

Thermal Tolerance and Growth Differences in Orangethroat Darter (*Etheostoma spectabile*) from Thermally Contrasting Adjoining Streams

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ABSTRACT.—Orangethroat darters *Etheostoma spectabile* occupying thermally contrasting streams within the same watershed were tested for differences in thermal responses by comparing their critical thermal maxima (CTM) and short-term growth. Individuals from a spring branch population had a lower CTM, better growth at low temperatures and more inhibited growth at higher temperatures compared to individuals collected from a thermally variable adjoining stream reach. The findings support the contention that intraspecific differences in thermal tolerance and growth may exist at relatively localized scales within a small watershed.

INTRODUCTION

The more variable the environments of fishes, the more tolerant species are to extremes in the ranges over which environmental parameters vary (Horwitz, 1978). For example, Matthews and Styron (1981) found that abrupt changes in dissolved oxygen, pH and temperature were better tolerated by headwater species (northern redbelly dace *Phoxinus oreas* and fantail darter *Etheostoma flabellare*) than by downstream species from physicochemically more consistent mainstream areas (five cyprinid and darter species). Likewise, riffle-dwelling fantail darters exhibited a greater thermal tolerance (measured as critical thermal maximum, CTM) than did johnny darter *Etheostoma nigrum* from more thermally consistent stream pools (Ingersoll and Claussen, 1984).

Correlations between habitat instability and fish tolerance to fluctuating environmental parameters have been documented repeatedly (Hubbs, 1964; Matthews, 1987; Cech *et al.*, 1990). The evidence that local adaptation influences tolerance within a species is more tenuous, especially regarding thermal tolerance. Of the two schools of thought regarding fish thermal physiology, proponents of the static view (that thermal tolerance is constant within a species regardless of location) find support in results from a diversity of species (*e.g.*, desert pupfish *Cyprinodon* spp., Brown and Feldmeth, 1971; red shiner *Notropis lutrensis*, Matthews, 1986; redbelly shiner *Lythrurus umbratilis*, bluntnose minnow *Pimephales notatus*, stoneroller minnow *Campostoma anomalum*, longear sunfish *Lepomis megalotis*, Smale and Rabeni, 1995a). Conversely, adherents of the labile view (that thermal tolerance is evolutionarily tuned to local environments) find support in the evidence of local evolutionary adaptation between populations within the same species (geanthroat darter *Etheostoma lepidum*, Hubbs, 1964; orangethroat darter *E. spectabile*, Hubbs and Armstrong, 1962; Feminella and Matthews, 1984). Where a species exhibits phenotypic plasticity it has been suggested that individuals may sort themselves along the prevailing thermal gradient. However, research has rarely found bimodality in thermal and physicochemical tolerances within populations (Matthews and Maness, 1979; Matthews and Hill, 1979). Instead, fish popula-

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tions are more apt to exhibit sharp unimodal selection peaks even in the presence of a range of thermal and chemical environments (Matthews, 1998).

In studies where species have been designated as "labile," sampled populations were from separate watersheds, or from locations within the same large watershed that were substantial distances apart. For the present study we attempt to alleviate this problem by testing for differences in thermal tolerance in orangethroat darter from close localities in the same small watershed that have different thermal regimes. We evaluated individual fish collected at two sites with two responses. First we measured the CTM of individuals and second, compared their short-term growth at 8 C and 24 C using different individuals from the same collection sites.

METHODS

Collection sites.—Adult orangethroat darter were collected using D-frame kick-netting 1 March–6 March 2000 from two localities in the Little Bonne Femme watershed located in southwestern Boone County in central Missouri. Our thermally more variable environment was a reach of Clear Creek where 25 individuals (presumably of both sexes) were collected. Our thermally more consistent environment, where we collected another 25 individuals, was the spring branch outflow of Devils Icebox Cave in Rock Bridge Memorial State Park. After surfacing from the mouth of the Devils Icebox Cave, the spring branch runs overland approximately 0.55 km before joining Gans Creek near its confluence with Clear Creek. The mouth of the spring branch was approximately 5.5 km stream distance from the Clear Creek collection site. Flows in both tributaries are sufficient to maintain a virtually continuous connection between the sites. Flows from the Devils Icebox have traveled underground for sufficient distances and time such that thermal equilibration is generally attained (Wicks, 1997).

To thermally characterize each collection site, water temperature histories were obtained for the 3-mo period that bracketed our collections (February–April 2000). At the Clear Creek site, recordings from a self-registering max-min thermometer (Taylor, model 5458) were obtained at 2- to 4-d intervals. Temperatures for the Devils Icebox spring branch were continuously recorded by an on-site thermometer and logger (courtesy Dr. R. Lerch, USDA-ARS), from which maxima and minima were extracted for date spans matching those for the Clear Creek sequence.

Fish maintenance.—After being transported to the laboratory in insulated containers and site water, the fish were allowed 24 h to acclimate to the ambient temperature of the laboratory (16 ± 0.5 C). Immediately thereafter fish from the two sites were allocated to separate 37-L aquaria containing coarse gravel substrates, aged tap water and under-gravel aerator/filters. Following an additional 14-d acclimation period, we then conducted our experimental trials over the succeeding 7-d period. Fish in the laboratory were maintained under a 12L:12D photoperiod and were fed *Chironomus* spp. larvae on alternate days. Feeding occurred after any CTM trials that were conducted on feeding days.

Critical thermal maxima.—We tested for differences in CTM as defined by Lowe and Vance (1955): "the arithmetic mean of the collective thermal points at which locomotory activity becomes disorganized and the animal loses its ability to escape from conditions that will promptly lead to its death." Our methodology for determining CTM values for the fish from each site followed Hutchinson (1961). Individual fish were placed in an 800-ml glass beaker containing 500 ml of dechlorinated water. Each fish was then subjected to a regime of increasing temperature of 1 C/min, from a starting point of 16–17 C, monitored by an electronic thermometer (Solomat 520c) using a 120-V rheostatic hotplate (Corning PC-35). The 1 C/min heating rate has been shown to be fast enough to prevent partial acclimation,

yet slow enough to prevent thermal shock and has been commonly used (Hutchinson, 1961; Maness and Hutchinson, 1980; Paladino *et al.*, 1980). Continuous aeration during each trial ensured even heat dispersal and prevented oxygen depletion; new water was used for each trial and all trials occurred between 1500 and 2100 h.

The end point of each trial was the temperature at which two responses occurred: final loss of equilibrium (LOE; inverted swimming accompanied by attempts at righting) and opercular spasms (OS; rapid flaring of the gill covers accompanied by gaping of the mouth and spastic body movements). Although some disagree as to which endpoint (LOE vs. OS) is most applicable in determining CTM (Hlohowskyj and Wissing, 1985; Ingersoll and Clausen, 1984), we found Hutchinson's (1961) argument for using both loss of equilibrium and body spasms compelling. We used *t*-tests (equal variance) to test for differences in each end point between locations. Before analysis, we confirmed data normality using normal probability plots, stem-and-leaf plots and box plots, and tested for variance homogeneity using Barlett's test (Zar, 1974). The test of significance is presented here with the understanding that the experimental design lacks true replication as discussed by Hurlbert (1984). However, recently some have defended the utility of applying inferential statistics to such designs [*see* Oksanen (2001) for discussion]. Following experimentation, the fish were returned to their respective source streams.

Growth experiment.—To further investigate possible between-site differences in response to temperature, we conducted a short-term growth experiment. In April 2000 we collected an additional 20 orangethroat darters (both sexes), each from the Clear Creek and Devils Icebox spring branch sites. The fish from each site were then randomly allocated into two groups (cool and warm).

Fish in the cool temperature groups (Clear Creek $n = 10$; Devils Icebox spring branch $n = 10$) were tested at a constant 8 ± 1 C in a temperature-controlled environmental chamber. Fish in the warm temperature groups (Clear Creek $n = 10$; Devil's Icebox spring branch $n = 10$) were tested at a constant 24 ± 1 C in a separate environmental chamber. Both maintained a 12L:12D photoperiod. The 8 C temperature was cooler than what is common in the Devils Icebox spring branch during the growing season, whereas the 24 C temperature is one which Clear Creek routinely experiences in summer, but which the spring branch likely never reaches.

Within each environmental chamber we prepared 10, 37L aquaria filled with aged tap water. Into each aquarium we placed two translucent Tupperware Readymade® containers, each of which contained coarse gravel similar to the substrate at both collection sites and were liberally perforated on all sides to permit continuous water exchange. After a 24-h period after capture in which site water was allowed to equilibrate with chamber temperature, two fish, one from each site selected randomly from those captured, were placed individually into the respective containers; in-tank location was assigned randomly—green and red marbles placed in the containers differentiated the Clear Creek and spring branch fish, respectively. By pairing the fish we sought to maximize the degree to which fish from the different sites shared similar maintenance conditions. The container translucency and in-tank separation minimized fish visibility to one another; tank aeration ensured oxygenation and homogenized any olfactory cues.

Once each day for 14 d, each fish was fed to satiation with frozen chironomid larvae; uneaten larvae were routinely removed from the containers. Keeping the fish separated minimized potential effects of aggression, territoriality and competition. Fish were weighed on the first and last day of the experiment. Fish were blotted and placed in a tared beaker of water and then weighed to the nearest 0.0001 g. To allow for gastric evacuation, fish were weighed 24 h after feeding. Differences among the four groups of fish were examined

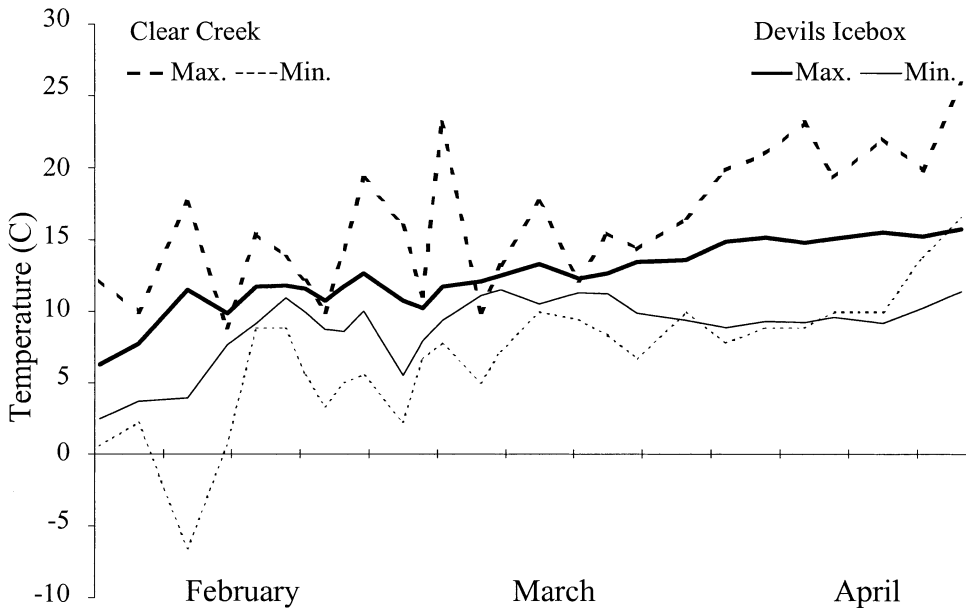


FIG. 1.—Maximum/minimum temperatures for Devils Icebox spring branch (solid, inner-most lines) and Clear Creek (dashed, outer-most lines) sample sites. The Clear Creek February sub-zero minimum was associated with a decrease in water levels

with an analysis of covariance (ANCOVA). Regression equations relating the starting weight to ending weight of each group were plotted to visualize the relationships within and among the site/temperature combinations.

RESULTS

Site temperature differences.—Clear Creek was subject to greater thermal fluctuation (6.7–16.0 C) than the Devils Icebox spring branch site (8.9–12.4 C) [Fig. 1]. As further evidence that the sites differed in thermal variability, coefficients of variation for the Clear Creek minima (67.1%) and maxima (29.1%) both significantly exceeded those for the spring branch (26.9 and 18.7%, respectively) when evaluated with a variance ratio test (minima $F = 4.912$, $df\ 26, 27$, $P < 0.05$; maxima $F = 3.230$, $df\ 27, 27$, $P < 0.05$; see Zar, 1974).

Between-site comparison of critical thermal maxima.—As water temperature approached the CTM, each of the 50 darters that we tested exhibited a characteristic behavior sequence: motionless resting on the beaker bottom, exploratory swimming above 29 C (sudden searching for a cooler location; see Hutchinson, 1961; Matthews *et al.*, 1982), erratic inverted swimming and loss of equilibrium (LOE) and then violent body spasms together with rapid opercular flaring (OS).

Orangethroat darters from the two collection sites differed in their respective CTM (Fig. 2). Fish from Clear Creek experienced LOE at temperatures higher than did those from Devils Icebox spring branch, 31.66 ± 0.10 C (mean \pm SE) vs. 30.97 ± 0.14 C ($t = 3.93$, $df\ 48$, $P = 0.0003$). The same Clear Creek fish similarly exhibited OS at temperatures higher than did the spring branch fish, 32.04 ± 0.10 C vs. 31.3 ± 0.12 C ($t = 4.83$, $df\ 48$, $P < 0.0001$).

Growth experiment.—Temperature differentially influenced growth in the fish from the two sites. For all four groups, fish weight at the end of the experiment (“ending weight,” EW) was positively and linearly related to weight at the beginning of the experiment (“starting weight,” SW):

Clear Creek 8 C:

$$EW = -0.09670 + 1.08117SW; \quad r = 0.991, \quad n = 8, \quad P < 0.0001;$$

Clear Creek 24 C:

$$EW = 0.13416 + 0.860336SW; \quad r = 0.978, \quad n = 10, \quad P < 0.0001;$$

Devils Icebox 8 C:

$$EW = -0.06375 + 1.09334SW; \quad r = 0.989; \quad n = 9, \quad P < 0.0001;$$

Devils Icebox 24 C:

$$EW = 0.06317 + 0.877336SW; \quad r = 0.974, \quad n = 10, \quad P < 0.0001.$$

In each case the high coefficient (r) provides evidence of an excellent regression fit. Fish from both sites tested at 8 C gained weight (both regression slopes exceed 1.00); in contrast, fish from both sites tested at 24 C lost weight (both regression slopes were less than 1.00). Given that weight gain or loss was proportional to the fish's starting weight, the differential is most apparent in the larger individuals (Fig. 3). The reduction in sample size for the 8 C trials is a consequence of the pathogen-related deaths of two Clear Creek and one Devils Icebox fish.

Statistical comparison of the four regressions revealed that their slopes differed significantly (ANCOVA slopes test, $F = 4.189$, df 3, 32, $P < 0.05$). Subsequent within-site comparisons of the two temperature regressions revealed that the weight gains at 8 C (slopes > 1.0) were different than the weight losses at 24 C for each population (slopes < 1.0) (Clear Creek, ANCOVA slopes test, $t = 2.037$, df 14, $P = 0.06$; Devils Icebox spring branch, ANCOVA slopes test, $t = 2.105$, df 15, $P = 0.055$; data \log_e -transformed to homogenize regression variances). The comparatively greater divergence of the Devils Icebox spring branch regressions suggests that the temperature effect was more pronounced in fish from that site.

Comparing the sites (Fig. 3), it is evident from the two uppermost regression lines that at 8 C the growth of fish from Devils Icebox spring branch exceeded that of fish from Clear Creek. Similarly, it is evident from the two lowermost regression lines that at 24 C the weight losses of fish from Clear Creek were less (*i.e.*, these fish fared better) than were those of fish from Devils Icebox spring branch. The results of pairwise covariance analyses support the interpretation that fish from each site grew differently when held at the same temperatures (8 C, ANCOVA slopes test, $t = 1.611$, df 17, 1-tailed $P = 0.06$; 24 C, ANCOVA slopes test, $t = -1.239$; df 14, 1-tailed $P = 0.12$; data \log_e -transformed to homogenize regression variances).

DISCUSSION

The results of our thermal tolerance experiment suggest that orangethroat darter should continue to be grouped among species designated as labile. Our results are also consistent with the findings of Feminella and Matthews (1984), in that fish inhabiting the thermally less variable spring branch exhibited a lower thermal tolerance than did ones inhabiting the more thermally variable stream reach. Our findings contribute to the growing appre-

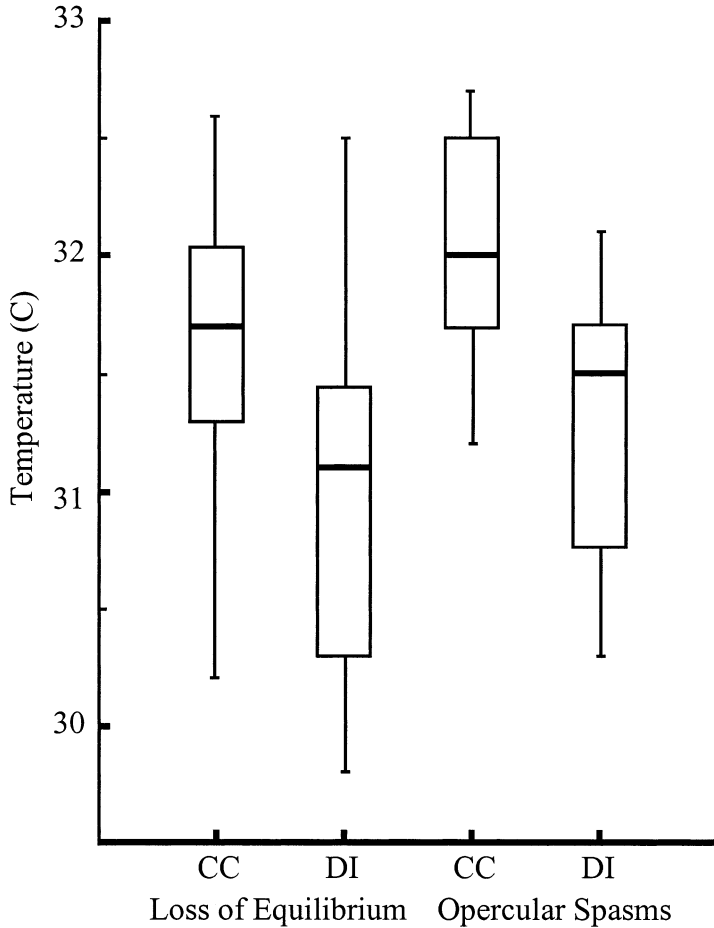


FIG. 2.—Box and whisker plots (mean, quartile, range) illustrating temperatures at which orangethroat darters from each site (CC = Clear Creek, DI = Devils Icebox spring branch) lost equilibrium (left) or exhibited opercular spasms (right). Clear Creek darters exhibited significantly higher loss of equilibrium and opercular spasm temperatures than did Devils Icebox fish ($P > 0.05$, see text for details)

ciation of the importance of spring branches as thermal refugia and unique habitats for fishes (Peterson and Rabeni, 1996).

The results of the short-term growth experiment complement those of the thermal tolerance tests: fish from Devils Icebox spring branch grew best at our lower test temperature and lost the most weight at our higher test temperature; in contrast, fish from Clear Creek grew less well at our lower test temperature and fared comparatively better at our higher test temperature. We found it interesting that fish from both sites lost weight at our higher test temperature as we expected the Clear Creek fish to maintain (if not gain) weight. Because 24 C was warmer than either collection site at the capture date, we interpret the weight loss as evidence of a thermally induced metabolic acceleration accompanied by a lag in feeding or digestion rate increases.

In past studies of fish thermal tolerance (*e.g.*, Hubbs and Armstrong, 1962; Hubbs, 1964;

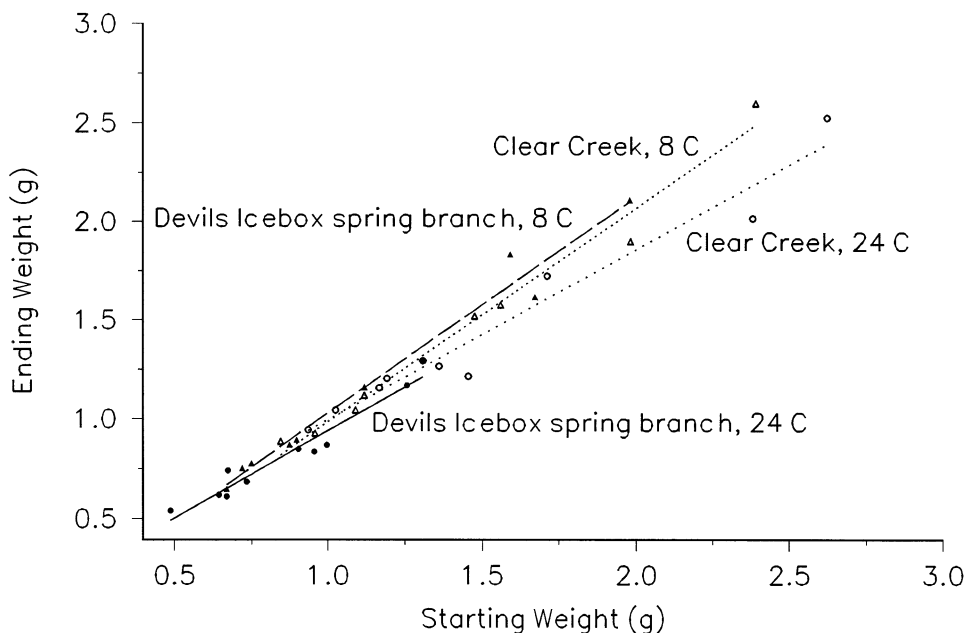


FIG. 3.—Regression relationships between starting and ending weight of Devils Icebox spring branch (solid and large dashed, outermost lines; filled symbols) and Clear Creek (small dashed, inner-most lines; open symbols) orangethroat darters held at 8 C (upper two lines; triangles) and 24 C (lower two lines; circles). See text for regression equations and covariance analysis results

Feminella and Matthews, 1984) the emphasis has been on ascertaining whether intraspecific differences occur at all. These studies used fish collected from widely separated populations between which gene flow would be minimal and infrequent. In contrast, the present study considered fish from sites separated by no physical dispersal barriers and by a smaller distance (<6 km) than has ever before been examined. Despite this proximity, we nevertheless identified between-site differences in critical thermal maxima and growth. The question arises whether the fish from these sites constitute evolutionarily significant units (sensu Waples, 1991, 1995; Moritz, 1994). Past studies testing physicochemical tolerance of fish species have been used to indicate presumed adaptations to local environments (Paladino *et al.*, 1980; Feminella and Matthews, 1984), while others have examined the genetic evidence related to differing physiological tolerances (Hirshfield *et al.*, 1980). Research on orangethroat darter has shown that local populations can and have diverged genetically (Echelle *et al.*, 1976; Wiseman *et al.*, 1978). The geographic distance and thermal differences between our two sample sites proved sufficient for differences in thermal tolerance and growth to be detectable. For other species, however, the scales (geographic, thermal, temporal) necessary for differences to occur are likely to differ. This may explain why Brown and Feldmeth (1971) could not detect differences in thermal tolerance between desert pupfish from thermally constant spring waters and thermally fluctuating environments, despite their long-term isolation. Species mobility may be an additional contributory factor, with populations of small benthic species (like darters) being more apt to become localized.

Finally, we recognize that thermal tolerance in some species may be an indirect response: work by Smale and Rabeni (1995a, b) demonstrated that fish thermal tolerance was more

strongly correlated to low dissolved oxygen levels rather than to maximum stream temperatures, and that the effects of elevated temperatures were only detectable when dissolved oxygen levels were sufficiently high. Further work will be required if the relative effects of temperature and oxygenation levels are to be partitioned.

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