

THERMAL SELECTION AND TEMPERATURE PREFERENCE OF THE AQUATIC SALAMANDER, *AMPHIUMA TRIDACTYLUM*

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Abstract.—The thermal biology of obligatory aquatic salamanders (e.g., Amphiumidae) is largely unknown. We therefore investigated thermal selection and temperature preference in the fully aquatic salamander, *Amphiuma tridactylum*. In a laboratory thermal gradient, *A. tridactylum* selected a mean water temperature of 26.3° C (SE = 0.29, n = 15, range = 24.3 to 27.9). We also investigated potential influences of body size on thermal selection and found no significant relationship between thermal selection and snout-vent length or body mass. The preferred selected temperature is higher than the preferred temperatures reported for other obligatory aquatic salamanders, a difference that may reflect *A. tridactylum*'s southern distribution in the U.S. We collected water and air temperatures at a site representative of *A. tridactylum* habitat to estimate when preferred temperature may be seasonally available in the field. Maximum water temperatures exceeded 25° C and thus would support activity of *A. tridactylum* near its preferred temperature during May to August.

Key Words.—Amphibian; *Amphiuma*; temperature; thermoregulation; preferred body temperature

INTRODUCTION

A species' thermal biology and capacity for temperature is central to its physiological and behavioral responses within an environment (e.g., Bietinger and Lutterschmidt 2011). Despite the pervasive underlying influence of temperature, the thermal biology of amphibians is much less understood than other groups of ectotherms (Rome et al. 1992). Much of our knowledge for amphibian-based thermal biology comes from anecdotal evidence, often the body temperature (T_b) of a few individuals in the field (e.g., Brattstrom 1963). For an ectotherm, variation in environmental temperature translates directly into variation in physiological performance. Each physiological system within an ectotherm has a unique optimum temperature for peak performance (Hillman et al. 2009), so only one such system is likely to be at its optimum at any given temperature. Thus, regulation of peak physiological performance is a challenge for poikilothermic ectotherms like amphibians because T_b may be constrained by the environment, including suitable temperature availability, risk of desiccation, risk of predation, etc. (Bohlin and Beiting 1979).

Preferred or optimal temperature of an amphibian, and whether that temperature is available in its habitat, are known for relatively few species. Particularly lacking is information for aquatic salamander families such as Amphiumidae. Hutchison and Dupré (1992) present an extensive review of laboratory studies investigating amphibian thermal preference, but they report no information for the genus *Amphiuma*. Duellman and Trueb (1994) summarized field T_b of North and Central

American amphibians; data for aquatic salamander families (i.e., Cryptobranchidae, Amphiumidae, and Sirenidae) are represented by only 12 individuals of five species. The Amphiumidae field T_b mean and range is 24° C, based on three individuals of *A. means* collected at the same time and locality (Brattstrom 1963, Feder et al. 1982). In addition, Cagle (1948) reported decreased activity of *A. tridactylum* at low temperatures (9–12° C) for 32 individuals collected in one night at 10° C; none was collected below 5° C. Here, we report the thermal preference of the large obligatory aquatic salamander, *A. tridactylum*, under controlled laboratory conditions. We also provide environmental temperatures from a suitable microhabitat site in the field to evaluate seasonal thermoregulatory opportunities available to these salamanders.

MATERIALS AND METHODS

Study animals.—We collected *Amphiuma tridactylum* (n = 15) by hand at night from East Baton Rouge Parish, Louisiana in April and May 1990. The animals were maintained unfed in the laboratory individually in 19-liter buckets at 25° C and a natural photoperiod for seven to 14 days. To reduce the potential confounding effects of digestive status on thermal preference, animals were allowed at least one week for clearance of the digestive tract prior to experimentation.

Laboratory thermal gradient.—The aquatic gradient (Hutchison and Dupré 1992) consisted of an aquarium (125 x 38 x 34 cm, L x W x H) divided into five water pool sections (25 x 38 x 9 cm) with four glass dividers

(9 cm tall), so that the pools were 9 cm deep but the aquarium perimeter walls were 34 cm tall. We chose the temperature gradient range to be 22–35° C because individuals in preliminary trials in a 20–35° C gradient chose only temperatures between 24–30° C. Prior to each experimental trial the aquatic thermal gradient was drained and rinsed with well water.

Water temperatures in the five pools within the thermal gradient were approximately 22, 26, 28, 31, and 35° C, which allowed salamanders to regulate body temperature by shuttling among pools. The warm end of the gradient was generated by a conventional aquarium heater and a 15 w heating coil mounted to the bottom of the glass aquarium. A cold water bath outside the aquarium's opposite end generated the cool temperature. Each pool was aerated to provide circulation of oxygenated water and to maintain an approximately uniform temperature within each pool. Conduction between the adjacent pools generated the temperature gradient. We kept the gradient in a dark room to eliminate possible light cues (Justy and Mallory 1985), as *A. tridactylum* is primarily nocturnal. However, when we entered the experiment room to record temperatures and observations, dim ambient light from the adjacent room allowed for visibility.

Assessing preferred temperature.—We used each salamander in two experimental trials conducted on separate days. We placed individual salamanders randomly (using a random number table) into one of the five pools to begin a 30-min habituation period prior to each experimental trial beginning between 1100 to 1700. During the habituation period, individuals demonstrated exploratory behavior among gradient pools. After this habituation period, salamanders commonly shuttled among only one or two pools.

Experimental trials allowed each salamander to be observed for 6–8 h during which we recorded behavior and near-body water temperatures ($n = 12$ to 16) every 30 min. We measured the near-body water temperature selected (T_{sel}) at mid-water depth within the loose or tight body coil of the salamander (Fontenot and Lutterschmidt 2011) using a mercury thermometer read to the nearest 0.5° C. Near-body temperature represents the surrounding water temperature as it is influenced by body temperature. More importantly, it is this surrounding water temperature that serves as the thermosensory modality for thermoregulation. We observed no apparent response by the salamander to this action, and thus assumed minimal or no disturbance. We did not measure cloacal temperature because this would disturb the animal by handling it or restricting its movement with the attachment of a thermocouple.

We calculated preferred selected temperature (preferred T_{sel}) for each salamander as the mean of the central T_{sel} values (i.e., excluding the lower and upper 25

percentiles) similar to the determination of set temperature T_{set} (Hertz et al. 1993, Row and Blouin-Demers 2006). Although we cannot directly infer preferred T_b from our experiments, this methodology for measuring T_{sel} (e.g., Lutterschmidt and Reinert 1990) and evaluating preferred environmental temperature is reliable and accepted (e.g., Sievert and Andreadis 2002, Rittenhouse et al. 2004). Water temperature is presumed a reasonable approximation of T_b because T_b is typically within 1.0° C of water temperature for aquatic salamanders in the field (Bohlin and Beiting 1979). Because there was a large range in body size, we tested for the possible effects of snout-vent length (SVL) and body mass (M_b) on preferred T_{sel} by model-II linear regression. Assumptions were tested and met.

Assessing available field temperatures.—To determine whether preferred T_{sel} was available in the environment, we measured water and air temperature available to *A. tridactylum* at a site in southern Louisiana. This site is located in Ville Platte, approximately 90 km west of where we collected the study salamanders. The habitat at this site is similar to that for *A. tridactylum* collection sites in the region (see Fontenot 1999), and there is little temperature variation in southern Louisiana over such a small distance. We recorded both air and water temperatures weekly throughout 1989 using minimum/maximum thermometers. We recorded air temperature in the shade 1.5 m above the ground. We also recorded water temperature in a tree-shaded area with a thermometer tethered to a post and submerged on the bottom of the deepest section (0.5 m) of a natural drainage channel. We did not include temperatures from the water thermometer when the channel was dry.

RESULTS

The mean preferred T_{sel} for *A. tridactylum* was 26.3° C (SE = 0.29, $n = 15$, range = 24.3–27.9). Individual measurements of T_{sel} were approximately normally distributed within a few degrees of the mean preferred T_{sel} (Fig. 1), and ranged from 24.0 to 32.0° C. We observed the near-body water temperature of 32.0° C ($n = 8$) within the 31° C pool, indicating that salamanders used the 35° C pool prior to being recorded in the 31° C pool. Mean snout-vent length (SVL) and body mass (M_b) were 54.2 cm (SE = 1.83, range = 43–63) and 435.7 g (SE = 50.26, range = 129.9–688.2), respectively. A model-II regression analysis indicated no association between preferred T_{sel} and SVL ($F = 0.124$; $df = 1, 13$; $P = 0.73$) nor between preferred T_{sel} and M_b ($F = 0.003$; $df = 1, 13$; $P = 0.96$).

Field temperatures at the Ville Platte study site showed that air temperatures were more variable than water temperatures, with cooling and warming trends synchronized (Fig. 2). Generally the lowest temperatures

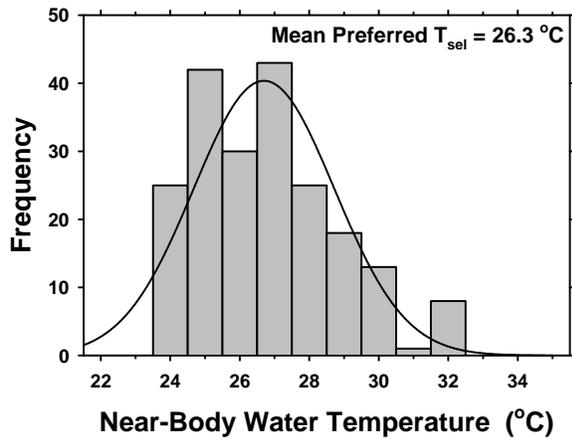


FIGURE 1. Frequency of all near-body water temperatures (T_{sel} ; $n = 205$) from all 15 *Amphiuma tridactylum* over both trials. Bars represent temperatures from 23.5 to 24.4° C, 24.5 to 25.4° C, etc. The black line represents the statistically fitted normal distribution for comparison. See text for methods on calculating mean preferred T_{sel} .

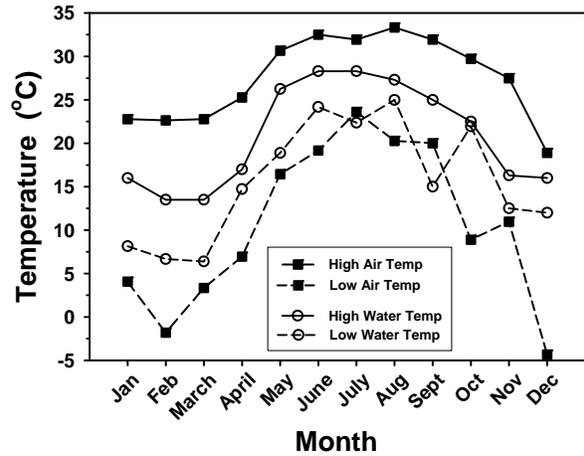


FIGURE 2. Monthly high and low air and water temperatures in 1989 at a site in Louisiana, USA, typical of *Aphiuma tridactylum* habitat.

occurred in March with the highest temperatures occurring in July or August.

DISCUSSION

The temperature preferences for two other large-bodied aquatic salamanders, *Cryptobranchus alleganiensis* and *Necturus maculosus*, have been reported using the same temperature acclimation regime (25° C) as the present study (Hutchison and Dupré 1992). The mean and modal temperature preferences for *Cryptobranchus alleganiensis* are 21.7 and 27° C, respectively ($n = 15$) and for *Necturus maculosus* are 20.2° C and 15° C, respectively ($n = 15$). Our reported mean preferred T_{sel} of 26.3° C for *A. tridactylum* is higher than the mean thermal preference of

either *Cryptobranchus* or *Necturus* (Table 1). Only *Ambystoma texanum* has a similar thermal preference to *A. tridactylum* (Table 1), which suggests that these more southern-distributed species have higher thermal requirements and tolerances. Such observations, where salamanders with more southern distributions have higher thermal preferences and tolerances, fit general macroecological patterns (Beitinger and Lutterschmidt 2011) and theory supporting thermal-dependent performance (Rome et al. 1992).

Compared to our available field temperatures (i.e., from a single location), the mean preferred T_{sel} (26.3° C) observed for *A. tridactylum* was available from May to August. Thus, *Amphiuma* may have the opportunity to reach its preferred temperature in the field during several months, although the opportunity for this is probably much greater temporally and spatially than the figure

TABLE 1. Mean temperature preferences for 13 salamander species acclimated at 25° C.

Species	N	Temperature Preference (°C)	Reference
<i>Cryptobranchus alleganiensis</i>	15	21.7	Hutchison and Hill 1976
<i>Necturus maculosus</i>	15	20.2	Hutchison and Hill 1976
<i>Ambystoma texanum</i>	10	26.5	Dupre and Petranka 1985
<i>Eurycea lucifuga</i>	11	19.3	Spotila 1972
<i>Plethodon caddoemis</i>	10	18.8	Spotila 1972
<i>Plethodon yonahlossee</i>	14	22.4	Spotila 1972
<i>Plethodon glutinosus</i>	10	18.7	Spotila 1972
<i>Plethodon jordani</i>	10	22.1	Spotila 1972
<i>Plethodon ouchitae</i>	10	18.2	Spotila 1972
<i>Plethodon c. cinereus</i>	15	15.9	Feder and Pough 1975
<i>Desmognathus quadramaculatus</i>	9	13.6	Sievert and Andreadis 2002
<i>Desmognathus monticola</i>	9	14.0	Sievert and Andreadis 2002
<i>Amphiuma tridactylum</i>	15	26.3	Present study

suggests. For example, summer temperatures in shallow water may reach 40° C, while burrows 30 cm underground are relatively constant at 18–20° C (Clifford Fontenot, unpubl. data). Thus, a large range of environmental temperatures is often available for thermoregulation and refuge from otherwise lethal environmental temperatures, which likely extends the time when salamanders may reach their preferred body temperature beyond the May to August period.

Although it appears reasonable that *A. tridactylum* has opportunities to reach its preferred T_{sel} of 26° C in the field, Major et al. (2011) showed that the protein complement system of *A. tridactylum* blood serum has peak activity at 30° C. While this temperature is substantially higher than our observed measures of preferred temperature in the laboratory, it may only be sought during times of immune system demand such as in fever (Hutchison and Erskine 1981). Current concerns surrounding the effects of global climate change on amphibian populations (e.g., Shoo et al. 2011) illustrate the need for studies that investigate temperature preference and temperature dependent activity and performance. The utility of the present study is that it provides a physiological performance measure for a species in a poorly studied group (i.e., obligate aquatic salamanders), so that future studies may address more theoretical and phylogenetic considerations of temperature preference.

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