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Preferred Temperature of Two Sympatric *Ambystoma* Larvae: A Proximate Factor in Niche Segregation?

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Acute preferred temperatures of both *Ambystoma jeffersonianum* and *Ambystoma maculatum* larvae varied with acclimation temperature (i.e., recent thermal history). The final preferred temperature of *A. jeffersonianum* was 25.2 C and for *A. maculatum*, 34.6 C. There was no significant ($P < 0.05$) difference between acute preferred temperatures at low acclimation temperatures, but preferred temperatures were significantly different when salamander larvae were acclimated to 30 C. It was postulated that temperature may be an important dimension along which the larvae segregate during the late spring and early summer months.

TEMPERATURE is one of the most important environmental parameters governing the metabolism, activity and behavior of aquatic poikilotherms (Fry, 1947; Stauffer, 1980). Much of the behavioral data collected to date have been associated with the effects of thermal discharges on the responses of fishes (Stauffer, 1980). Amphibians do not normally encounter temperatures that threaten their survival (Bogert, 1952; Keen and Schroeder, 1975), with the possible exception of toad embryos (Volpe, 1957), salamander embryos (Anderson, 1968) and some juvenile salamanders (Pough and Wilson, 1970). However, various thermal regimes may influence activities such as digestion rates (Skoczylas, 1970), conversion efficiencies (Brett, 1971) and growth (Licht et al.,

1969). Certainly, benefits derived from maintaining a preferred body temperature through behavioral means are constrained by metabolic costs (Lillywhite, 1971).

Compared to those on fish, studies of temperature selection of amphibians are relatively rare. Lillywhite et al. (1973) identified the need to determine the ecological significance of temperature selection in all ectothermal groups, and Brattstrom (1979) reviewed thermal regulation studies of amphibians. In a recent study, Thompson and Gates (1982) noted that breeding pools used solely by *Ambystoma jeffersonianum* tended to have cooler water temperatures than those with *Ambystoma maculatum*, a species with which it is often syntopic. They hypothesized that one of the dimensions along which the two

species segregate might be temperature. The purpose of this study was to 1) test their hypothesis by comparing the preferred temperatures of larval *A. jeffersonianum* and *A. maculatum* and 2) to provide data to support the hypothesis that sympatric larval *A. jeffersonianum* and *A. maculatum* use temperature as a proximate factor for niche segregation.

Thompson et al. (1980) and Thompson and Gates (1982) described the breeding habitat distribution and segregation of both *A. jeffersonianum* and *A. maculatum*. Walters (1975) discussed larval interactions of syntopic populations. Several authors (Mohr, 1931; Bishop, 1941; Anderson and Graham, 1967; Gatz, 1971) noted that *A. jeffersonianum* breeds earlier and develops more rapidly than *A. maculatum*, but Thompson and Gates (1982) stated that syntopic larvae may be in competition for space, food, and/or other resources.

MATERIALS AND METHODS

Egg masses of both species were collected from a single breeding site in Allegany County, Maryland, and transported to the holding facilities of the Appalachian Environmental Laboratory. Eggs were held between 15 and 18 C until larvae hatched. Larvae were acclimated to the following temperatures at a rate which did not exceed 1 C day⁻¹: 6, 12, 18, 24 and 30 C. Salamanders were held at their respective temperatures (± 1 C) for a minimum of 5 days prior to testing. Larvae were fed brine shrimp once per day between 0800 and 0900 h, except for days on which tests were conducted; thus, specimens which were tested had not been fed for at least 24 h. Photoperiod was 12L:12D throughout the study. Illumination was provided by Vita-lites (fluorescent lights emitting wave lengths similar to solar radiation). Water obtained from the city water supply was passed through a filtration system to remove chlorine and iron.

Twenty-five salamander larvae (five at each acclimation temperature) of each species were tested individually in a horizontal preference trough modeled after the one described by Meldrim and Gift (1971) which provided a temperature gradient above and below the acclimation temperature. The trough ($3.6 \times 0.203 \times 0.254$ m) was constructed of aluminum and covered with a non-toxic epoxy paint. Cold water introduced at one end was heated as it flowed down the trough by heat lamps positioned under the trough and set at increasing intensities.

Water level in the trough was approximately 3 cm deep. A perforated air hose was extended down the length of the trough and the resultant air stream prevented any vertical thermal stratification. Although the unit was a flow-through system, current was negligible. The entire trough was enclosed, and mirrors were positioned so that we could view the test organisms without disturbing them. Twenty-one thermistors were spaced equally throughout the unit at a depth of 1.5 cm so that we could record the temperature at the position of the test organism. After a 40 min orientation period, the water temperature at the organism's position was recorded every 15 s for 20 min. The mean of these observations was the acute preferred temperature for that particular test (Stauffer et al., 1976). All tests at a particular acclimation temperature and species were conducted between 0900–1600 h to minimize the potential of any diel effects.

Preference temperature was regressed against acclimation temperature using first, second, third, and fourth degree polynomials (Stauffer, 1981). Stepwise regression analysis was used to determine which model best described the data. This equation was then solved for the point at which acclimation temperature equalled preference temperature to determine the final temperature preferendum (Fry, 1947).

Once the "best fit" regression model was determined, data from both species were analyzed as though all the data were from one population. Residuals for each species were then calculated by subtracting the observed preference from the predicted preference, which corrected for the effect of acclimation temperature. The residuals were then analyzed by a nested analysis of variance with the 80 observations for each individual salamander nested within each of the 25 salamanders for each species.

Specimens tested ranged in size from 13–58 mm total length. No specimens showed any signs of metamorphosis; and, with the exception of those acclimated to 12 C, there was no significant ($P \leq 0.05$) difference in size between species at any acclimation temperature.

RESULTS AND DISCUSSION

Acclimation temperature influenced the preferred temperature of both salamander species. The mean preferred temperature of *A. jeffersonianum* was greater than the acclimation temperature at 6, 12, 18 and 24 C. The mean

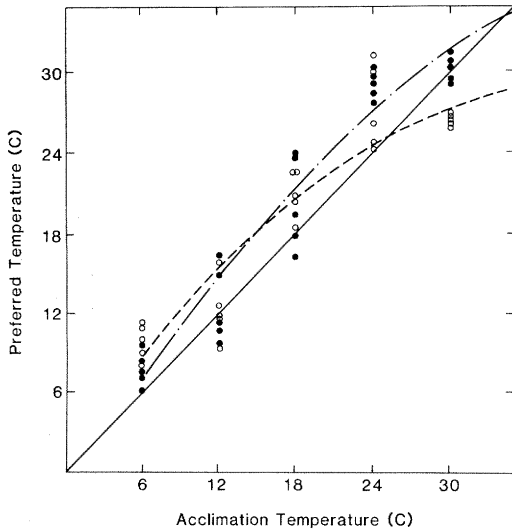


Fig. 1. Temperature preference responses of *Ambystoma jeffersonianum* (---; ○) and *Ambystoma maculatum* (—; ●). The point at which these curves cross the line with a slope of 1 (—) is the final preferred temperature.

preferred temperature for *A. jeffersonianum* acclimated to 30 C was 26.3 C (Fig. 1). The equation $P = -0.019A^2 + 1.469A + 0.232$ ($r^2 = 0.87$), where P = preference temperature and A = acclimation temperature, best described the data. Solving this equation for the point where $P = A$ yielded a final preferred temperature of 25.2 C. The mean preferred temperature of *A. maculatum* for each acclimation temperature was greater than the acclimation temperature at all temperatures. The model $P = -0.012A^2 + 1.462A - 1.608$ ($r^2 = 0.92$) best described the data (Fig. 1). Solving this equation for the point where $P = A$ yielded a final preferred temperature of 34.6 C.

When all the data were analyzed simultaneously, the equation $P = -0.015A^2 + 1.467A - 0.700$ resulted. This equation was

used to calculate the residuals for each species. A nested ANOVA of these residuals indicated that there was no significant difference ($P < 0.05$) between the species. These results may have been produced because of the similarity of the responses at the lower acclimation temperatures (Fig. 1). Therefore, a separate nested ANOVA was calculated for those salamanders acclimated to 6, 12, 18 and 24 C and one for salamanders acclimated to 30 C. These analyses indicated that while no significant difference ($P < 0.05$) occurred at the lower acclimation temperatures, there was a significant difference ($P < 0.05$) between the two species' responses at the 30 C acclimation temperature (Table 1).

Amphibians are capable of regulating body temperature through behavioral means such as microhabitat selection (Prosser, 1911; Licht and Brown, 1967; Clausen, 1973; Lillywhite et al., 1973; Heath, 1975; Keen and Schroeder, 1975). As stated previously, the final temperature preferendum can be calculated from acute temperature preference data by solving the regression equation for the point where preference temperature equals acclimation temperature. Theoretically, this is the highest temperature an organism will prefer if exposed to an expanded thermal gradient for an indefinite time period. For example, the *A. maculatum* which were acclimated to 6 C preferred temperatures ranging from 6–10 C. If these temperatures were available to them, they would eventually become acclimated to them and subsequently prefer a higher temperature. This sequence of preference-acclimation-higher preference would continue until the final temperature preferendum was reached. The substantially higher final temperature preferendum of *A. maculatum* indicates that in situations where *A. maculatum* and *A. jeffersonianum* are syntopic, *A. maculatum* would seek higher temperatures.

An acute temperature preference extrapolated from curves presented by Keen and Schroeder (1975) for *A. maculatum* (17.3 mm

TABLE 1. NESTED ANALYSIS OF VARIANCE OF RESIDUALS OF TEMPERATURE PREFERENCE RESPONSES OF *Ambystoma jeffersonianum* AND *Ambystoma maculatum* ACCLIMATED TO 30 C.

Source	SS	df	MS	F
Among groups	2,904.36	1	2,904.36	27.85*
Among subgroups within groups	417.05	4	104.26	153.24*
Within subgroups	535.29	790	0.68	

* Significant at $P < 0.05$.

snout-vent length) acclimated to 5 C and maintained in a temperature gradient trough for approximately four or five hours ranged between 22–26 C. Lucas and Reynolds (1967) stated that *Ambystoma tigrinum* selected a preferred temperature of 25 C (range = 16–31 C) in June, but did not select any specific temperature range during July. Heath (1975) reported adult *A. tigrinum* rarely selected temperatures above 28 C. The above results agree reasonably with data generated for *A. jeffersonianum*. *Ambystoma maculatum* demonstrated a much higher preference response than previously reported for other *Ambystoma* larvae and, as stated earlier, preferred a significantly ($P < 0.05$) higher temperature than *A. jeffersonianum* when acclimated to 30 C.

An attempt to acclimate *A. jeffersonianum* to temperatures higher than 33 C resulted in complete mortality; thus, suggesting a critical thermal maximum (CTM) between 30 and 33 C. Gatz (1971) reported a CTM of adult *A. jeffersonianum* of 34.0 C, when populations collected from Pennsylvania were acclimated to 10 C. A lack of specimens prohibited an attempt to acclimate *A. maculatum* to higher temperatures. However, CTM's of *A. maculatum* reported in the literature range from 34.8 to 39.2 C (Hutchison, 1961; Sealander and West, 1969; Pough and Wilson, 1970; Gatz, 1971). These high CTM's are consistent with the high preferred temperature of *A. maculatum*, which was observed in this study.

Differences in temperature selection may have significant ecological consequences. It may be that temperature selection acts as a proximate factor in niche segregation of syntopic larval amphibian populations during the late spring and early summer months (i.e., when water temperatures increase). Pool temperatures as high as 30 C were recorded by Gates and Thompson (1982), and temperatures of 36 C are not uncommon (Gill, 1978). Thompson and Gates (1982) generally observed *A. jeffersonianum* in deeper pools than *A. maculatum*. These deep pools often had differences of several degrees Celsius between surface and bottom water temperatures during June. Both larvae are benthic in their habits and would be at the temperature of their pool bottom. Because of greater variation in depth, large pools would typically have a greater range of bottom temperatures than small pools. Pools in which both species were syntopic were in fact larger than pools where the species were allotopic. This situation should

facilitate spatial segregation by the two species using pool temperature alone. Our results predict that as pool temperatures increase in late spring and early summer, there should be increased spatial segregation by the two species with *A. jeffersonianum* in cooler regions and *A. maculatum* in warmer regions of the pool. If temperature selection is an important dimension along which larvae segregate, competition for various resources would be reduced, as well as interspecific predation.

As mentioned previously, our tests were conducted between 0900 and 1600 h in an attempt to minimize any potential diel effects. While no information could be found concerning diel changes in preferred temperatures of amphibians, several authors have suggested that fishes demonstrate diel changes in selected temperatures (Barlow, 1968; Brett, 1971; Stauffer, 1980). Should this occur with these larvae, habitat selection may differ markedly throughout any given 24 h period.

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