

## Effects of Temperature on Egg Production and Early Life Stages of the Fountain Darter

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**Abstract.**—Studies were conducted to determine the effects of water temperature on egg and larval production, larval survival, and juvenile growth of the endangered fountain darter *Etheostoma fonticola*. Adult fish were exposed to water temperatures of 14, 17, 20, 23 (control), 25, 27, and 29°C for 33 d. Egg production was significantly higher ( $P \leq 0.05$ ) for fish held at 14, 17, 20, 23, and 25°C than for fish held at 27 and 29°C. Percent hatch was significantly higher ( $P \leq 0.05$ ) at 17, 20, and 23°C than at 14, 25, 27, and 29°C. Larval production was significantly higher ( $P \leq 0.05$ ) at 14, 17, 20, and 23°C than at 25, 27, and 29°C. Estimated low and high temperatures to produce 50% mortality of larvae (24–72 h old) over a 24-h period were 3.8 and 31.9°C, respectively. Low survival and substantial variation in growth of controls prevented a meaningful assessment of temperature effects on juvenile growth.

### Introduction

The fountain darter *Etheostoma fonticola* is an endangered fish endemic to the San Marcos (Hays County) and Comal (Comal County) rivers of central Texas. Reduced spring flow from the Edwards Aquifer into the rivers is considered a threat to the fountain darter because of the potential for alteration in the thermal regime of the rivers (U.S. Fish and Wildlife Service 1995). The water temperature in the springs and upper reaches of the San Marcos and Comal rivers is 22–24°C during years of normal and above normal precipitation. However, drastically altered water temperatures may occur because of increased pumping and droughts that decrease spring flows. Information is needed on the effects of altered temperature regimes on the fountain darter in order to predict the effects of reduced spring flow on fountain darter populations.

Existing information on the effects of temperature on fountain darter fecundity and growth is limited. Schenck and Whiteside (1977) reported year-round spawning of fountain darters in the San Marcos River and indicated that food availability, light intensity, and light duration had no observed

effects on spawning. However, they suggested that water temperature and decreases in flow may influence spawning. Strawn (1956) also reported year-round spawning by the fountain darter in the San Marcos River but provided no data to support his conclusion.

Brandt et al. (1993) spawned fountain darters at temperatures between 3 and 30°C in the laboratory. Few eggs were obtained at 3 and 30°C, and these were not viable. Larvae were hatched from eggs produced between 6 and 27°C and incubated at approximately 22°C. However, observed differences between egg and larval production at the different temperatures were not statistically tested. Daily egg production by individual females at four test temperatures (9, 15, 21, and 27°C) was found to vary substantially (0–60 eggs), as did the number of days out of 14 that an individual female released eggs (0–10 d). Brandt et al. (1993) also determined that the critical thermal maximum for adult fountain darters was 34.8°C and that fish held at approximately 24°C for 146 d grew an average 0.2 mm/d.

The objectives of this study were to determine the effects of temperature on fountain darter egg and larval production, the lower and upper 24-h temperature lethal to 50% (TL50) of larval foun-

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tain darters, and the effect of temperature on the growth of juvenile fountain darters.

### Methods

In all, 117 male (with clearly visible red and black bands on the dorsal fin) and 93 female (with a colorless dorsal fin) fountain darters were collected with dip nets from five areas of the Comal River between 8 February and 12 July 1995. All fish kept were between 27 and 35 mm total length (TL) and were assumed to be mature ( $>26$  mm; Brandt et al. 1993). The lengths of the fish when captured were not recorded. Total lengths were recorded for all subsequent fish in this study. The fish were transported to the National Fish Hatchery and Technology Center (NFHTC), San Marcos, Texas, and treated for 1 h in formalin (250 mg/L) for external parasites.

Fish were placed in 9-L flow-through glass aquaria located on top of either 650-L or 840-L fiberglass tanks (Living Stream models LS-700 and LS-900, Frigid Unit, Toledo, Ohio) that were used as reservoirs for the water recirculated through the aquaria (exchange rate, once every 10 min). Electric pumps (0.5 hp, 373 W) were used to circulate water between the reservoirs and aquaria. The standard Frigid Unit foam rubber and charcoal baskets were used to filter the water, and 0.5-hp chiller/1,000-W heater units (ACRY-TEC, Inc., San Diego, California and Universal Marine Industries, Inc., San Leandro, California) were used to maintain desired water temperatures ( $\pm 1.0^\circ\text{C}$ ). A diagram of a similar recirculation system used in an earlier study at the NFHTC is illustrated in an article by Anderson et al. (1998). Well water (total hardness, 270 mg/L as  $\text{CaCO}_3$ ; pH, 7.1; Ogden et al. 1985) for the tanks was pumped from the Edwards Aquifer. Photoperiod for all trials was 12 h light and 12 h dark.

**Temperature effects on reproduction.**—Because of equipment limitations, two separate trials were conducted to determine the upper and lower temperature effects on reproduction. In trial 1, eight groups of five males and eight groups of five females were randomly selected and placed in separate 9-L flow-through glass aquaria. At time of stocking, the water temperature in the aquaria was  $23^\circ\text{C}$ . Four of the aquaria, two containing five males each and two containing five females each, remained at  $23^\circ\text{C}$  for the duration of the study. Water temperature in the remaining 12 aquaria was increased  $1^\circ\text{C}/\text{d}$  until the target temperature for an individual treatment was reached. For example, after 2 d, four of the aquaria, containing a total of

10 males and 10 females, had reached  $25^\circ\text{C}$  and remained at this temperature for the duration of study. Following the same method, groups of 10 males and 10 females were raised to 27 and  $29^\circ\text{C}$  and held at those temperatures. Once the final groups of fish were at  $29^\circ\text{C}$ , all fish were acclimated at their test temperatures (23 [control], 25, 27, and  $29^\circ\text{C}$ ) for 11 d. Thus, the total male–female separation period was 17 d. The fish were then redistributed in the aquaria within a treatment temperature so that two males and two females were in each of five aquaria at each of the four test temperatures. However, at  $27^\circ\text{C}$ , two aquaria were excluded from the study because each aquarium was mistakenly stocked with one greenthroat darter *Etheostoma lepidum*. In trial 2 (started 60 d after the completion of trial 1), fish were acclimated in the same manner as in trial 1, except that the test temperatures were 23 (control), 20, 17, and  $14^\circ\text{C}$  and the final acclimation period was for 7 d.

Spawning mops (Brandt et al. 1993) were placed in each aquarium. Eggs were removed from each spawning mop and from aquaria surfaces three times a week for 33 d. All eggs were classified as either healthy or fungus infected.

All healthy eggs collected from each aquarium were placed in separate 0.7-L incubation jars (Anderson et al. 1998) within the same recirculation system in which the eggs were produced. The incubation jars received a constant flow of water to roll the eggs. Eggs were incubated until all eggs hatched or became infected with fungus. The incubation jars were attached to 12-L collection buckets to allow hatched larvae to swim from the incubation jar into the collection bucket. Fungus-infected eggs and healthy larvae were removed and counted three times a week. On two occasions, at  $14^\circ\text{C}$  and at  $17^\circ\text{C}$ , eggs from one aquarium were placed into the wrong incubation jar within the same test temperature. The total number of misplaced eggs (approximately 50, less than 1% of total egg production for each test temperature) was deemed negligible.

Fish were fed black worms (Aqualife, Friant, California) ad libitum, supplemented with plankton harvested from hatchery ponds (Graves and Morrow 1988). One mortality (male) occurred at  $23^\circ\text{C}$ , and the dead adult was removed promptly and replaced by a male preconditioned in the same manner as the test fish. Dissolved oxygen and temperature (YSI model 58 dissolved oxygen meter, Yellow Springs, Ohio), pH (UniFET FieldLAB-100, San Diego, California), and percent saturation of total gases (Sweeney Aquametrics Satrometer

model DS-1B, Stoney Creek, Connecticut) were monitored three times a week.

Differences in mean number of eggs, mean number of larvae, and mean percent hatched produced from the separate runs at 23°C were tested by an independent *t*-test ( $P \leq 0.05$ ; Wilkinson et al. 1992). Significant differences in mean number of egg, mean number of larvae, and mean percent hatched for all test temperatures were determined by analysis of variance (ANOVA; Cagnon et al. 1994). The mean number of eggs and mean percent hatched for all test temperatures were analyzed for significant difference from the control by a Dunnett one-tailed test (Cagnon et al. 1994). Before statistical analysis was performed for differences in mean percent hatch, all percentage data were arcsine transformed. Because the mean number of larvae produced at each temperature were from different numbers of eggs produced at each temperature, the mean number of larvae were analyzed for difference from the control by Dunnett two-tailed test to determine the optimum temperature for maximum larval production (Cagnon et al. 1994).

*Temperature tolerance.*—Ten 9-L aquaria were each stocked with five male–female pairs of fish that were maintained at 23°C. Each aquarium contained one spawning mop. Healthy eggs deposited on the spawning mops and aquarium surfaces were removed daily and placed in 0.7-L incubation jars that also received 23°C water.

To determine lower 24-h TL50 of larval fish, a preliminary test was conducted to establish an approximate TL50. During the preliminary test, 200 larvae that were 24–72 h old and held at 23°C were randomly selected from the collection buckets. Ten larvae were placed into each of four aquaria without acclimation at each test temperature (23 [control], 19, 15, 11, and 7°C). Twenty flow-through glass aquaria (9-L working volume) were used. The larvae were held at the test temperatures for 24 h, and then the numbers of fish alive and dead in each aquarium were counted. Once the approximate TL50 was determined, a second group of larvae that were 24–72 h old and held at 23°C were divided without acclimation among 12 glass aquaria (9-L; 10 fish per aquarium) and held for 24 h at 23 (control), 7, 5, and 3°C. The larvae held at 23, 7, and 5°C (at three aquaria per temperature) were in flow-through systems. Because of chiller limitations, larvae held at 3°C were placed in three static aquaria inside a Percival Environmental Chamber (model 1–35LL, Percival Manufacturing, Boone, Iowa). The aquaria were

supplied with aeration and maintained at the same photoperiod as the other test fish. After 24 h of exposure to the test temperatures, numbers of alive and dead fish in each aquarium were counted.

To determine the approximate upper 24-h TL50 of larval fish, a group of 200 larvae (24–72 h old; 10 larvae per aquarium) were exposed for 24 h in flow-through aquaria (9-L; four aquaria per temperature) to temperatures of 23 (control), 27, 31, 35, and 39°C. At the end of the exposure period, numbers of alive and dead fish were counted. Based on the approximate upper 24-h TL50, a second group of 200 larvae (24–72 h old; 10 fish per aquarium) were held for 24 h at 23 (control), 31, 32, 33, and 34°C. After 24 h of exposure to the test temperatures, numbers of alive and dead fish in each of the aquarium were counted.

Percent mortality for each aquarium at each test temperature was determined. A nonlinear regression (bounded monotonic response function curve; Wilkinson et al. 1992), was fitted, based on the percent mortality for both the upper and lower trials, to estimate the 24-h TL50 for the larvae.

*Temperature effects on growth.*—Two separate trials were conducted to determine temperature effects on growth. In both trials, larval production was conducted similar to the 24-h TL50 study. Healthy larvae were collected daily and each day's collection was placed in a separate aquarium. When approximately 500 larvae had been collected within a 7-d period, the fish were combined into one aquarium. Twenty-five randomly selected fish were measured to obtain an estimated initial mean length. A dissecting microscope with a calibrated micrometer ocular lens was used to measure fish length. Twenty groups of 20 fish were randomly selected from the remaining fish and placed into 20 aquaria (9 L; flow through) at 23°C. During trial 1, five aquaria remained at 23°C (control) for the duration of the test. Four each of the remaining aquaria were acclimated to 20, 17, and 14°C, following the same method as in the reproduction trials. In trial 2, fish were handled in the same manner as trial 1, except that test temperatures were 23 (control), 25, 27, and 29°C.

Fish were fed zooplankton (harvested from hatchery ponds; primarily cladocerans and copepods) each day. Zooplankton was screened through a 180- $\mu$ m mesh sieve, collected on a 53- $\mu$ m mesh sieve, and distributed in each aquarium to provide a concentration of 4,000 organisms/L. When zooplankton numbers were below the target concentration (36,000 per aquarium), brine shrimp *Artemia* sp. were added to obtain the desired food

concentration. After 35 d, all surviving fry were removed, euthanized, and lengths were measured.

Percent survival, final length, and growth rate were determined. The means for initial fish length for trials 1 and 2 were tested for a significant difference by an independent *t*-test ( $P \leq 0.05$ ; Wilkinson et al. 1992) to determine if the growth of control fish in both trials was similar. Significant differences in mean final lengths of fish reared at the different temperatures were tested by ANOVA, and when appropriate, multirange comparisons were made to determine specific significant differences among groups by Tukey's honestly significant difference test (Wilkinson et al. 1992).

## Results and Discussion

### Temperature Effects on Reproduction

The test temperature ( $^{\circ}\text{C}$ ), actual mean temperature ( $^{\circ}\text{C}$ ), SD, and range (test [actual, SD, range]) for each trial were as follows: 14 $^{\circ}\text{C}$  (14.2, 0.26, 13.5–14.6); 17 $^{\circ}\text{C}$  (17.7, 0.21, 17.4–18.1); 20 $^{\circ}\text{C}$  (20.1, 0.34, 19.3–20.2); 23 $^{\circ}\text{C}$  (22.7, 0.50, 22.2–23.8); 23 $^{\circ}\text{C}$  (22.6, 0.26, 22.1–23.0); 25 $^{\circ}\text{C}$  (25.5, 0.52, 25.1–26.2); 27 $^{\circ}\text{C}$  (27.4, 0.14, 27.1–27.5); 29 $^{\circ}\text{C}$  (29.5, 0.20, 29.1–30.0). For both trials, dissolved oxygen concentration ranged from 6.8 to 10.3 mg/L and the pH ranged from 8.1 to 8.7. Approximately 1 month after fountain darters were collected and before the trials began, mortalities ranged from 0 to 3 fish/d. Examination of fish revealed parasites (nematodes and monogenetic flukes), dermal emphysema, or exophthalmus. Air supersaturation in the aquaria was measured at 105% or greater. It was assumed that supersaturation of nitrogen, which can cause gas bubble disease, contributed to the demise of these fish. Water-degassing columns were installed. Gas supersaturation level in the tanks decreased below 101%, and the mortality rate decreased. Saturation level was maintained between 97% and 101% during all trials. In later observations not associated with this study, chronic exposure to supersaturation above approximately 102% seemed to increase the mortality rate.

There were no significant differences between egg and larval production of the two 23 $^{\circ}\text{C}$  tests for each trial; therefore, data for the egg and larval production at 23 $^{\circ}\text{C}$  for both trials were combined. Fountain darters produced eggs at each temperature tested between 14 $^{\circ}\text{C}$  and 27 $^{\circ}\text{C}$  but not at 29 $^{\circ}\text{C}$  (Table 1).

Brandt et al. (1993) reported egg production at 30 $^{\circ}\text{C}$  by fountain darters, but the darters had been

TABLE 1.—Mean (SD) numbers of fountain darter eggs and larvae produced by two males and two females per aquarium held for 33 d; there were five aquaria at each temperature. Asterisks denote means significantly different from the corresponding 23 $^{\circ}\text{C}$  control group ( $P \leq 0.05$ ).

| Trial and temperature ( $^{\circ}\text{C}$ ) | Mean (SD) number of eggs | Mean (SD) number of larvae | Mean (SD) percent hatch <sup>a</sup> |
|--|--------------------------|----------------------------|--------------------------------------|
| Trial 1                                      |                          |                            |                                      |
| 14   | 738 (210)                | 208 (86)                   | 30 (12)*                             |
| 17   | 617 (76)                 | 319 (29)                   | 52 (5)*                              |
| 20   | 965 (406)                | 432 (250)                  | 43 (8)                               |
| 23 <sup>b</sup>                              | 696 (331)                | 288 (104)                  | 44 (13)                              |
| Trial 2                                      |                          |                            |                                      |
| 23 <sup>b</sup>                              | 824 (318)                | 302 (110)                  | 38 (8)                               |
| 25   | 527 (179)                | 67 (30)*                   | 13 (5)*                              |
| 27 <sup>c</sup>                              | 189 (253)*               | 0 (0)*                     | 0 (0)*                               |
| 29   | 0 (0)*                   | 0 (0)*                     | 0 (0)*                               |

<sup>a</sup> Means were derived from arcsine-transformed data.

<sup>b</sup> Control group; combined totals for egg, larvae, and percent hatch produced at 23 $^{\circ}\text{C}$  were used for the analysis of variance. The combined totals (mean and SD) produced at 23 $^{\circ}\text{C}$  were as follows: number of eggs, 757 (314); number of larvae, 295 (101); percent hatch, 41 (11).

<sup>c</sup> Three aquaria at this temperature.

spawning at 27 $^{\circ}\text{C}$  for 2 weeks before the water temperature was raised to 30 $^{\circ}\text{C}$ . Only a few eggs were produced during the 2 weeks the fish were held at 30 $^{\circ}\text{C}$ , and these eggs were probably brought to the ripe egg stage while at 27 $^{\circ}\text{C}$  and sequentially released at 30 $^{\circ}\text{C}$ .

Maximum egg production occurred at 20 $^{\circ}\text{C}$ . No significant differences from the control (23 $^{\circ}\text{C}$ ) in egg production were found among test temperatures of 14, 17, 20, and 25 $^{\circ}\text{C}$ . Egg production at 27 and 29 $^{\circ}\text{C}$  was significantly lower than egg production at 23 $^{\circ}\text{C}$ .

In comparing the effect of temperature on fountain darter egg production with the effect on another central Texas warmwater stream fish, the greenthroat darter has a much narrower optimum temperature range (20–23 $^{\circ}\text{C}$ ) for egg production (Hubbs and Strawn 1957). Strawn (1956) observed that greenthroat darters stopped producing eggs at temperatures between 24 and 27 $^{\circ}\text{C}$ . Brungs (1971) determined that fathead minnows *Pimephales promelas* did not spawn in water temperatures above 32 $^{\circ}\text{C}$  and that egg production at 26 $^{\circ}\text{C}$  was significantly lower than egg production at 20–23 $^{\circ}\text{C}$ .

In this study, percent hatch of fountain darters at 14, 25, 27, and 29 $^{\circ}\text{C}$  was significantly lower than the control (Table 1). Percent hatch of fountain darters at 17 $^{\circ}\text{C}$  was significantly higher than the control. Eggs produced at 17 $^{\circ}\text{C}$  produced the highest percent hatch (52%), which may be the result of a decrease in fungus infection on eggs at

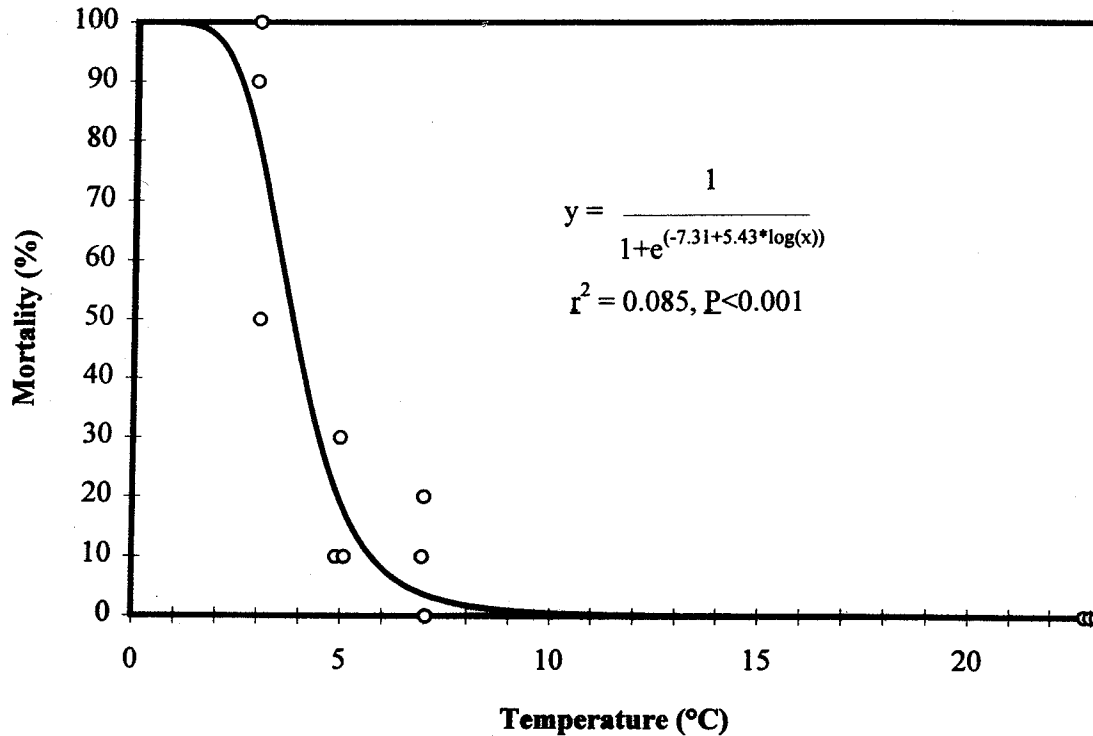


FIGURE 1.—Percent mortalities ( $y$ ) for larval fountain darters (24–72 h old) maintained at temperatures ( $x$ ) between 3°C and 23°C for 24 h. Open circles represent percent mortalities per aquarium (10 fish/aquarium).

temperatures of 17°C or lower. Brandt et al. (1993) found the maximum percent hatch at 18°C (42%).

Percent hatch was similar to the results of Brandt et al. (1993), except at 23 and 27°C. At 23°C in this study, percent hatch (41.2%) was higher than found by Brandt et al. (1993) at 24°C (28.6%). In addition, Brandt et al. (1993) had 24% hatch from eggs produced at 27°C, whereas in this study, percent hatch was 0%. In their study, the eggs were produced at 27°C but incubated at approximately 22°C. Cooler temperatures can inhibit fungus growth, which could explain the higher percent hatch and the differences between studies.

Our findings with the fountain darter are similar to those reported for bluegill *Lepomis macrochirus* and the fathead minnow. Banner and Van Arman (1973) reported maximum percent hatch for bluegill at 22°C to be 44%. Although significance of differences was not studied, they found that percent hatch was fairly constant at temperatures tested between 22 and 34°C. At 36°C, Banner and Van Arman (1973) found that percent hatch decreased to 4%. Brungs (1971) determined that temperatures between 23.5 and 30°C had no apparent effect on percent hatch for fathead minnows but that at

test temperatures above 32°C, larvae failed to hatch. Brungs (1971) maintained a high hatch rate (64–96%) from fathead minnow eggs produced and incubated at temperatures between 23.5 and 30°C by using prophylactic treatments of fungicide. Methods to control fungus on fountain darter eggs should be investigated.

Larvae were produced at all temperatures except 27°C and 29°C (Table 1). Larval production at 25, 27, and 29°C was significantly lower than the control. Maximum number of larvae was produced at 20°C; however, there was no significant difference in larval production among temperatures of 23, 20, 17, and 14°C. Mean number of larvae produced is useful in determining the range of temperatures for maximum larval production. However, because unequal numbers of eggs were used to determine the number of larvae produced for each temperature, results cannot be used to determine temperature effects between treatments.

#### Temperature Tolerance

In the first trial to determine the range of the lower TL50 for larval fountain darters, all larvae placed in temperatures of 23, 19, 15, 11, and 7°C

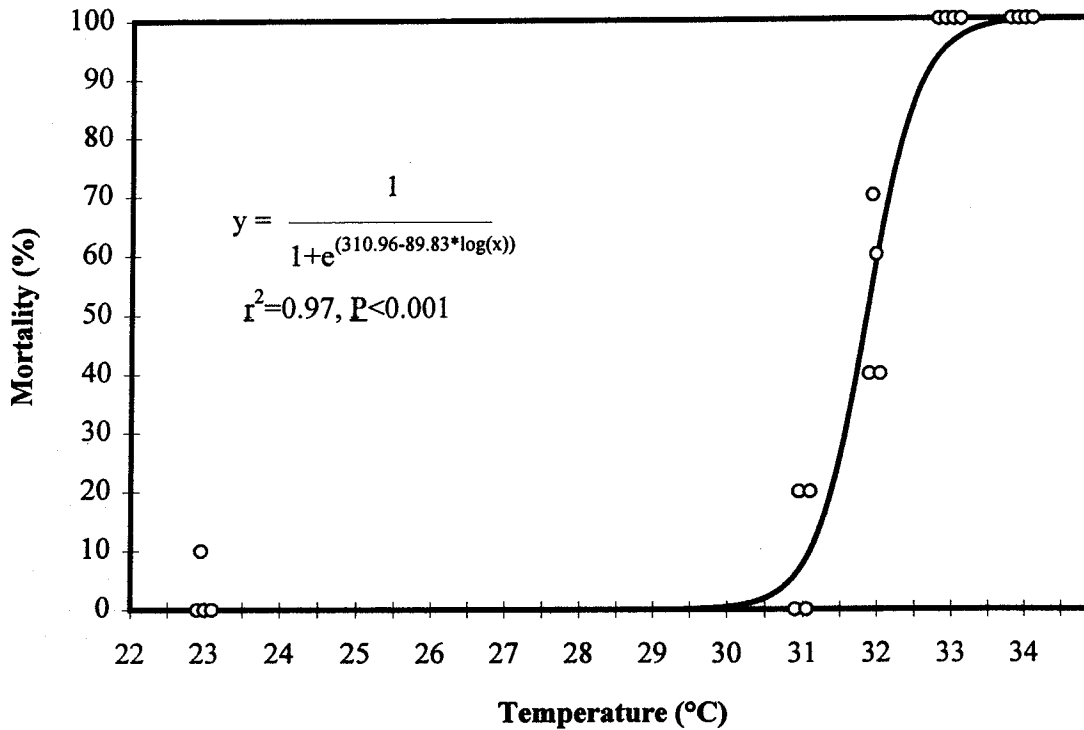


FIGURE 2.—Percent mortalities ( $y$ ) for larval fountain darters (24–72 h old) maintained at temperatures ( $x$ ) between 23°C and 34°C for 24 h. Open circles represent percent mortalities per aquarium (10 fish/aquarium).

survived for 24 h. For the second trial, larvae placed at 23, 7, 5, and 3°C had mean mortalities of 0, 10, 16.7, and 80%, respectively (Figure 1). The lower TL50 was estimated to be 3.8°C ( $r^2 = 0.85, P = 0.001$ ).

In the third trial to determine the approximate upper TL50, larvae placed in temperatures of 23,

27, 31, 35, and 39°C had mean mortalities of 0, 0, 12.5, 100, and 100%, respectively. For the fourth trial, larvae placed in temperatures of 23, 31, 32, 33, and 34°C had mean mortalities of 2.5, 10, 62.5, 100, and 100%, respectively (Figure 2). The upper TL50 was estimated to be 31.9°C ( $r^2 = 0.97, P = 0.001$ ).

The lethal temperature tolerances of fountain darter larvae were similar to other warmwater fish larvae. Banner and Van Arman (1973) reported the lower and upper TL50 for larval bluegills to be 4.7 and 33°C. McCormick and Wegner (1981) reported the upper TL50 for larval largemouth bass *Micropterus salmoides* to be 32°C. At an acclimated temperature of 20°C, the lower and upper TL50s were 2 and 32°C for the fathead minnow, 4 and 31.9°C for the golden shiner *Notemigonus crysoleucas*, and 2.5 and 32.8°C for the channel catfish *Ictalurus punctatus* (Strawn 1958). It is unknown if these last three pairs of TL50s were for larvae or adults.

#### Temperature Effects on Growth

The mean initial lengths and SDs for larval fountain darters were  $6.3 \pm 0.58$  mm for trial 1

TABLE 2.—Percent survival, total length, and growth rate for larval fountain darters (20 fish/aquarium; 5 aquaria/treatment) held for 35 d at various temperatures. Larval mean starting length for both trials was 6.3 mm. Within a trial, length values sharing a common letter were not significantly different ( $P > 0.05$ ).

| Temperature (°C) | Survival (%) | Total length (mm) |      | Growth rate (mm/d) |
|------------------|--------------|-------------------|------|--------------------|
|                  |              | Mean              | SD   |                    |
| Trial 1          |              |                   |      |                    |
| 14               | 24           | 10.1 y            | 1.10 | 0.11               |
| 17               | 33           | 11.7 z            | 1.31 | 0.15               |
| 20               | 26           | 11.8 z            | 1.64 | 0.16               |
| 23               | 41           | 11.0 zy           | 1.75 | 0.13               |
| Trial 2          |              |                   |      |                    |
| 23               | 55           | 9.7 y             | 0.92 | 0.10               |
| 25               | 49           | 10.6 z            | 1.40 | 0.12               |
| 27               | 34           | 9.5 y             | 1.11 | 0.09               |
| 29               | 11           | 10.5 zy           | 1.18 | 0.12               |

and  $6.3 \pm 0.96$  mm for trial 2. These mean values were not significantly different. The mean final lengths for the two test temperatures of 23°C were significantly different, so the two trials could not be combined to test significance between fish held at all test temperatures. In trial 1, the mean length of fountain darter fry held at the test temperature of 14°C was significantly different from the mean lengths at test temperatures of 17 and 20°C but not from those at 23°C (Table 2). In trial 2, the mean length of fish held at 25°C was significantly different than the mean lengths of fish held at 23 and 27°C but not from that at 29°C (Table 2).

Low survival of the fry at all temperatures (11–55%) and substantial differences in survival (41–55%) and final length (11.0 and 9.7 mm) of controls in the two trials prevented a meaningful assessment of temperature effects on larval growth. A previous attempt to determine growth of fountain darters produced similar results. Brandt et al. (1993) found low survival (14.9% at approximately 22°C) within the first 60 d. According to the growth equation calculated by Brandt et al. (1993), 35-d-old fish held at room temperature (approximately 22°C) should be 11.2 mm TL. The fish in trial 1 reared at 23°C were close to this length (11.0 mm mean TL), while the fish in trial 2 that were reared at 23°C were 13% shorter (9.7 mm mean TL). Fountain darter culture techniques need to be refined before more consistent larval survival and growth rates can be obtained. Until this is done, the effects of temperature on larval growth rates cannot be properly evaluated.

Based on the wide temperature range for maximum egg and larval production and larval survival of fountain darters found in this study and the critical thermal maximum for adult fish found by Brandt et al. (1993), fountain darters have temperature tolerances similar to those of other species that have wider geographic and thermal distribution (Strawn 1956, 1958; Hubbs and Strawn 1957; Brungs 1971; Banner and Van Arman 1973; McCormick and Wegner 1981). Constant temperatures between 22 and 24°C do not seem to be necessary for the short-term survival of fountain darters. However, constant temperatures in the 22–24°C range may be important indirectly to the fountain darter by affecting invertebrate populations, plant growth and plant composition, and the growth and reproduction of other fish species in the Comal and San Marcos rivers.

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