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Intraspecific differences in thermal tolerance of the diamondback watersnake (*Nerodia rhombifer*): effects of ontogeny, latitude, and sex

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Abstract

Ontogenetic shifts in microhabitat use are widespread among taxa and can result in drastic shifts in thermal habitat among age classes. Likewise, geographic variation in climate along latitudinal gradients can cause differences in thermal environments among populations of a species. Using a common garden design, we examined four populations of a single species of semi-aquatic snake, *Nerodia rhombifer*, to determine whether ontogenetic shifts in habitat use (and/or body size) and latitudinal differences in ambient temperature have resulted in evolutionary changes in thermal tolerance. We found ontogenetic differences in thermal tolerance for all populations, with neonates tolerating temperatures 2 °C higher than adults, a pattern that is consistent with ontogenetic shifts in body size and microhabitat use in this species. There were differences in thermal tolerance among latitudes in neonates, suggesting genetic differences among populations, but adults showed no latitudinal differences. In combination, the increased thermal tolerance of neonates and the age-specific response to latitude suggest individuals may be most sensitive to selection on thermal tolerance as neonates. Although latitudinal differences exist in neonates, their tolerances were not ranked according to latitude, suggesting the effects of some other local factor (e.g., microclimate) may be important. Lastly, among neonates, females tolerate higher temperatures than males.

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1. Introduction

Understanding the evolution of thermal tolerance in ectothermic organisms has become a central theme in evolutionary and ecological physiology (Huey and Kingsolver, 1993; Angilletta et al., 2002). For species that are not suitable for laboratory selection experiments (i.e., most vertebrate organisms), comparative methods generally must be used to infer evolutionary changes in thermal tolerance as a response to changes in thermal environments associated with habitat use or geographic variation in climate (Huey and Kingsolver, 1993; Bennett and Lenski, 1999; Feder et al., 2000; Angilletta et al., 2002). In particular, comparative approaches that use a single species from different habitats and geographically separated populations offer a better alternative than interspecific comparisons, because among-species comparisons may be confounded by variables other than habitat differences (Pearson et al., 2002).

Ontogenetic shifts in microhabitat use are widespread among taxa and can sometimes result in drastic shifts in thermal habitat among size or life history stage classes (Middendorf and Simon, 1988; Paulissen, 1988a; Krebs et al., 1998). For example, most larval amphibians are constrained to their natal aquatic habitat, which can limit thermoregulatory options and at times be vastly hotter than other microhabitats available to the more terrestrial adults (Lillywhite, 1970; Tracy, 1976; reviewed in Ultsch et al., 1999). Similarly, larvae and pupae of *Drosophila* are constrained to thermally stressful necrotic fruit, while adult

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flies have the ability to minimize thermal stress through behaviors such as microhabitat selection (Krebs et al., 1998). In both of these cases, the larval stages, which are constrained to the more thermally stressful temperatures, have higher heat tolerance than the adult stages (Delson and Whitford, 1973; Berkhouse and Fries, 1995; Krebs et al., 1998; Ultsch et al., 1999). In aquatic snakes (e.g., *Nerodia*) and fish ontogenetic shifts in microhabitat use can result from ontogenetic changes in prey resource use or predator avoidance (McCauley and Huggins, 1979; Mushinsky et al., 1982; Plummer and Goy, 1984; Scott et al., 1989; Jobling, 1994; Lind and Welsh, 1994; Olson, 1996; Savitzky and Burghardt, 2000). In some freshwater fish there is a corresponding ontogenetic change in heat tolerance and selected body temperature, with smaller fish selecting and/or tolerating higher temperatures (McCauley and Read, 1973; Cox, 1974; Reynolds and Casterlin, 1978; McCauley and Huggins, 1979; Jobling, 1994). Aside from microhabitat differences in environmental temperature, species that exhibit ontogenetic changes in body size might be expected to exhibit ontogenetic changes in body temperatures because of their basic biophysical properties (Stevenson, 1985). Smaller ectotherms usually have faster heating and cooling rates than larger ectotherms (Hutchison, 1961; Ballinger et al., 1970; Stevenson, 1985; Carrascal et al., 1992; Tosini and Avery, 1993), which may make them more susceptible to thermal stress (Stevenson, 1985). As a result, increased heat tolerance might be adaptive in smaller/younger ectotherms.

On a larger scale, there can also be significant differences in thermal environments across a species' geographic range. By comparing populations that occur across a range of climatic regimes, such as along an altitudinal or latitudinal gradient, it is possible to determine whether a species has responded to historical differences in thermal habitat through local genetic adaptations in thermal tolerance. There are a limited number of studies that have demonstrated within species variation in heat tolerance among vertebrate populations (e.g., Hutchison, 1961; Brattstrom, 1968; 1970; Delson and Whitford, 1973; Miller and Packard, 1977; Hoppe, 1978; Hertz et al., 1979; Garland and Adolph, 1991; Meffe et al., 1995; Schwarzkopf, 1998). However, few studies have performed common garden experiments designed to distinguish between genetic and acclimation-induced differences in physiology (Garland and Adolph, 1991). Furthermore, most studies have examined organisms from populations in close proximity to each other (i.e., along an altitudinal gradient), which may have high rates of gene flow that could decrease the likelihood of interpopulational differences evolving (Lenormand, 2002).

We examined four populations of a single species of semi-aquatic snake, *Nerodia rhombifer*, to determine how it has evolved to deal with local variation in its thermal environment associated with both ontogenetic shifts in body size and habitat use and differences in environmental temperature along a latitudinal gradient. We predicted that thermal tolerance would exhibit a latitudinal gradient in both neonates and adults, with snakes from lower latitudes having higher thermal tolerance than snakes from higher latitudes. We also predicted that neonates would have higher heat tolerance than adults as a mechanism to cope with ontogenetic differences in heating rates (i.e., body size) and thermal environments. *N. rhombifer* spend most of their time in or near the water or basking in branches above the water (Gibbons and Dorcas, 2004). They exhibit ontogenetic shifts in diet and microhabitat use, using shallow (warmer) habitats to forage for smaller fish and perhaps to avoid predators as neonates and juveniles, but shifting to deeper (cooler) habitats to forage on larger fish as adults (Mushinsky et al., 1982; Plummer and Goy, 1984; Savitzky and Burghardt, 2000). Because we found strong ontogenetic differences in thermal tolerance we also examined the effects of body size on thermal tolerance, to see if smaller snakes (within age class) had higher thermal tolerance. Lastly, we examined the effects of neonatal sex on heat tolerance.

2. Materials and methods

2.1. The species

Diamondback watersnakes (*N. rhombifer*) are well-suited for studies of ontogenetic and latitudinal variation in thermal tolerance. First, operative temperatures, as measured using snake models, frequently exceed 45 °C in microhabitats where *N. rhombifer* are found, suggesting that elevated thermal tolerances may be ecologically important in this species (Keck, unpublished data for a population in central Texas). Second, nearly all *Nerodia* exhibit strong ontogenetic variation in habitat and food resource use and thus offer an excellent opportunity to determine if ontogenetic variation in heat tolerance exists in reptiles (Mushinsky et al., 1982; Plummer and Goy, 1984; Scott et al., 1989; Savitzky and Burghardt, 2000). Third, *N. rhombifer* are geographically widespread, very abundant, and easily captured in many aquatic habitats (particularly lentic systems) throughout much of their range, which includes 14 states in the central United States and northern Mexico (Gibbons and Dorcas, 2004). In contrast, most terrestrial snakes are extremely secretive and difficult to systematically capture in large numbers during a short time period, as was required for this study. Lastly, *N. rhombifer* are easy to maintain in laboratory conditions, which is a necessary requirement for common garden experimental designs.

2.2. Collection and animal care

During May and early June of 2000, we collected adult male and adult pregnant female *N. rhombifer* from