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Laboratory Spawning and Rearing of the Endangered Fountain Darter

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Abstract.—Survival of the fountain darter (*Etheostoma fonticola*), a U.S. federally listed endangered species, may depend on captive propagation. Studies were conducted to determine the effect of temperature on spawning and to develop methods for culture. The fountain darter spawned and produced viable offspring in aquaria at 27, 24, 21, 18, 15, 12, 9, and 6°C. The fish also spawned at 3 and 30°C but did not produce viable eggs. Daily egg production of individual fish held at 27, 21, 15, and 9°C was variable. The mean critical thermal maximum for the fountain darter was 34.8°C. Early life stages, 4–14 mm long, were offered a variety of live protozoans, rotifers, and microcrustaceans. Food selection varied with fish size and food size. Fountain darters reached sexual maturity in about 180 d when maintained at 21°C. Three-year-old darters produced viable offspring, and several lived longer than 4 years. Tricaine methanesulfonate was an effective anesthetic at 60 mg/L but was fatal to subadults at 100 mg/L.

The fountain darter (*Etheostoma fonticola*), an endangered species (U.S. Office of the Federal Register 35[13 October 1970]:16047), occurs only in the thermally constant (21–24°C) upper reaches of the San Marcos and Comal rivers, Texas. Both river populations depend on headwater springs; the springs, in turn, depend on the water level of the Edwards Aquifer (Ogden et al. 1985). Cessation of flow from Comal Springs during a drought in the mid-1950s contributed to the demise of the Comal River fountain darter population (Schenck and Whiteside 1976). No fountain darters were captured during intensive sampling (>300 h) of the Comal River in 1973, 1974, and 1975. The Comal River was restocked with fountain darters from the San Marcos River in 1975 (Schenck and Whiteside 1976).

Flows from Comal and San Marcos springs are closely monitored by the U.S. Geological Survey, which allows fountain darters to be collected and transported to a refugium when flows drop too low. When spring flows resume and habitat is reestablished, the darters can be returned. Previous studies have provided information on the biology of adult fountain darters: spawning behavior (Strawn 1956); habitat (Schenck and Whiteside 1976); spawning periodicity, fecundity, sexual dimorphism, sex ratio, and ova description (Schenck and Whiteside 1977a; Hubbs 1985); feeding behavior and food habits (Schenck and Whiteside 1977b); and some limited information on spawning and rearing (Strawn 1955, 1956; Strawn and Hubbs 1956). No information on the larval and juvenile development or the early life history of this species has been reported. This study was designed primarily to provide information on the effect of temperature on the laboratory culture of the fountain darter. The objective was to refine culture techniques so that in the event of a threat to the natural habitat, fountain darters can be successfully bred and reared in captivity until conditions enable fish to be restocked.

Methods

Fish collection.—Fountain darters were collected immediately downstream of the confluence of Sessom Creek with the San Marcos River and just below Rio Vista Park Dam. The two collection sites correspond to Schenck and Whiteside's (1976)

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sampling stations D and F. Fish were collected on 3, 4, and 24 September 1986 with a 30- by 45-cm hand-held dip net. After collection, the fish were transported to the National Fish Hatchery and Technology Center (NFHTC), San Marcos, Texas, and placed in 40- and 70-L glass aquaria. Males, females, and subadults were placed in separate aquaria. All fish were treated for possible external parasites with formalin (250 mg/L) for 1 h.

Spawning temperature range.—Eight 38-L glass aquaria were each stocked with two male and four female fountain darters. The aquaria were housed in two Percival Environmental Chambers (model 1-35LL, Percival Manufacturing, Boone, Iowa), four aquaria per chamber. The chambers were set to maintain a temperature of 21°C and a 12-h-light:12-h-dark daily cycle. Well water pumped from the Edwards Aquifer (total hardness, 270 mg/L as $CaCO_3$; pH 7.1; Ogden et al. 1985) was used in the aquaria. The aquaria were equipped with air-lift sponge filters. Each aquarium also was equipped with two nylon yarn spawning mops (Simon 1987). A spawning mop consisted of forty 15-cm-long strands of nylon yarn that were anchored to the center of one-half of a 5-cm styrofoam ball. The styrofoam was attached to a plastic-encased weight. Live organisms—primarily amphipods (scuds), aquatic oligocheates (black worms), and zooplankton (mainly cladocerans and copepods)—were offered as food for the darters. The amphipods were collected from native waters, the black worms were from native waters and commercial sources, and the zooplankton were collected from hatchery ponds according to methods described by Graves and Morrow (1988). The aquaria were cleaned as needed by wiping the sides and bottom with a plastic scouring pad and then siphoning off the waste material.

Both environmental chambers were operated at 21°C for 7 d. On day 8, the temperature in chamber 1 was lowered slowly over 6 h to 18°C and maintained at 18°C for 2 weeks. The temperature in chamber 1 was then lowered to 15°C for 2 weeks, to 12°C for 2 weeks, to 9°C for 3 weeks, to 6°C for 3 weeks, and to 3°C for 2 weeks; then it was raised back to 6°C for 2 weeks. The temperature in chamber 2, on day 8, was raised slowly to 24°C for 2 weeks, to 27°C for 2 weeks, and to 30°C for 2 weeks; then it was lowered to 27°C for 3 weeks.

The spawning mops in the eight aquaria were inspected twice weekly. Numbers of viable and nonviable eggs removed from the mops and from the walls of each aquarium were recorded. Nonviable eggs were distinguished by cloudiness within the egg or the presence of fungus. Viable eggs removed from the mops were incubated in plastic petri dishes at room temperature. The number of eggs that hatched and the appearance of the larvae (normal or not) were recorded.

Egg production by individual females.—On 24 April 1987, three of the 38-L aquaria were removed from chamber 2 and were replaced with four 10-L aquaria. Each 10-L aquarium was stocked with one male and one female fountain darter. The remaining darters from the three 38-L aquaria were stocked into the one remaining 38-L aquarium in the chamber. The temperature in chamber 2 was maintained at 27°C for 2 weeks, dropped slowly to 21°C and held there for 2 weeks, dropped to 15°C for 2 weeks, and finally dropped to 9°C for 2 weeks. The two spawning mops in each of the 10-L aquaria were inspected daily for eggs. After eggs were removed, the spawning mops were placed in a drying oven at 50°C for 24 h to ensure no viable eggs were transferred from one aquarium to another. Two sets of mops were maintained for each aquarium; while one set was in the aquarium, the other was in the oven. The numbers of viable and nonviable eggs removed daily from each aquarium were recorded; however, the eggs were not incubated, and hatchability was not studied. The darters were offered live food, generally in excess of what they could consume. All aquaria were cleaned as described earlier, and the water level was adjusted as needed.

An analysis of variance of the actual numbers of eggs collected at the four temperatures and of the square root of the numbers plus one was used to determine if differences existed among treatments at the 5% probability level (Zar 1984).

Critical thermal maximum.—Nine male and nine female fountain darters were used to determine the critical thermal maximum (CTM) (Cowles and Bogert 1944). Two screened baskets were placed in a Blue M Water Bath (model MW-1140 A-1, Blue M Electric Company, Blue Island, Illinois). Three males were placed in one basket, and three females were placed in the other. Initial water temperature of the unit was 19.2°C. Water temperature of the unit was raised at the rate of about 0.5°C per min until all fish displayed a critical response.

Loss of equilibrium or muscle spasms are often used as critical responses in determining CTM (Bonin et al. 1981). However, these responses could not be used for the fountain darter because they could not always be detected. Fountain darters are sedentary, and in preliminary trials they generally remained stationary on the bas pectoral fins outstretched. Mus never detected and the pectoral in place when equilibrium was l librium could only be detected if to be swimming at the time, a r the preliminary trials. A respon cernable and repeatable was th operculum and mouth. In preli flaring seemed to occur just be was lost. When a fish did flare i respiring. Water temperatures w ery 60 s. The temperature at the period in which the opercular fl the temperature recorded as the trials were repeated three times. of variance was used to determi ferences (Zar 1984).

TABLE 1.—Species, sizes, and per 13 mm total length in trial 1 and 7– per species in trial 1 and 100 organi

Species
Ceriodaphnia quadrangula
Kurzia latissima
Simocephalus vetulus
Diaptomus pallidus
Copepodid
Nauplius
Cyclops vernalis
Copepodid
Nauplius
Trichotria tetractis
Euchlanis sp.
Polyarthra dilochoptera
Hydrachna sp.
Chaoborus sp.

Fry food selection trials.—In fountain darters 4–13 mm in t held in a 38-L aquarium. Zoop several known types, sizes, and r to the aquarium (Table 1). Dart to feed for 1 h, after which 50 we a siphon, euthanized in a 50-mg/ caine methanesulfonate (MS-22 ical Laboratories, Redmond, W preserved in 4% buffered formali yses consisted of removing the e tinal tract and teasing out the con ton prey in the gut were identifie their total length and diameter at were measured. Fish total lengt before the gut was removed. Stra index of food selection was used t

the presence of fungus. Viable eggs
ı the mops were incubated in plastic
room temperature. The number of
ıed and the appearance of the larvae
ıt) were recorded.
tion by individual females.—On 24
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chamber 2 and were replaced with
ıuaria. Each 10-L aquarium was
one male and one female fountain
maining darters from the three 38-L
ıtocked into the one remaining 38-L
the chamber. The temperature in
ıs maintained at 27°C for 2 weeks,
y to 21°C and held there for 2 weeks,
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another. Two sets of mops were
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TABLE 1.—Species, sizes, and percentages of zooplankton offered as prey to fountain darter early life stages, 4–13 mm total length in trial 1 and 7–14 mm in trial 2. Sizes were determined from measurements of 40 organisms per species in trial 1 and 100 organisms per species in trial 2.

Species	Total length (mm) in trial:		Maximum diameter (mm) in trial:		Percentage in trial:	
	1	2	1	2	1	2
Ceriodaphnia quadrangula	0.5	0.5	0.2	0.2	68	27
Kurzia latissima	0.4	0.4	0.3	0.3	5	8
Simocephalus vetulus	1.0		0.7		2	
Diaptomus pallidus	0.8	0.9	0.3	0.3	13	1
Copepodid	0.5		0.3		1	
Nauplius	0.2		0.1		3	
Cyclops vernalis		1.2		0.5		14
Copepodid		0.5		0.3		12
Nauplius		0.2		0.1		4
Trichotria tetractis	0.1		0.1		2	
Euchlanis sp.	0.1		0.1		6	
Polyarthra dilochoptera		0.2		0.1		8
Hydrachna sp.		0.3		0.2		19
Chaoborus sp.		0.7		0.1		7

remained stationary on the basket bottom with pectoral fins outstretched. Muscle spasms were never detected and the pectoral fins held the fish in place when equilibrium was lost. Loss of equilibrium could only be detected if the fish happened to be swimming at the time, a rare occurrence in the preliminary trials. A response that was discernable and repeatable was the flaring of the operculum and mouth. In preliminary trials, the flaring seemed to occur just before equilibrium was lost. When a fish did flare it seemed to stop respiring. Water temperatures were recorded every 60 s. The temperature at the start of the 60-s period in which the opercular flare occurred was the temperature recorded as the CTM. The CTM trials were repeated three times. A nested analysis of variance was used to determine sex or trial differences (Zar 1984).

Fry food selection trials.—In feeding trial 1, fountain darters 4–13 mm in total length were held in a 38-L aquarium. Zooplankton prey of several known types, sizes, and ratios were added to the aquarium (Table 1). Darters were allowed to feed for 1 h, after which 50 were collected with a siphon, euthanized in a 50-mg/L solution of tricaine methanesulfonate (MS-222; Argent Chemical Laboratories, Redmond, Washington), and preserved in 4% buffered formalin. Stomach analyses consisted of removing the entire gastrointestinal tract and teasing out the contents. Zooplankton prey in the gut were identified to species, and their total length and diameter at maximum width were measured. Fish total length was measured before the gut was removed. Strauss' (1979) linear index of food selection was used to determine prey preference. This index—$L = 100\ (r_i - p_i)$; r_i and p_i are proportions of prey i in the gut and aquarium, respectively—ranges from -100 (strong aversion) to 100 (strong preference). Index values were tested for significant departures from zero with t-tests (Strauss 1979) at the 5% probability level.

Trial 2 took place 10 d after the first trial; fry remaining from the first trial were used. Fry total lengths ranged from 7 to 14 mm. Methods were the same as those in trial 1. The species composition and sizes of zooplankton prey offered in trial 2 are listed in Table 1.

Growth, maturity, and sexual longevity.—On 11 February 1987, 149 larvae, hatched at the NFHTC between 29 January and 3 February 1987, were stocked into a 38-L aquarium. The aquarium was maintained at room temperature. Live zooplankton or black worms were offered generally 5 d a week in excess. All fish in the aquarium were sexed based on color patterns, and individual total lengths were obtained at 30- to 60-d intervals initially and then periodically thereafter. Two spawning mops were placed in the aquarium and inspected daily until eggs were discovered for the first time. After initial discovery of eggs, the mops were inspected occasionally for eggs until all female darters in the study had died. The tank was cleaned and water level was adjusted in the manner described earlier. An equation was developed to describe growth from hatching until the first eggs were collected. Data were collected on length of fish and viability of eggs until all fish had died.

Tricaine methanesulfonate (MS-222) study.—Juvenile offspring of darters used in the growth

TABLE 2.—Mean number of fountain darter eggs per aquarium collected from spawning mops at temperatures ranging from 30 to 3°C. Each of four aquaria was stocked with four females and two males.

Temperature (°C)	Number of weeks	Number of eggs		Normal larvae produced (%)	
		Mean	Range	Mean	Range
30	2	10.5	0–42	0	
27	2	115.5	66–161	24.8	13.6–49.4
24	2	337.7	154–472	28.6	13.7–60.1
18	2	400.5	195–505	47.6	18.2–82.0
15	2	295.5	187–392	32.2	15.5–42.6
12	2	197.8	88–269	61.6	48.4–70.3
9	3	166.0	76–215	24.0	12.6–33.5
6	3	72.7	7–143	24.4	1.2–85.7
3	2	0		0	

study were removed from their parental aquarium on 7 October 1988. One group of 5 fish (the control) and five groups of 10 fish were each stocked into 1 L of 21°C well water containing MS-222 at concentrations of 0, 10, 20, 30, 40, or 50 mg/L. The fish were held for about 10 min, measured for total length, and placed in recovery aquaria, one aquarium per treatment. A second trial was conducted in the same manner with groups of 10 fish being treated with MS-222 at 60, 70, 80, 90, and 100 mg/L. A third trial was conducted with groups of 10 fish exposed to MS-222 at 60, 80, and 100 mg/L. In the third trial, the fish were held in the test solutions for a timed 15–18-min period, measured, and placed in recovery aquaria for 3 d. Total lengths, effect of treatments on immobilization of fish, and survival were recorded.

Results and Discussion

Spawning Temperature Range

Fountain darters deposited eggs at all temperatures tested between 3 and 30°C, but they only deposited eggs on the mops between 6 and 30°C (Table 2). The deposition of eggs on surfaces other than the mops was not anticipated and affected the accuracy of the egg production data. The aquaria were contained within environmental chambers, which limited viewing of the contents of the aquaria to one end of each aquarium. The transparency and small size of the eggs also hindered their detection. We felt that we could accurately collect all eggs deposited on the mops but not from the rest of the aquarium; therefore, we decided to incubate only those eggs collected from the mops. We also felt that removing the eggs from the mops was far less stressful to the eggs than removing them from the rest of the aquarium. Many eggs were physically damaged when we attempted to remove them from the aquarium corners. We do not know if the eggs collected from the walls at 3°C would have hatched. Egg deposition on the walls was variable and substantial. At 3°C, the eight eggs collected were all taken from the walls. From 6 to 30°C, egg deposition on the walls composed 7.8–48.5% of the total number of eggs collected and averaged 21.5%. The egg collection data in Table 2 represent only eggs collected from the mops and do not represent the total numbers of eggs produced.

Eggs were deposited on the mops at all temperatures tested between 6 and 30°C (Table 2). Egg production was substantially reduced at the lowest and highest temperatures. Maximum egg production seemed to occur in the 15–24°C temperature range, but egg production was so variable among aquaria within the same treatment that no conclusion can be drawn concerning an optimum spawning temperature.

Normal larvae were produced over the wide temperature range of 6–27°C (Table 2). We attempted to hatch the eggs collected at 30°C, but all eggs developed fungus during incubation. Percentage of fry surviving varied among aquaria at each temperature. There was no clear temperature effect on percentage of normal larvae produced.

The deposition of eggs on surfaces other than the spawning mops substantially lowered the accuracy of the egg production data. The results do demonstrate, however, that fountain darters are intermittent spawners that produce viable eggs between 6 and 27°C. Furthermore, substantial variation exists in the number of ova produced by individual fish.

Egg Production by Individual Females

The mean numbers of eggs produced by individual females at four temperatures for 14-d trials are presented in Table 3. Because aquaria housing the darters were smaller than in the spawning temperature range study (10 L versus 38 L), the entire aquarium could be viewed. This permitted a more complete collection of eggs from the mops, aquarium walls, and aquarium bottoms. Values reported in Table 3 are for all eggs removed from the aquaria. Total egg production over the test period seemed to increase as temperature increased, but statistical analysis showed no significant differences between actual or transformed values. This was attributed to substantial variation within treatments.

TABLE 3.—Egg production by fou
four temperatures.

Measure	Te 9	
Number of eggs produced per female per 2 weeks		
Mean	17.2	6
Range	0–40	0
Number of eggs produced per female per day, range	0–40	0
Number of days eggs were produced, per female		
Mean	0.8	1
Range	0–1	0

Substantial variation was als
number of days when eggs wer
daily number of eggs released
both within and between trea
Maximum number of eggs relea
ual female over a 24-h period
imum number of mature ova
fountain darters examined by Sc
side (1977a) was in the mid-60
74 fish, which had a mean of 1
our study, the mean number of
days when eggs were released,
the course of these trials, the me
produced by an individual fish
was 94–471. The number of d
individual deposited eggs average
from 5 to 27. Because egg produ
ual fountain darters was so varia
to an estimate of the number
annually by an individual was r

Critical Thermal Maxima

The fountain darter's mean
(SD, 0.474). Sex of the darter di
affect this value. The value of 34
tain darter is similar to CTM va
four populations of orangethroa
toma spectabile) collected from f
ferent habitats in Oklahoma (Fe
thews 1984). The CTMs for or
populations varied from 32.8°C
tion inhabiting an 18°C cons
spring, to 35.4°C, for a popula
creek where water temperature
had been recorded.

Fry Food Selection Trials

Food habit analyses of the e
several etheostomid darters ha

em from the rest of the aquarium. ere physically damaged when we at- emove them from the aquarium cor- not know if the eggs collected from 3°C would have hatched. Egg depo- walls was variable and substantial. ght eggs collected were all taken from om 6 to 30°C, egg deposition on the sed 7.8–48.5% of the total number of d and averaged 21.5%. The egg col- in Table 2 represent only eggs col- the mops and do not represent the s of eggs produced.

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TABLE 3.—Egg production by four fountain darters at four temperatures.

Measure	Temperature (°C)			
	9	15	21	27
Number of eggs produced per female per 2 weeks				
Mean	17.2	64.0	68.2	110.6
Range	0–40	0–96	0–139	16–228
Number of eggs produced per female per day, range	0–40	0–60	0–42	0–51
Number of days eggs were produced, per female				
Mean	0.8	3.8	4.6	4.6
Range	0–1	0–7	0–10	1–9

Substantial variation was also observed in the number of days when eggs were released and in daily number of eggs released by individual fish both within and between treatments (Table 3). Maximum number of eggs released by an individual female over a 24-h period was 60. The maximum number of mature ova contained within fountain darters examined by Schenck and Whiteside (1977a) was in the mid-60s; they examined 74 fish, which had a mean of 19 mature ova. In our study, the mean number of eggs released, on days when eggs were released, was 19.3. During the course of these trials, the mean number of eggs produced by an individual fish was 260; the range was 94–471. The number of days out of 54 an individual deposited eggs averaged 13.5 and ranged from 5 to 27. Because egg production by individual fountain darters was so variable, extrapolation to an estimate of the number of eggs produced annually by an individual was not possible.

Critical Thermal Maxima

The fountain darter's mean CTM was 34.8°C (SD, 0.474). Sex of the darter did not significantly affect this value. The value of 34.8°C for the fountain darter is similar to CTM values obtained for four populations of orangethroat darter (*Etheostoma spectabile*) collected from four thermally different habitats in Oklahoma (Feminella and Matthews 1984). The CTMs for orangethroat darter populations varied from 32.8°C, for the population inhabiting an 18°C constant-temperature spring, to 35.4°C, for a population inhabiting a creek where water temperatures as high as 39°C had been recorded.

Fry Food Selection Trials

Food habit analyses of the early life stages of several etheostomid darters have been reported (Braasch and Smith 1967; Scalet 1972; Schenck and Whiteside 1977b; Cordes and Page 1980). Young darters feed primarily on microcrustaceans, especially cladocerans and copedods. However, prey preference by species or size of prey was not addressed. Zooplankton preference by species and size is reported here to provide more specific information on prey organisms required to sustain young fountain darters, 4–14 mm long, in intensive culture.

Ninety-two percent of the stomachs examined in the first feeding trial contained food. Fish 4–13 mm long generally selected for the small cladoceran *Kurzia latissima* (Table 4). The smallest larvae, 4–8 mm, selected against the calanoid copepod *Diaptomus pallidus,* whereas 10–13-mm larvae showed a positive selection for this copepod. *Ceriodaphnia quadrangula,* a small cladoceran, was eaten only by the 4–5-mm larvae. The large cladoceran *Simocephalus vetulus* and water mites (*Hydrachna* sp.) generally were avoided.

Prey species offered in trial 2 were essentially the same as in trial 1 with the addition of a cyclopoid copepod (*Cyclops vernalis,* adults and juveniles) and chironomids (mainly *Chironomus* sp.). In trial 2, 97% of the stomachs examined contained food. Fry (7–14 mm) in trial 2 showed a preference for adult *C. vernalis* and *Chironomus* sp. over adult *K. latissima* (Table 4). Fry also generally avoided copepodites (juvenile copepods) and nauplii (larval copepods) of both copepods species.

Prey sizes ingested during both trials were related to size of larvae: prey depth (mm) = 0.205 + 0.008 × fish total length (mm); $P < 0.001$, $r^2 = 0.119$. Consumption of *K. latissima* by the smallest larvae was probably related to this prey's small size as well as to its comparatively slow movement along the bottom of the aquarium. Vertical movement of the larvae more than 6–8 mm off of the aquarium bottom, even in pursuit of prey, was rare, especially for the smallest larvae. Prey of suitable size, approximately 0.2–0.4 mm in diameter, in close proximity to the bottom tended to be the most vulnerable. The dietary preference of 7–15-mm fry for adult copepods and chironomids over other zooplankters of similar size was probably a response to visual cues. Response to prey movement by darters has been reported by Roberts and Winn (1962), Scalet (1972), and Schenck and Whiteside (1977b). Early life stages of all sizes seemed to be attracted to the almost constant movement of the copepods and chironomids, but only the larger larvae were suc-

TABLE 4.—Linear forage index values for zooplankton offered as prey to fountain darter early life stages, which were 4–13 mm total length in trial 1 and 7–14 mm in trial 2. Asterisks indicate significant differences from zero at the 5% probability level.

Zooplankton species	Fry length (mm)										
	4	5	6	7	8	9	10	11	12	13	14
Trial 1											
Ceriodaphnia quadrangula	18*	5	−14*	−68*	20*		−32*	−21*	−24*	−60*	
Kurzia latissima	6*	22*	37*	81*	−5*		12*	13*	8*	−5*	
Simocephalus vetulus	−2*	−2*	2*	−2*	−2*		1*	4*	−2*	−2*	
Diaptomus pallidus	−10*	−13*	−13*	1	−1		16*	16*	24*	70*	
Copepodids	−1*	−1*	−1*	−1*	−1*		1	−1*	−1*	−1*	
Hydrachna sp.	−4*	−4*	−4*	−4*	−4*		10*	−4*	2*	4*	
Trial 2											
Ceriodaphnia quadrangula				−27*	−27*	−27*	−27*	−27*	−27*	−27*	−27*
Kurzia latissima				−8*	−6*	−8*	−8*	−8*	−7*	−8*	−8*
Diaptomus pallidus				−1*	−1*	−1*	−1*	−1*	−1*	−1*	−1*
Cyclops vernalis				24*	31*	51*	17*	30*	30*	30*	35*
Copepodids				8*	−4*	−3*	−5*	10*	−2*	−3*	−9*
Nauplii				6*	−4*	−4*	13*	8*	−4*	−4*	−4*
Hydrachna sp.				11*	12*	−10*	5*	−17*	−10*	−2*	−14*
Chironomus spp.				−7*	7*	10*	7*	13*	28*	28*	33*

cessful in capturing and ingesting them. Even though copepodites and nauplii displayed the same movements as adult copepods, they were generally avoided, probably because they were not as conspicuous. Their limited pigmentation and small size probably also contributed to their low selection. Both adult copepod species possessed far more pigmentation than the copepodite stages. Attraction to *Chironomus* sp. may also have been related in part to pigmentation.

Inconsistent selectivity patterns, as seen with *C. quadrangula* and *Hydrachna* sp. in the first trial, may be explained by individual fish consuming large numbers of a single prey species. This presumably occurred when fry encountered clustered zooplankton. On several occasions in this study, fry were observed actively feeding on swarming zooplankton.

The use of *C. vernalis* as a food source in this study was not wise because of the possibility of injury to or predation on the fry. Cyclopoids are predatory, and several of the *C. vernalis* introduced into the aquarium approached 2 mm in length. Although *C. vernalis* was not observed to

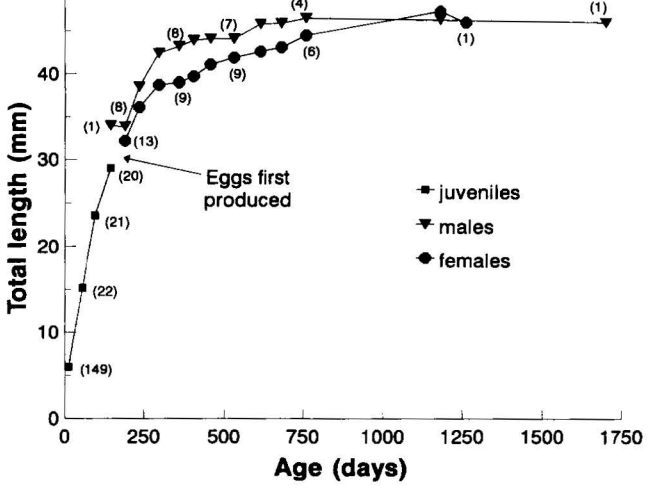

FIGURE 1.—Laboratory growth curve for fountain darters. Number of surviving fish used to generate each point is given in parentheses.

TABLE 5.—Effect of tricaine met
222) on mobility and survival of juv
ers. Five control fish were tested and
at the various MS-222 levels. Expo
and 2 was approximately 10 min; i
18 min.

Trial	MS-222 (mg/L)	Fish immobilize
1	0, 10, 20, 30, 40	No
1	50	Partially
2	60, 70, 80	Yes
2	90	Yes
2	100	Yes
3	60	Yes
3	80	Yes
3	100	Yes

attack fry in this study, it was c
live fry in a petri dish.

Growth, Maturity, and Sexual I

During their first 146 d of life
in this study grew an average of
1). The equation, $Y = 5.2 + 0.1$
relation between length (Y) and
over this 146-d period ($r^2 = 0.9$

Survival during the first 60 d
vae were stocked into the aqua
lower, 14.9%, than we normally
fountain darters held in aquari
day 60 through the first year wa
not determine the causes of m
showed no signs of parasite-, ba
related problems. We do not kno
ed dying of old age. The last fem
after start of study and the last

The first eggs were collected w
about 6 months old and averaged
Schenck and Whiteside (1977a)
24.4-mm female contained 18
size fish in our study would hav
months old. Three-year-old fem
our study produced viable eggs
normal offspring. Within 3 mont
were collected, all females had
tempt was made to collect eggs
months that the females were al

MS-222 Study

Exposure to MS-222 at levels
40 mg/L for about 10 min did
juvenile *E. fonticola* (Table 5).
fish were partially immobilized,

o fountain darter early life stages, which
dicate significant differences from zero at

(mm)				
10	11	12	13	14
−32*	−21*	−24*	−60*	
12*	13*	8*	−5*	
1*	4*	−2*	−2*	
16*	16*	24*	70*	
1	−1*	−1*	−1*	
10*	−4*	2*	4*	
* −27*	−27*	−27*	−27*	−27*
* −8*	−8*	−7*	−8*	−8*
* −1*	−1*	−1*	−1*	−1*
* 17*	30*	30*	30*	35*
* −5*	10*	−2*	−3*	−9*
1* 13*	8*	−4*	−4*	−4*
)* 5*	−17*	−10*	−2*	−14*
)* 7*	13*	28*	28*	33*

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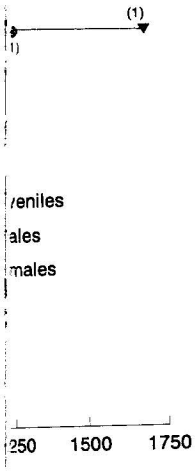

of surviving fish used to generate each point

TABLE 5.—Effect of tricaine methanesulfonate (MS-222) on mobility and survival of juvenile fountain darters. Five control fish were tested and 10 fish were tested at the various MS-222 levels. Exposure time in trials 1 and 2 was approximately 10 min; in trial 3 it was 15–18 min.

Trial	MS-222 (mg/L)	Fish immobilized	Survival (%)	Mean total fish length (mm)
1	0, 10, 20, 30, 40	No	100	21.3
1	50	Partially	100	22.0
2	60, 70, 80	Yes	100	22.8
2	90	Yes	80	22.8
2	100	Yes	30	20.7
3	60	Yes	100	22.4
3	80	Yes	90	22.1
3	100	Yes	0	22.3

attack fry in this study, it was observed to attack live fry in a petri dish.

Growth, Maturity, and Sexual Longevity

During their first 146 d of life, fountain darters in this study grew an average of 0.2 mm/d (Figure 1). The equation, $Y = 5.2 + 0.17X$, describes the relation between length (Y) and time in days (X) over this 146-d period ($r^2 = 0.96$).

Survival during the first 60 d after the 149 larvae were stocked into the aquarium was much lower, 14.9%, than we normally experience with fountain darters held in aquaria. Survival from day 60 through the first year was 77%. We could not determine the causes of mortality. The fish showed no signs of parasite-, bacteria-, or virus-related problems. We do not know when fish started dying of old age. The last female died 3.4 years after start of study and the last male 4.7 years.

The first eggs were collected when the fish were about 6 months old and averaged 32 mm in length. Schenck and Whiteside (1977a) reported that a 24.4-mm female contained 18 mature ova. This size fish in our study would have been about 3.5 months old. Three-year-old females and males in our study produced viable eggs that hatched into normal offspring. Within 3 months after these eggs were collected, all females had expired. No attempt was made to collect eggs during the last 3 months that the females were alive.

MS-222 Study

Exposure to MS-222 at levels between 10 and 40 mg/L for about 10 min did not immobilize juvenile *E. fonticola* (Table 5). At 50 mg/L the fish were partially immobilized, and at 60 to 100 mg/L they were completely immobilized. Fish exposed to 70 mg/L or less all survived, but all fish exposed to 100 mg/L of MS-222 for 15–18 min died. In this study, 60-mg/L solutions of MS-222 immobilized the fish without causing mortality. During the course of this project, we routinely used MS-222 at 60 mg/L with excellent results. Fountain darter juveniles appear to be more sensitive to MS-222 than juvenile channel catfish (*Ictalurus punctatus*), which Plumb et al. (1983) found able to withstand 100 mg/L for 20 min without adverse affects.

Epilogue

During the years of the longevity study, the Edwards Aquifer level dropped to a dangerously low level and the decision was made to collect fountain darters from the Comal River to be held in refugium. Just after the fish were collected, heavy rains recharged the aquifer to a less critical level, but low spring flows into the Comal River prevented returning the fish to that system for 9 months. Much of the information presented above was used in determining how the refugium was to be designed and managed.

Acknowledgments

Glenn Longley and Clark Hubbs provided much background information and contributed substantially to the designs of parts of this project; their help was crucial. Roy Jones, Jacob Morrow, and Joe Fries, on several occasions, contributed physically and mentally to the completion of this project; their help is appreciated.

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Evaluation of

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