

Evaporative Water Loss in Two Natricine Snakes, *Nerodia fasciata* and *Seminatrix pygaea*

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Integrating knowledge of physiological adaptations and constraints of animals is crucial for a deeper understanding of their behavior and ecology (Lillywhite, 1987; Lillywhite and Smits, 1992). For instance, physiological characteristics are often correlated with a species' ecology, and, thus, they may act as constraints on terrestrial activity and habitat use (e.g., Lillywhite, 1987). One such physiological characteristic, evaporative water loss, has long been documented to correlate with habitat use in ectotherms and may be a constraining factor operating on their behavior and spatial distributions (reviewed in Mautz, 1982; see below). Evaporative water loss (EWL) is the water lost by evaporation, through the surface of the skin (cutaneous water loss) and respiratory passages (respiratory water loss); EWL does not include body water lost through excretory processes (Mautz, 1982). Physiological variables [e.g., shedding cycle and peripheral circulation (Cohen, 1975), body size (Gans et al., 1968; Mautz, 1982), and acclimation (Kattan and Lillywhite, 1989)], as well as behavioral characteristics [e.g., activity levels (Gans et al., 1968), and exposed surface area and body posture (Cohen, 1975)] are known to influence EWL in reptiles. Abiotic factors, such as temperature, humidity, and wind speed also may be significant factors affecting EWL (Cohen, 1975; Mautz, 1982).

Contrary to popular belief, reptile scales are not major inhibitors of EWL in snakes (Licht and Bennett, 1972; Bennett and Licht, 1975). Rather, the concentration of lipids in the epidermal layer appears to be the single variable most responsible for limiting EWL (Roberts and Lillywhite, 1980; Lillywhite and Maderson, 1982; Baeyens and Roundtree, 1983). In snakes, the rate of cutaneous water loss is the major contributor to both overall rates of EWL and between-species differences in EWL; the contributions of respiratory water loss appear to be relatively equal among species (Prange and Schmidt-Nielsen, 1969; Dmi'el, 1972).

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TABLE 1. Size of *Nerodia fasciata* and *Seminatrix pygaea* used in the unconstrained and constrained experiments. The results of one-way ANOVAs are based on log-transformed data; mean and SE reported are untransformed.

N:	Unconstrained experiment		Constrained experiment	
	<i>N. fasciata</i> 9	<i>S. pygaea</i> 15	<i>N. fasciata</i> 8	<i>S. pygaea</i> 14
Mass (g)				
Mean	18.55	14.42	21.37	13.57
SE	2.25	1.27	2.87	1.01
	$F_{1,22} = 2.04, P = 0.1670$		$F_{1,20} = 5.31, P = 0.0321$	

In this paper, we compare the total EWL (respiratory and cutaneous water loss combined) of two natricine snakes (Colubridae: Natricinae): the black swamp snake (*Seminatrix pygaea*) and the banded watersnake (*Nerodia fasciata*). *Seminatrix pygaea* is among the most aquatic of the natricine snakes, rarely leaving aquatic habitats unless under extreme duress (e.g., Dodd, 1993; Seigel et al., 1995), whereas *N. fasciata* frequently leaves aquatic habitats to bask (Hebrard and Mushinsky, 1978; Ernst and Barbour, 1989) and disperse overland to other suitable aquatic habitats (Seigel et al., 1995). Because of a well-demonstrated positive correlation between terrestriality and decreased EWL among ectotherms (Gans et al., 1968; Prange and Schmidt-Nielson, 1969; Elick and Sealander, 1972; Cohen, 1975; Mautz, 1980; Baeyens and Roundtree, 1983), we expected *N. fasciata* to exhibit lower rates of EWL than the more aquatic *S. pygaea*. In addition to quantitative comparisons of EWL, we briefly discuss the effects of activity, death, and posture (coiled vs. uncoiled) on rates of EWL in these species.

All *S. pygaea* and *N. fasciata* used in the following experiments were captured from Ellenton Bay, a 10-ha Carolina bay located on the Savannah River Site in Aiken County, South Carolina (Gibbons and Semlitsch, 1991; Seigel et al., 1995), from 26 May to 21 June 1999, using unbaited minnow traps set along the margin of the bay. Adult *N. fasciata* are much larger than *S. pygaea* [adult lengths in this population range from 32–167 cm snout-vent length in the former species (unpubl. data) and up to 48.5 cm in the latter (Kean and Tuberville, 1995)]. Because the ratio of surface area to volume is usually positively correlated with EWL (Gans et al., 1968; Mautz, 1982), we used only juvenile *N. fasciata* of a size class similar to the distribution of adult *S. pygaea* in the Ellenton Bay population (Table 1). Both sexes were used in the experiments.

To prevent differences in EWL resulting from acclimation differences (Kattan and Lillywhite, 1989), snakes were housed individually in polyethylene shoeboxes (33.0 × 18.4 × 10.5 cm) filled to 1 cm with unfiltered wellwater; to provide refugia, paper towels were placed in the water on one end of each shoebox. Water and paper towels were changed daily to deter algal and bacterial colonization within the shoeboxes. The snakes were maintained at temperatures between 26°C and 28°C. Once a week, each snake was fed one *Ambystoma talpoideum* larva, weighing approximately 10% of the snake's mass. However, prior to data collection, food was withheld from the snakes for 10 days to ensure that all individuals were postabsorptive during experiments.

We performed two experiments. In the first experiment (hereafter, the "unconstrained" experiment), all animals had been held in the laboratory for a minimum of 18 days. This ensured that each individual ate at least twice in captivity, and, thus, all snakes were assumed to be in good health. Because shedding can increase EWL (Cohen, 1975), shedding snakes were excluded from the experiment. On the day of the unconstrained experiment, the initial mass of each snake was recorded to 0.001 g using an electronic balance, after being blotted dry to remove surface moisture. Each individual used in the experiment was then placed inside a cylindrical steel mesh cage (88.8 mm long, 60 mm diameter, mesh openings 3.25 mm) that was large enough to permit both coiling and limited movements. The mass of both the cage and the snake together was recorded. Immediately following the determination of masses, each snake was placed into a lighted environmental chamber (Percival Scientific, model 1-37LLVL) set at 27°C and 50% humidity. We determined the mass of each snake plus its cage every hour for 6 h; the loss of mass over each measurement interval was attributed to EWL. In addition, we made behavioral observations every 15 min to determine relative activity of each snake within the environmental chambers. Observations were made through a window so as to minimize disturbance. During each observation, the position of each individual within its cage was noted, and a score was assigned as follows: "resting" if it was motionless and its body overlapped at more than one point (= 0 points); "slightly active" if it was motionless but had its head elevated (= 1 point); "moderately active" if it was moving but not actively pushing against the cage with its nose (= 2 points); or "very active" if it was actively pushing against the cage with its nose (= 3 points). We ranked individuals according to the sum of the activity observations over the 6-h testing period and used a Mann-Whitney *U*-test to evaluate differences in activity between the species, as higher rates of activity might increase respiratory water loss, thus increasing total EWL.

At the conclusion of the unconstrained experiment, snakes were returned to their shoeboxes and fed an appropriate sized *A. talpoideum* larva; all snakes ate within 24 h and were assumed to be in good health. Ten days later, after the snakes were assumed to again be postabsorptive, the experiment was repeated with modification. In this second experiment (hereafter referred to as the "constrained" experiment), after an initial mass was recorded, each snake was placed in a steel mesh cylinder (mesh openings 3.25 mm as

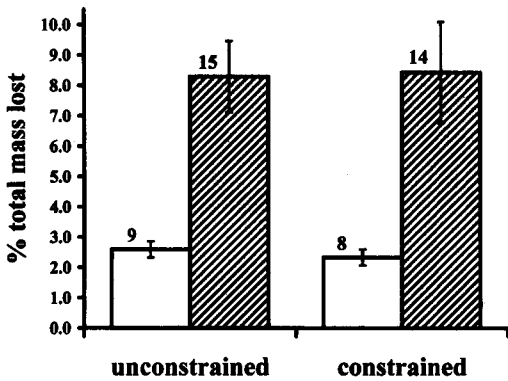


FIG. 1. Percent body mass lost attributable to evaporative water loss after exposure to 50% RH for 6 h in *Nerodia fasciata* (open bars) and *Seminatrix pygaea* (stippled bars); the samples sizes are indicated above each bar. The difference in the unconstrained experiment is significant at $P = 0.0005$, and at $P = 0.0226$ for the constrained experiment. Error bars represent 1 SE.

above) that required it to maintain a straight, elongated position. Placing the snakes inside the cylinders required extra handling time, resulting in lower initial masses relative to those of the unconstrained experiment. After the mass of the snake and the cylinder together was recorded, the snakes were placed into the environmental chamber under the same conditions as described above. Mass of each snake was recorded as above; however, because the snakes were forced to maintain an elongated position, no behavioral data were collected. All data, for both the unconstrained and constrained experiments, were collected between 1030 and 1630 during July 1999.

We sacrificed an individual of each species (by a lethal dose of ketamine) and immediately after death, recorded mass at the same intervals as with the live snakes. This was done to determine the extent to which EWL in these species is a function of structural components (i.e., epithelial lipid deposits) rather than physiological or behavioral processes (i.e., respiratory water loss).

We measured EWL as the percent total mass lost over the testing period (initial mass-final mass/initial mass); this value was angularly transformed (arcsine of the square root) to meet the assumptions of parametric tests. Mass values were otherwise log-transformed prior to analyses to increase additivity and normality.

Preliminary analyses demonstrated that there were no significant differences in size between the sexes in either species [analysis of variance (ANOVA); *N. fasciata*: $F_{1,7} = 0.3677$; $P = 0.5634$; *S. pygaea*: $F_{1,13} = 1.3655$; $P = 0.2636$], and thus we pooled the sexes within each species in all subsequent analyses. Because the *N. fasciata* were generally larger than the *S. pygaea* (Table 1), when analyzing water loss data, we used body mass as a covariate in analysis of covariance (ANCOVA) with species as the sole main effect (Packard and Boardman, 1987). Finally, these preliminary analyses also demonstrated that the interaction between mass

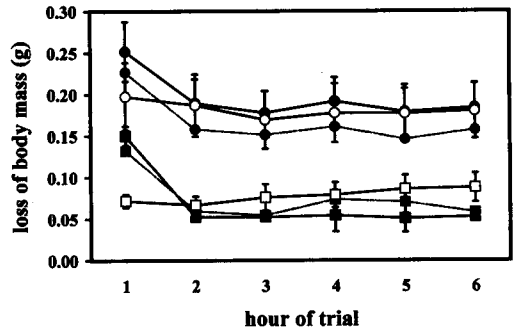


FIG. 2. Body mass lost each hour of exposure to 50% RH. Squares represent *Nerodia fasciata* and circles represent *Seminatrix pygaea*. For each species, closed symbols (mean \pm 1 SE) are used for the unconstrained experiment, open symbols (mean \pm 1 SE) are used for the constrained experiment, and gray symbols with broken lines are data collected from the single sacrificed individual. Sample sizes are as indicated in Figure 1.

and species (i.e., test for equality of slopes) was not significant in either experiment (unconstrained ANCOVA: $F_{1,20} = 0.16$; $P = 0.6924$; constrained ANCOVA: $F_{1,18} = 0.19$; $P = 0.6647$), and therefore this term was omitted from the subsequent analyses.

Seminatrix pygaea lost water at a rate nearly three times as great as that of *N. fasciata* in the unconstrained experiment (ANCOVA, Type III Sums of Squares; $F_{1,21} = 9.67$; $P = 0.0053$; Fig. 1). This result obtained despite the fact that *N. fasciata* were relatively more active than *S. pygaea* during the testing period [*N. fasciata*: $N = 9$, mean activity score (SE) = 2.28 (0.21); *S. pygaea*: $N = 14$, mean (SE) = 1.45 (0.23); Mann-Whitney U-test: $U_{9,14} = 104$; $P = 0.001$]. Restricting movement did not affect the loss of mass, as the difference between species was consistent between constrained and unconstrained experiments (constrained ANCOVA, Type III Sums of Squares; $F_{1,19} = 6.15$; $P = 0.0226$; Fig. 1). Circulation, peripheral vasomotor responses also appear to have no effect on EWL; sacrificed individuals of each species demonstrated rates of EWL comparable to the means of live snakes in the unconstrained experiment (Fig. 2). This suggests that respiratory water loss is a minor component of total EWL in these species, at least under the test conditions used. These results are expected if lipid deposition in the epithelial tissue serves as the single greatest barrier to EWL rather than some (active) physiological mechanism. Accordingly, we suggest that the observed differences between EWL in *N. fasciata* and *S. pygaea* are primarily a result of variation in epidermal lipid barriers (see Lillywhite and Maderson, 1982).

Our results are consistent with a general correlation between habitat and EWL (Gans et al., 1968; Prange and Schmidt-Nielsen, 1969; Elick and Sealander, 1972; Cohen, 1975; Mautz, 1980; Baeyens and Roundtree, 1983). *Seminatrix pygaea* is a semiaquatic snake that is rarely found far from permanent water sources (Dorcas et al., 1998), whereas *N. fasciata* is semiaquatic and commonly exhibits terrestrial activity (Hebrard and Mushinsky, 1978; Ernst and Barbour, 1989). Unlike *N.*

fasciata, terrestrial activity in *S. pygaea* generally only occurs on rainy nights (Dorcas et al., 1998), a time when rates of EWL may be greatly reduced, or when aquatic habitats dry (Dodd, 1993; Seigel et al., 1995). Also, in contrast to other natricines, *S. pygaea* avoids direct insolation (Dowling, 1950; Ernst and Barbour, 1989; J. W. Gibbons, pers. comm.). These behaviors would be expected of a species with a high rate of EWL. We submit that differences in microhabitat affinities and behavior in the two species under study may be attributable to the extremely high rate of EWL exhibited by *S. pygaea* relative to *N. fasciata*.

Cohen (1975) suggested that the higher rates of EWL exhibited by species living in more aquatic habitats may simply be a relaxation of the selective pressures of xeric habitats. Cohen reasoned that aquatic snakes would be wasting energy creating an effective water barrier when it is not needed, and, thus, species should only restrict EWL to the degree that is necessary. Although this mechanism is a likely cause of higher EWL rates in more aquatic snakes, we would like to suggest an additional possible adaptive advantage to the highly permeable skin of *S. pygaea*.

Highly permeable skins, characteristic of other highly aquatic snakes, such as the Australian file snakes (*Acrochordus* spp.) and sea snakes (e.g., *Hydrophis*), are often permeable enough to facilitate substantial cutaneous respiration (Heatwole and Seymour, 1978; Lillywhite and Ellis, 1994). Cutaneous respiration has been observed to account for as much as 24% of oxygen uptake and 76% of carbon dioxide loss in *A. granulatus* (Lillywhite and Ellis, 1994). Other, less aquatic snakes (e.g., *Constrictor*, *Nerodia*, *Thamnophis*), also have been shown to exhibit some degree of cutaneous respiration (Standaert and Johansen, 1974; Gratz, 1978; Costanzo, 1989). Thus, there may be a trade-off between having relatively impermeable skin for terrestrial life (to resist desiccation) or relatively permeable skin for aquatic life (to facilitate cutaneous respiration). Permeable skin allowing relatively high amounts of cutaneous respiration would reduce the energetic costs of pulmonary respiration and concomitant surfacing, as well as the costs of maintaining a lipid barrier to water (Roberts and Lillywhite, 1980; Lillywhite and Maderson, 1982; Baeyens and Roundtree, 1983). It could also serve to allow longer dive times for foraging (Seymour, 1982; Lillywhite and Ellis, 1994), especially when the diet consists entirely of aquatic prey (Mills et al., 1999). Possibly more important, cutaneous respiration may also facilitate the submerged hibernation habits of *S. pygaea* (Seymour, 1982; Costanzo, 1989). In winter, when the oxygen concentration of water increases as a function of decreased temperature, there is virtually no surface activity in *S. pygaea* (J. W. Gibbons, pers. comm.). For terrestrial animals, however, having permeable skin would be costly because it could decrease activity times available for foraging, mate searching, or dispersion and increase the likelihood of desiccation. This hypothesis hinges on the assumption that cutaneous gas and water permeability are positively correlated in reptiles, an assumption for which there are only limited data (e.g., Lillywhite and Maderson, 1982).

A comparison of the results of the unconstrained versus constrained experiments, although not conclusive, is contrary to Cohen's (1975) conclusion regard-

ing EWL and coiling behavior. Cohen found that sidewinders (*Crotalus cerastes*) that were allowed to coil exhibited significantly lower rates of EWL than those prevented from coiling. A difference in methodologies may, in part, underlie the differences in our conclusions. Rather than relying on an environmental chamber with only a fixed temperature and humidity, Cohen used an air-flow desiccation system housed within an environmental chamber with a much lower humidity of $5\% \pm 3\%$. Under Cohen's experimental conditions *C. cerastes* readily coiled, providing an ideal situation to compare EWL in coiled versus uncoiled snakes. However, when given the opportunity to coil, most of our study animals spent the majority of their time in some form of activity, leading us to believe that *N. fasciata* and *S. pygaea* do not modify rates of EWL by changing body position. In light of the ecological differences of these natricine and crotaline snakes, these apparent differences in coiling behaviors are not surprising. *Crotalus cerastes* is a desert species that rarely, if ever, has access to bodies of water. Thus, although the desert-dwelling *C. cerastes* appears to use coiling to limit EWL, the semiaquatic *N. fasciata* and *S. pygaea* appear to select habitats that preclude the need for water-conserving coiling.

In conclusion, we have demonstrated that *S. pygaea* has an EWL rate three times as high as *N. fasciata*. We suggest this difference in physiology could be a proximate cause of infrequent terrestrial activity in *S. pygaea*, as compared to *N. fasciata* and other watersnakes. In addition, we offer the hypothesis that the operating mechanism that increases EWL rates in highly aquatic snakes, such as *S. pygaea*, facilitates cutaneous respiration at the cost of constraining terrestrial activity. This hypothesis requires that gas and water permeability are positively correlated in *S. pygaea*, an assumption for which we have no data. Future research will be needed to detect and quantify rates of cutaneous respiration *S. pygaea*, as well as the importance of this phenomenon to the overall ecology of *S. pygaea*. In this regard, studies of cutaneous respiration in *S. pygaea* under hibernation conditions would be especially enlightening (e.g., see Costanzo, 1989), as *S. pygaea* routinely spend their winter submerged or buried beneath mats of vegetation in well-oxygenated water (Carr, 1940).

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