestrially nesting marbled salamanders, nest elevation is likely important to reproductive success because it is directly related to the time of hatching. We tested the hypothesis that females choose nest sites based on elevation and its correlates by controlling the availability of nesting cover, a potentially important factor in nest site selection which often covaries with elevation. Breeding adults were confined to field enclosures in which natural nesting cover had been removed and replaced with equal proportions of artificial cover in each of three elevation zones. In four enclosures that spanned from lowest to highest areas of a wetland breeding site, females used artificial nesting cover most frequently at low elevations. These results contrast with other studies in which intermediate elevations had highest nest densities, but are consistent with a conceptual model in which opposing selective forces result in locally adapted nest site selection.

**Introduction**

Nest site selection is an important component of adult reproductive fitness in many organisms and should be favored by natural selection if benefits to offspring survival outweigh the costs to adults (Resetarits, 1996). For example, eggs should be placed in a microenvironment favorable for embryonic development and inaccessible to predators. Additionally, because abiotic conditions affect the sex (Janzen and Paukstis, 1991), size (Packard and Packard, 1988), growth rate (Brooks et al., 1991), and locomotor performance (Shine et al., 1997) of offspring, variation in nest site preferences can influence nest success and offspring fitness (Janzen, 1995; Sadeghi and Gilbert, 1999).

Egg placement can contribute to reproductive success in amphibians (e.g., Resetarits and Wilbur, 1989). For example, individuals are likely to oviposit in areas with few predators and parasites (Kiesecker and Skelly, 2000). Also, water temperature, vegetation, litter, and elevation may provide cues that amphibians use to select breeding and oviposition sites (Petranka and Petranka, 1981a; Seale, 1982). Terrestrially nesting amphibian species may be especially likely to exhibit adaptive nest site selection because of the vulnerability of amphibian eggs to desiccation.

Marbled salamanders (*Ambystoma opacum*) breed and nest terrestrially during late summer and autumn in seasonal wetlands in the eastern United States. Females choose nest sites beneath logs, leaf litter, or vegetation in which they oviposit and then remain with their clutches for variable durations, usually from a few weeks to 3 months (see Petranka and Petranka, 1981a, b; Jackson et al., 1989). Embryos hatch and become aquatic larvae when nests are flooded by fall and winter rains (Petranka et al., 1982). They are ready to hatch after 9 to 40 days of development (Petranka, 1998; J.S. Doody, pers. comm.).

Petranka and Petranka (1981a) proposed a conceptual model to explain the evolution of nest site selection in marbled salamanders. They reasoned that optimal nest placement along an elevational gradient depends on opposing biotic and abiotic selective forces (fig. 2 in Petranka and Petranka, 1981a). Nesting at very...
high elevations should not be favored because eggs are less likely to be inundated early in the larval period, if at all. Conversely, nests placed at very low elevations are likely to be temporarily flooded by heavy rains early in the season only to subsequently dry, resulting in complete hatching mortality. Thus, nest sites are expected to be clumped at intermediate elevations. However, their model also posits that intraspecific competition among aquatic larvae may promote a dispersed distribution of nests and that the actual pattern of nest distribution reflects an equilibrium between these opposing pressures.

Petranka and Petranka (1981a) and Petranka (1990) tested their nest site selection model at ponds in Alabama and North Carolina (depths ranging from 40 to 100 cm). They observed that nest distributions were generally clumped, with highest densities at intermediate elevations. Graham (1971) reported similar results in New Jersey. However, Jackson et al. (1989) found that nests were concentrated at the lowest elevations of a Carolina bay in South Carolina where cover was most abundant. Both Jackson et al. (1989) and Petranka (1990) noted that cover availability, as well as elevation, may be an important determinant of nest site selection at some breeding ponds.

To separate the influence of nesting cover availability from elevation (and correlated variables) on nest site selection in marbled salamanders, experimental manipulations are needed to test the ideas generated by observational studies. The purpose of this study was to test the hypothesis that female marbled salamanders choose nest sites based on elevation and its correlates by controlling the availability of nesting cover across an elevation gradient. We built field enclosures spanning the range of elevations within our study site, replaced natural nesting cover with artificial cover, and observed the patterns of nest site selection by females that were added to the enclosures.

Materials and methods

Study site

This study was conducted at Ginger’s Bay (GB; 33°18′55″N, 81°46′24″W), a small (0.8 ha), shallow Carolina bay (Sharitz and Gibbons, 1982) in Aiken County, South Carolina, USA that typically fills with water in late autumn and dries by midsummer (Scott, 1990). The bay consists of three elevation zones that may be recognized by vegetation patterns (Jackson et al., 1989). Our field enclosures spanned each of the three zones. The predominant vegetation was knotweed (*Polygonum* sp.) in the low zone (0-15 cm above the depression bottom), sedge (*Scirpus cyperinus*) in the intermediate zone (16-27 cm), and red maple (*Acer rubrum*) and loblolly pine (*Pinus taeda*) trees along with greenbriar (*Smilax* sp.) in the high zone (28-82 cm). Jackson et al. (1989) showed that salamanders use each of these elevation zones for nesting at GB.

Field experiment

In September 2000 we expanded four previously constructed field enclosures (see description in Scott, 1990). The old enclosures, which were used for manipulations of aquatic larvae, were located in the low elevation zone and constructed of 91.4 cm high buried aluminum flashing. Before this study, no experiments with adult marbled salamanders had been performed within these enclosures. The additional enclosure walls were made from 24-cm high aluminum flashing buried 3-7 cm below the soil surface. The expanded enclosures spanned the range of available elevations at GB, from the low knotweed zone to the high zone of trees and greenbriar. The total available area for nesting varied among enclosures (from 112 to 223 m²) and among elevation zones within single enclosures. In each of the four wedge-shaped enclosures, the high elevation zone was the largest, followed by the intermediate zone, and the smallest low elevation zone. This geometric relationship is also true of the entire wetland (e.g., the low zone is approximately 12% of the total wetland area). Although the area of the elevation zones differed, we created an equal proportion of artificial cover in each zone so that cover availability was constant among enclosures and elevation zones. Artificial nesting cover, 31 by 124 cm particle boards, was present at a ratio of one unit of cover area for each 27 units of area in each elevation zone of the four enclosures. In each zone, one board was cut into a smaller piece to adhere precisely to this ratio. To encourage salamanders to use the boards for nesting, we placed leaf litter around the margins of each board. All other leaves, logs, and other debris that could be used for nesting were removed. We used a weed trimmer to remove all herbaceous vegetation, making ground level nesting cover nearly identical across elevation zones. Cover boards were not placed near the margins of the elevation zones but were clustered into three distinct groups, one each at the center of the low, intermediate, and high elevation zones.

From 24 October to 9 November 2000, during the autumn nesting season, we stocked the enclosures with an
equal sex ratio of 484 total salamanders ($\bar{x} = 121$ salamanders, or 60.5 females, per enclosure) that had migrated to GB in October and November. Animals were released at the highest ends of the enclosures, which simulated their normal orientation upon arrival at the wetland. Breeding populations of >10,000 individuals are known from GB, so enclosure densities were not unusually high. Enclosures were stocked with 10.76 females per square meter of total artificial cover. In November, boards were checked for nesting females daily. Clutches and attendant females were removed immediately after discovery and used in other studies. We recorded the total number of nests located under artificial cover in the three elevation zones for each of the four enclosures.

Statistical Analysis. We expressed the data as the number of nests per square meter of artificial cover. Because the data did not conform to a normal distribution, we used the non-parametric Kruskal-Wallis test in the NPAR1WAY procedure in SAS (SAS Institute, 2003) to detect differences in the density of nests occurring under artificial cover among the elevation zones. If cover were the primary influence on nest site selection (as opposed to elevation and its correlates), then we would have expected similar nest densities in the three elevation zones. Subsequently, we performed all pairwise comparisons with the same Kruskal-Wallis test to determine which zones were different from one another.

Results

We located 43 nests under artificial cover in the four enclosures, an average of 10.75 per enclosure. Of these, 37 were in the low elevation zone ($\bar{x} \pm SD = 9.25 \pm 2.63$) and the remaining six nests were in the intermediate zone ($\bar{x} \pm SD = 1.50 \pm 1.73$). No salamanders nested in the high zone. Of the 242 females originally stocked in enclosures, 199 (82.23%) did not nest under the artificial cover. The difference in numbers of nests per unit area cover among the elevation zones was statistically significant overall ($H = 9.43$, df = 2, $P < 0.01$). Specifically, the low zone was preferred over the intermediate zone ($H = 5.33$, df = 1, $P < 0.05$) and the high zone ($H = 6.05$, df = 1, $P < 0.05$); the intermediate zone was also preferable to the high zone ($H = 3.94$, df = 1, $P < 0.05$).

Discussion

Our data show that female marbled salamanders prefer to nest in the lowest areas of Ginger’s Bay when cover availability is held constant. Significantly more salamanders (37 of 43) nested beneath artificial cover in the low elevation zone than in the intermediate or high zones. In fact, no salamanders used the experimental cover for nesting in the highest parts of our field enclosures. These results are in accordance with those of Jackson et al. (1989) who observed more nests in the lowest areas of this site, but differ from other studies which found that nests occur more frequently at intermediate elevations of breeding ponds (Graham, 1971; Petranka and Petranka, 1981a; Petranka, 1990).

Because we experimentally controlled the relative availability of artificial nesting cover in the enclosures and elevation zones, this study shows that female marbled salamanders select low elevations for nesting and do not rely solely on areas of high cover availability. However, because we could not control other covariates of elevation, our results do not necessarily show that salamanders choose nest sites based on elevation alone. Numerous other variables are correlated with elevation and may have been important cues used by salamanders to select preferred areas for nesting. For example, informal field observations indicated that soil moisture was negatively correlated with elevation in our enclosures; the low elevation zone had highest soil moisture. Other unmeasured gradients, such as temperature, soil organic matter, fine root biomass, and soil texture may have also varied with elevation.

In our experimental design the primary objective was to standardize cover type and availability throughout the three elevation zones. Instead of natural cover objects (i.e., Polygonum mats at low elevation, sedge clumps at intermediate, and logs and leaves at high), we used a cover type (i.e., particle board) that prior observations of wetland cover board transects had shown to provide suitable cover at all elevations. The cover boards created nesting condi-
tions comparable to those beneath natural cover objects a few meters outside of our enclosures, and non-nesting males and females routinely took refuge under the boards in all elevation zones. Although removal of litter in the enclosures may have decreased soil moisture away from the cover boards, it probably did not affect our results. The nesting period occurred entirely during the last three weeks of November, during which 8.9 cm total rain fell on eight different days (Savannah River Technology Center, 2000). The rich organic humus layer retained moisture throughout the study. Thus while the cover boards themselves served as islands of suitable nest and refuge habitat across elevation zones, we are confident that environmental conditions remained moist enough to allow for nocturnal movements among zones and the possibility for nest site choice.

Social interactions among the female salamanders probably did not affect the distribution of nests within the enclosures. Each cover board was checked daily for nesting females which were removed immediately upon discovery, diminishing much of the social influence of previous nesters. Additionally, observational studies by Graham (1971) and Petranka (1990) have shown that communal nesting is infrequent in marbled salamanders when nesting cover is prevalent. Territoriality among nesters, if present, would have resulted in greater dispersion of nests among the elevation zones and a more conservative interpretation of the results.

We only found 17.8% of the stocked females nesting under cover boards. A majority of the remaining females probably either escaped the enclosures or died. Some portions of each enclosure were as short as 18 cm and could have been traversed, especially if salamanders were highly motivated to escape immediately after release into the cleared enclosures. In addition, natural mortality during the breeding season can be high; many breeding immigrants at a nearby wetland (range of 11-72%, $\bar{x} = 39\%$, $n = 25$ years of study at Rainbow Bay) never return to the terrestrial habitat (D.E. Scott, unpublished data). The cleared portions of the enclosures may have increased predation by raccoons, opossums, and skunks, which had access to the enclosures and can prey on adult *A. opacum* (Scott, 2005).

Our finding that females most frequently nested at low elevations in GB contrasts with a preference for intermediate elevations reported by other studies (Graham, 1971; Petranka and Petranka, 1981a; Petranka, 1990). The depth of our field enclosures (82 cm) was comparable to the depths of the ponds in these studies. The varied results can be explained by the unique characteristics of different breeding sites and are nonetheless consistent with a nest site choice model (Petranka and Petranka, 1981a) in which opposing selective forces result in locally adapted nest site selection. An implicit assumption of the model is that suitable nesting cover is available across the elevation gradient. At our study site, cover happens to be most prevalent at low elevations (Jackson et al., 1989) but other sites have very little cover in their lowest areas (Petranka, 1990).

Given suitable nest cover, other selective forces operate to determine nest distribution as conceptualized in Petranka and Petranka’s model (1981a). The most important factor in opposition to low elevation nesting is temporary pooling in low areas, followed by subsequent rapid drying and hatchling death (Petranka and Petranka, 1981a). But this hydrologic pattern rarely occurs at GB, resulting in little or no selection against low elevation nesting. Other wetland habitats may be more likely to pool temporarily than Carolina bays because of differences in soil properties. The sites studied by Petranka (1990) and Petranka and Petranka (1981a) often fill temporarily.

As Petranka and Petranka (1981a) pointed out, selection also acts to limit nest placement at high elevations where nest success may be decreased by freeze damage, failure of nests to become inundated, reduced growth period of larvae, and increased interspecific competition, most of which occur at GB (Jackson et al.,
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1989; Boone et al., 2002; Scott, 2005). GB and other Carolina bays often fill gradually, allowing earlier hatching for nests at low elevations. Boone et al. (2002) found that early-hatching *Ambystoma opacum* larvae were larger at metamorphosis, had higher survival, and metamorphosed sooner than late-hatching larvae. Overall, at the GB site we have observed strong selection against nesting at higher elevations, but little or no selection against low elevation nests.

Thus, the contrasting results of several studies of nest site selection in marbled salamander populations suggest local adaptation to unique suites of opposing selective forces. Hydrologic patterns influence nesting via their varying effects on hatching probability, time of hatching, potential hatching mortality caused by temporary filling, and interspecific competition. Additionally, females undoubtedly show considerable behavioral plasticity in using their nesting habitat.

Our study is the first to investigate the potential effects of elevation on nest site selection in *Ambystoma opacum* while experimentally controlling cover availability. Most females nested in the lowest parts of our study site, few nested in the intermediate elevation zone, and none used the high zone. Salamanders may have used elevation, soil moisture, other correlated factors, or some combination as a cue in assessing potential nest sites. Further experiments are needed to separate the effects of these variables. Geographic differences in nest placement may reflect local adaptation and/or opportunistic use of available nesting habitat.

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