Fountain Darter Laboratory Study: Reproductive Response to Parasites and Temperature Fluctuations

Final Report
Variable Flow Study: Project 802, Task 18
Southwest Texas State University, San Marcos, Texas

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EXECUTIVE SUMMARY

Fountain darters (*Etheostoma fonticola*) occupy areas that are strongly influenced by springflows and nearly constant water temperatures. In both the Comal and San Marcos Rivers, fountain darters are most abundant in headwater areas where temperatures range from 22 to 25°C. Consequently, the species appears to have adapted to a relatively narrow temperature range. Downstream of spring inputs, at the edges of the fountain darter range, water temperature exhibits some fluctuation. During summer months, particularly when springflows are reduced, these fluctuations can result in temperatures that may influence fountain darter reproduction. Previous laboratory research has indicated a decline in fecundity at 27°C and above (Brandt et al. 1993, Bonner et al. 1998) but these studies did not account for daily fluctuations in water temperature of approximately 2°C in the wild. We hypothesized that egg and larval production at fluctuating temperatures (2°C oscillation within 24 hours that incorporates optimum or near optimum spawning temperatures for at least half of the 24 hours) will not differ from egg and larval production at a constant optimum temperature (24°C). Specifically, we sought to determine if compensatory reproduction was possible for the fountain darter.

The following study was conducted by Dr. Timothy Bonner (Southwest Texas State University) to examine this question with a controlled laboratory experiment. Dr. Bonner examined the effect of fluctuating temperatures and cercariae (parasite) infestation on total egg, healthy egg, and larval production. Temperatures included a constant 24°C, fluctuating 24 to 26°C, fluctuating 26 to 28°C, and fluctuating 28 to 30°C; all fluctuations were on a 12h day, 12h night cycle. These temperatures were chosen because the lowest level falls within the range of temperatures commonly found in fountain darter habitats. The next two treatments had temperatures that may potentially be reached during low-flow/high temperature periods (~26°C); the last treatment included a range above the "worst-case scenario" to evaluate the potential improvement with the 2°C diel fluctuation. Dr. Bonner's report detailing his methods and results is attached.

The first analysis revealed that parasites did not have an effect on egg or larval production, so darters with and without parasites were lumped for all other analyses. This is an important finding of the study. Parasites are a concern in the Comal Springs ecosystem because of high rates of infestation, particularly in areas where the fountain darters are most abundant (old channel). Parasites do not appear to cause mortality directly in the wild (T. Brandt, USFWS, personal communication) but have been assumed to have sub-lethal effects (cause greater stress, reduce predator avoidance, reduce reproductive ability). These results suggest that, at least in the early stages of infestation, there is little effect on reproductive capability. It is possible; however, that long-term infestation or greater parasite loads may have negative effects that were not observed here.

There were significant differences observed between temperature treatments in the number of healthy eggs and larvae produced; the 24°C treatment resulted in significantly more eggs overall, healthy eggs and larvae than any other trial. This is not surprising since this temperature is within the range of mean temperatures found in areas where fountain darters are abundant. The two treatments with increasingly higher temperatures (24-26°C and 26-28°C) had similar numbers (not significantly different) of total eggs and healthy eggs produced, but the number of larvae produced was lower in the 26-28°C treatment. The 28-30°C treatment resulted in virtually zero eggs

produced. Although standardizing healthy eggs and larvae produced by percentage of total eggs produced did reveal less significant differences between treatments, the large decline in total egg production between treatments suggests that temperatures in the ranges tested here do have an important effect on reproduction in darters.

Dr. Bonner notes that combining temperature results from this study and from Bonner et al. (1998) would suggest that fountain darter egg production is reduced at temperatures between 25 and 26°C and larval production decreases between 24°C and 25°C. However, this assessment is based solely on laboratory evidence. As part of the Variable Flow Study conducted by BIO-WEST for the Authority, fountain darter sampling has occurred seasonally and water temperature monitoring (using remote data loggers) has occurred continuously since August/September 2000. During that time, water temperature has remained above 25°C for extended periods in the area with the highest fountain darter density, the old (natural) channel of the Comal River downstream of Landa Lake, yet dip netting has consistently produced fountain darters of 5-15mm long (<58 days old) in that area. This evidence of recent reproduction has occurred during times when temperature exceeded 25°C and approached 26°C (temperatures have rarely exceeded 26°C during the Variable Flow Study), including one sample in late August 2001 in which 19 of 55 (35%) captured fountain darters were <15mm long and should have hatched in early June (according to the growth formula given by Brandt et al. [1993]). Another sample, one month later, resulted in 14 of 68 (21%) fountain darters <15mm long; these should have hatched in early August when temperatures were still near 26°C in the old channel reach. Samples in late summer 2000, when temperatures approached (and exceeded on 3 days) 26°C, also had relatively high numbers of small (<15mm) fountain darters, but the data loggers were not in place 58 days prior to these samples to assess temperature at the calculated time of hatching.

Overall, these results do not confirm our initial hypothesis. It appears that, in the laboratory, egg and larval production do not benefit greatly from the 2°C diel fluctuation that fountain darters experience in the wild. As in previous studies, lower egg and larval production was observed at temperatures higher than in controls (23°C and 24°C). Although the percentage of healthy eggs and larvae produced showed some encouraging results, these observations may be greatly outweighed by significantly lower total egg production at higher temperatures. Regardless of findings in the laboratory, the results do not necessarily translate directly into conditions occurring in the wild. As noted by Bonner et al. (1998), the temperature ranges for maximum egg and larval production found in these laboratory studies are similar to those of other species that "have wider geographic and thermal distributions." This suggests that some factors related to egg and larvae production are not accurately represented in laboratory studies, otherwise, species such as the ubiquitous fathead minnow with significantly lower egg production at 26°C than at 20-23°C (Brungs 1971) would be confined to habitats similar to fountain darters. One problem that may affect laboratory trials evaluating temperature is the development of fungus (Brandt et al. 1993, Bonner et al. 1998) which reduces egg viability and possibly affects spawning. The presence of fungus increases with higher temperatures in artificial laboratory habitat and may prevent initiation of spawning by adults or force them to divert reproductive energy toward resisting infection. Other unknown factors may also affect fountain darter health and reproductive capability in the laboratory with increasing temperatures differently than conditions experienced in the wild. Therefore, laboratory results and available field data should be considered jointly to evaluate temperature effects on fountain darter reproduction.

Final Report

The effect of fluctuating temperature and exotic nematode infestation of spawning potential of the endangered fountain darter.

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Study Objectives

The objectives of this study were to determine the effect of fluctuating temperatures and cercariae infestation on total egg, healthy egg, and larval production of the fountain darter Etheostoma fonticola. For the fountain darter, optimum temperatures range between 14 and less than 27°C for egg production and between 14 and less than 25°C for larval production (Bonner et al. 1998). Here, we test the hypothesis that egg and larval production at fluctuating temperatures (2°C oscillation within 24 hours that incorporates optimum or near optimum spawning temperatures for at least half of the 24 hours) will not differ from egg and larval production at a constant optimum temperature (24°C). The premise of the study was derived from field observations where temperatures may exceed optimum temperatures during the day but decrease at night to optimum or near optimum temperatures. Specifically, we sought to determine if compensatory reproduction was possible for the fountain darter.

In addition, we sought to determine if an exotic digenetic trematode, Centrocestus formosanus, negatively affects fecundity and natality rates of the fountain darter. The nematode was first observed in the San Antonio River basin in 1990 (Knott and Murray 1991) and in fountain darters from Comal River in 1996 (Mitchell et al., In press). The trematode encysts in the gill lamellae of its host thereby producing extensive gill damage, respiratory difficulties, and death (Blazer and Gratzer 1985, Lo and Lee 1996, and Alcaraz et al. 1999).

Methods

Fountain darters (n=78) were collected from a raceway at the National Fish Hatchery and Technology Center (NFHTC), San Marcos, Texas. Apparently, these darters were descendents of some that accidentally were stocked in the raceway during a previous aquatic vegetation study. In addition, wild fish (n=66) were collected from the San Marcos River. All fountain darters were captured by seines or dipnets and treated for 1 h in formalin (250 mg/L) for external parasites. Only fish between 28 and 35 mm total length (TL) were used in this study because each exceed the minimum length for sexually maturity (>26 mm; Brandt et al. 1993).

We used a randomized block design to test for differences in number of total eggs, healthy eggs, and larvae produced at four temperature treatments: constant 24°C, fluctuating 24 to 26°, fluctuating 26 to 28°C, and fluctuating 28 to 30°C. Temperatures for fluctuating treatments cycled 12 h at each lower target temperature and 12 h at each higher target temperature within a 24 h period. Replication (n = 3) was through time so time was the block.

For each block, 24 males and 24 females were randomly selected and half of the males and females were infected with cercariae (n ~ 500) at a level similar to that observed in wild fish from the Comal River (Mitchell et al., In press). Half of the fish remained without parasites.

Infected and uninfected fish were distributed among 16, 9-L flow-through glass aquaria located on top of four, 650-L fiberglass tanks (Living Stream model LS-700, Frigid Unit, Toledo, Ohio) held at 24°C. Males and females remained in separate aquaria. An electric pump (0.5 hp) was used to circulate from the fiberglass tanks through the aquaria at an exchange rate of once every 10 min. Water temperatures were maintained by using 0.5-hp chiller/1,000-W heater units (ACRY-TEC, Inc., San Diego, California and Universal Marine Industries, Inc., San Leandro, California) and controlled electronically by Delta System Controller (DIVCOM, Austin, Texas). Photoperiod for all trials was 12h light and 12h dark.

Each fiberglass tank was randomly assigned a temperature treatment. Temperatures were raised 1°C a day until each target temperature was obtained in each fiberglass tank. On top of each fiberglass tank, infected and uninfected pairs were redistributed randomly among six 9-L, flow-through glass aquaria so that each aquarium had one male/female pair of infected or uninfected fish. For 21-d period, eggs were removed from aquarium sides and spawning pipe every three days, enumerated, and classified as healthy or with fungus. Healthy eggs were placed in 9-L flow-through aquarium next to each aquarium with the breeding pair. From this, larvae were removed daily and enumerated. Larvae were counted 5 d after the completion of the replication to allow time for all larvae to hatch.

These procedures were used for three replications (blocks) and new fish were used each time. During all replications, fish were fed black worms (Aqualife, Friant, California) to satiation, and dead broodstock fish were removed promptly and replaced by a preconditioned fish. Dissolved oxygen and temperature (YSI model 58 dissolved oxygen meter, Yellow Springs, Ohio), pH (YSI model 95 pH meter, Yellow Springs, Ohio), and percent saturation of total gases (Sweeney Aquametrics Saturometer model DS-1B, Stoney Creek, Connecticut) were monitored daily throughout the study.

Total egg, healthy egg, and larval production were compared among temperature and parasite treatments with a two-factor analysis of variance ($\alpha = 0.05$). Using a two factor analysis of variance, cercariae infestation did not significantly affect total egg ($\underline{P}$ = 0.78), healthy egg ($\underline{P}$ = 0.23), or larval production ($\underline{P}$ =0.11). Thus, response variables were combined across infection status and tested for differences among temperature treatments with a one-factor analysis of variance followed by Fisher's LSD for mean separation ($\alpha = 0.05$). Responses variables were $\log_{10}(n+1)$ transformed to improve normality and sphericity.

In addition, percent healthy eggs from total eggs produced were compared among temperature treatments with one-factor analysis of variance. Percent healthy eggs and were averaged within blocks. Percent data were arcsin transformed for analyses to improve normality.

Results and Discussion

Total egg production differed ($F_{11,57} = 8.4$, $P < 0.0001$) among temperature regimes (Table 1). Total egg production was greatest at 24°C and significantly decreased ($P < 0.05$) by 42% (within block average) at temperature regime 24 – 26°C, 65% at temperature regime 26 – 28°C, and 99.6% at temperature regime 28 – 30°C (Figure 1). Likewise, healthy egg production differed ($F_{11,57} = 7.42$, $P < 0.0001$) among temperature regimes. Healthy egg production was greatest at 24°C and significantly decreased ($P < 0.05$) by 51% at temperature regime 24 – 26°C, 89% at temperature regime 26 – 28°C, and 100% at temperature regime 28 – 30°C. Total and healthy egg productions were not significantly different ($P > 0.05$) between temperature regimes 24 – 26°C and 26 – 28°C, but differed from those at temperature regime 28 – 30°C.

These results indicated that total and healthy egg production of the fountain darter significantly decreased once temperatures exceeded their optimum spawning range even though half of the darters time for temperature regime 24 – 26 was spent within optimum temperature limits. Thus, fountain darters cannot compensate for egg production losses incurred during periods when water temperatures exceed optimum ranges.

Furthermore, these results refined spawning temperature requirements of the fountain darter. No difference was found in egg production between a constant 23°C and constant 25° but a 75% decrease in fountain darter egg production occurred at 27°C (Bonner et al. 1998). Also, a decrease in egg production occurs at temperatures greater than 25°C but less than 27°C. Here, we report that egg production significantly decreased by 42% between a constant 24°C and fluctuating 24 – 26°C. By combining the results of these two experiments, we concluded that significant decrease in egg production occurs at temperatures greater than 25°C but less than 26°C.

Larval production differed ($F_{11,58}$=7.34, $P < 0.0001$) among temperature regimes. Larval production was greatest at 24°C and significantly decreased ($P<0.05$) by 63% at temperature regime 24 – 26°C, 99.9% at temperature regime 26 – 28°C, and 100% at temperature regime 28 – 30°C. These results were similar to Bonner et al. (1998) where they found highest larval production at temperatures 14 – 23°C and significant decreases by 77% at 25°C and 100% at 27 and 29°C. By combining the results of these two experiments, we concluded that significant decrease in larval production occurs at temperatures greater than 24°C but less than 25°C.

Overall, the production of total eggs, healthy eggs, and larvae decreased when water temperatures fluctuated ≥ 2°C from 24°C. However, fewer healthy eggs and larvae produced at higher temperatures were dependent upon fewer total eggs produced initially. Thus, we tested for differences in percent healthy eggs to determine if relative numbers of healthy eggs and larvae differed among treatments. Percent healthy eggs differed ($F_{8,31}$=2.75, $P = 0.022$) among temperature regimes excluding 28 – 30°C since few eggs were initially produced. Percent healthy eggs from within block means ranged from 21 – 84% at 24°C, 27 – 33% at 24 – 26°C, and 14 – 22% at 26 – 28°C. Significance difference ($P<0.05$) was found only between 24°C and 26 – 28°C. Likewise, we assess differences in percent larvae among temperature treatments; statistical analyses were not conducted since few larvae were produce at temperatures >24°C. However, percent larvae followed a similar trend as percent healthy eggs with relatively fewer larvae produced at higher temperatures. Percent larvae from within block means ranged from 56 – 67% at 24°C, 20 – 33% at 24 – 26°C, and 2% from only one breeding pair at 26-28°C. Thus, percent number of healthy eggs and larvae were greatest at 24°C and generally decreased at higher temperatures.

We failed to detect differences in cercariae infestation of the fountain darter. Our results suggest that fountain darter egg and larval production was not affected by cercariae within 21 days of attachment. However, long-term effects of cercariae infestation on fountain darter reproduction have yet to be assessed.

In summary, temperature results suggest that the number of eggs and larvae are reduced at temperatures between 25 and 26°C. Since fountain darters spawn year round (Schenck and Whiteside 1977), water temperatures between 25 and 26°C can negatively affect fountain darter egg and larval production over 21 days even if a temperatures fall within their optimum spawning range during the night. Thus, 1 – 2°C water temperature increase above 24°C decreases fecundity and natality rates of the fountain darter.

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Table 1. Number of total eggs, healthy eggs, and larvae produced over 21 days among four temperature treatments. Replication was conducted through time (block). The letter “N” represents the number of breeding pairs.

Temperature regime (°C)	Block	N	Number of eggs		Number of healthy eggs		Number of larvae	
			Mean	SD	Mean	SD	Mean	SD
24	1	6	220.7	121.05	189.2	105.02	122.3	87.22
	2	5	67.8	143.81	24.2	51.91	14.4	30.55
	3	6	94.3	62.73	66.8	67.16	46.7	59.52
24-26	1	6	111.7	96.43	62.2	82.75	28.0	45.37
	2	6	13.3	29.29	7.2	17.07	3.8	9.39
	3	4	99.3	115.88	55.5	80.67	28.0	56.00
26-28	1	6	64.3	61.82	13.5	16.81	0.5	1.22
	2	6	14.2	33.25	3.8	9.39	0.0	0.00
	3	6	50.5	107.14	6.7	14.42	0.0	0.00
28-30	1	6	2.3	3.14	0.0	0.00	0.0	0.00
	2	6	0.0	0.00	0.0	0.00	0.0	0.00
	3	6	0.0	0.00	0.0	0.00	0.0	0.00

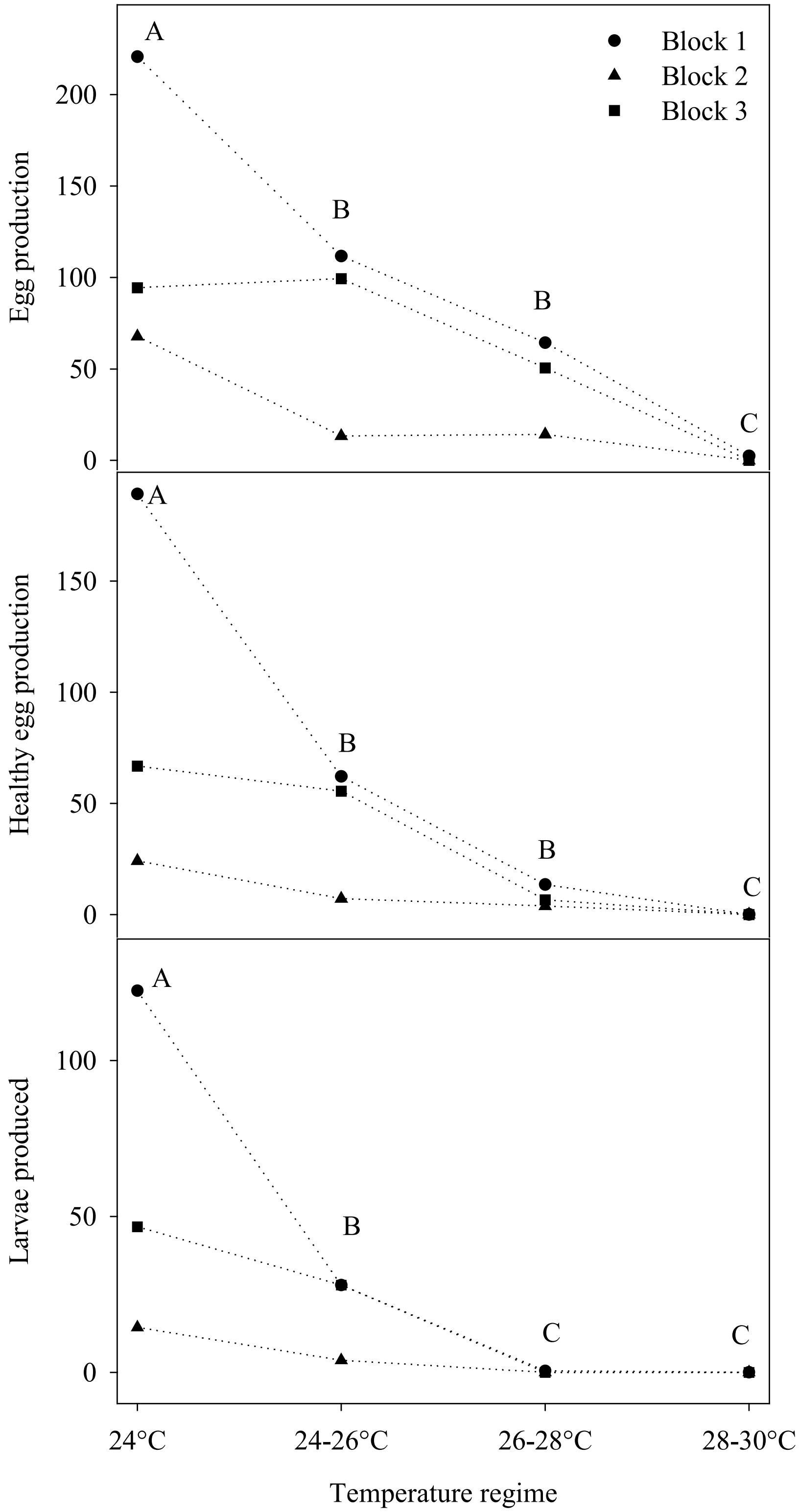

Figure 1. Mean number of total egg, healthy egg, and larvae produced within blocks for each block among four temperature treatments. Same letters represent no significant differences between treatments.