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Effects of Fluctuating Temperatures and Gill Parasites on Reproduction of the Fountain Darter, *Etheostoma fonticola*

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ABSTRACT

We assessed the effects of fluctuating temperature and gill parasitism on egg and larval production of the endangered fountain darter (*Etheostoma fonticola).* Fountain darters, with and without the exotic digenetic trematode *Centrocestus formosanus,* were exposed in the laboratory to constant (24°C) and fluctuating (24 to 26°C, 26 to 28°C, and 28 to 30°C) water temperatures for 21 d. No differences were detected between the number of eggs produced ($P = 0.78$) or number of larvae produced ($P = 0.11$) between fountain darters with and without trematodes. Total egg production was greatest at 24°C and decreased ($P < 0.05$) by 42% at fluctuating temperature of 24 to 26°C, 65% at fluctuating temperature of 26 to 28°C, and 99.6% at fluctuating temperature of 28 to 30°C. Likewise, larval production was greatest at 24°C and decreased ($P < 0.05$) by 63% at 24 to 26°C, 99.9% at 26 to 28°C, and 100% at 28 to 30°C. Water temperature (24 to 26°C) that fluctuated within previously considered optimum temperature (< 27°C) reduced the number of eggs and larvae produced by the fountain darter. Results of this study refined maximum optimum temperature requirements of the fountain darter reproduction with water temperatures ≥ 26°C reducing egg production and water temperatures ≥ 25°C reducing larval production.

INTRODUCTION

The federally endangered fountain darter (*Etheostoma fonticola)* is a continuous spawning, oviparous fish that is endemic to the thermally constant headwater reaches of the San Marcos River and the Comal River (Schenck and Whiteside 1976, Brandt et al. 1993). Constant water temperatures in these headwater reaches are dependent upon flows from spring discharges of the Edwards Aquifer (U.S. Fish and Wildlife Service 1996, Saunders et al. 2001). At normal flows (50 year mean discharge rate = 4.81 m^3/sec for San Marcos Springs and 8.0 m^3/sec for Comal Springs), main channel water temperatures in the upper reaches of the San Marcos River and the Comal River are approximately 21 and 24°C, respectively (Linam et al. 1993, Groeger et al. 1997, Saunders et al. 2001), whereas backwaters and other areas peripheral to the main channel can fluctuate ± 2°C. With diminished flows, main channel water temperature can deviate > 2°C through ambient heating (Ogden et al. 1985, Hubbs 1995, Saunders et al. 2001).

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Laboratory studies have demonstrated that water temperatures > 27°C reduced fountain darter egg and larval production (Brandt et al. 1993, Bonner et al. 1998). However, these studies only tested egg and larval production at constant temperatures, which are not the conditions which darters experience during many summer months. Spring flows generally decline during the summer months when air temperatures generally drop to between 22 and 26°C at night and rise to 35 to 39°C during the day. Water temperatures at night generally return to near spring outflow temperatures, while daytime water temperatures increase several degrees. One purpose of this study was to assess egg and larval production at temperature regimes that reflect natural, fluctuating temperatures associated with ambient heating in the periphery of the main channel, exceeding 27°C within a 24-h period.

The second purpose of this study was to assess the effects of an introduced parasite on fountain darter reproduction. In 1996, fountain darters collected from Comal River had *Centrocestus formosanus* metacercariae encysted in their gill cartilage and lamellae (Mitchell et al. 2000). Upon further investigation during 1997 and 1998, 3% of the fountain darters collected from the San Marcos River and all of the fountain darters collected from Comal River were infected with *C. formosanus*. This digenetic trematode and its first intermediate host, *Melanoides tuberculatus*, are native to Southeast Asia (Faust and Nishigori 1926, Cheng 1964); *M. tuberculatus* was discovered in Texas in 1964 (Murray 1964). The cercarial stage of *C. formosanus* targets non-specific fish gills as a second intermediate host (Alcaraz et al. 1999). The cercariae penetrate and encyst in the gill lamella, impairing blood flow and disrupting respiration and ionic exchange in the fish (Balasuriya 1988, Blazer and Gratzek 1985, Roberts 1978, Salmon 2000, Yamaguti 1975). Disrupted respiration causes the fish to swim at or near the water surface to gulp air, thereby precariously exposing the fish to avian and mammalian predators, definitive hosts of the trematode (Cheng 1964, Ribelin and Migaki 1975).

Fish mortality is severe at times in aquaculture ponds with *C. formosanus* (Mohan et al. 1999, Paperna 1996, Subasinghe 1992, Yanohara and Kagei 1983, Zeng and Liao 2001). Mortality in wild populations is suspected but undocumented (Mitchell et al. 2000). If not lethal, trematode infestations indirectly affect fish populations by lowering reproductive output, attributed to energy expenditures in physiological defense responses (Meakins 1974, Wootton 1990). A lowered reproductive output is potentially detrimental to an endangered fish with a narrow geographical range, such as the fountain darter. Thus, we compared numbers of eggs and larvae produced between fountain darters with and without the trematode to assess potential effects on the fish population.

MATERIALS AND METHODS

Fountain darters were collected initially from a raceway at the National Fish Hatchery and Technology Center, San Marcos, Texas. These darters were descendents of fish that had been stocked in the raceway during an earlier unrelated study. These were supplemented with wild fountain darters (66% of the fish used in this study) collected from the San Marcos River. All fountain darters were captured by dip nets and treated for 1 h in formalin (250 mg/L) for external parasites. Only sexually mature fish were used (> 26 mm in total length; Brandt et al. 1993) and were inspected visually for gill inflammation prior to experimental use.

We used a randomized block experimental design to test for differences in number of total eggs and larvae produced at the following four temperature treatments: constant 24°C, fluctuating 24 to 26°C, fluctuating 26 to 28°C, and fluctuating 28 to 30°C. Temperatures for each fluctuating treatment cycled between 10 h at the lower target temperature and 10 h at the higher target temperature within a 24 h period to mimic a natural diel temperature cycle. Between the extremes, temperatures increased or decreased at a rate of 1°C/h. Replication ($N = 3$) was through time (block effect). For

each replicate trial, 24 males and 24 females were randomly selected, and half of the males and females were artificially infected with cercariae (target mean = 500) by methods described by Lo and Lee (1996) at a level similar to what Salmon (2000) used to impact the health of fountain darters during laboratory sealed-jar hypoxia tests. Fish were infected seven (trials 1 and 3) to 12 (trial 2) days before the start of each trial.

At the start of each trial, three infected and three uninfected fish of each sex (total N of fish = 12) were transferred to one of four separate holding aquarium systems at 24°C. Each aquarium system consisted of a thermostatically-controlled water reservoir and six 9-L flow-through glass aquaria with a 10 min water exchange rate between aquaria and reservoir. Each aquarium system was randomly assigned a temperature treatment. Water temperatures were raised 1°C per day in each aquarium system, if necessary, until the lower target temperature of each treatment was reached. Initially, fish were segregated by sex and infection or non-infection and housed in separate aquaria. Once treatment temperatures were reached, males and females were paired so that six aquaria held one breeding pair of either infected or uninfected fish. For a 21-d period, eggs were removed from aquarium sides and spawning substrate every three days, enumerated, and classified as healthy or with fungi. Those with fungi were counted and discarded. Healthy eggs were placed in new aquaria, one for each breeding pair. As eggs hatched, larval fish were counted and removed daily. Larvae were also counted five days after completion of each trial (i.e., at day 26) to allow time for all viable eggs to hatch.

These procedures were repeated three times (trials 1 through 3) and new fish were used each time. During each trial, fish were fed black worms (Aqualife, Friant, California) to satiation, and dead darters (N = 13 among three trials) were removed daily and replaced by a preconditioned fish. Photoperiod throughout the experiment was 12 h light and 12 h dark. Dissolved oxygen and temperature (YSI model 58 meter, Yellow Springs, Ohio), pH (YSI model 95 meter), and percent saturation of total gases (Sweeney Aquametrics Saturometer model DS-1B, Stoney Creek, Connecticut) were monitored daily throughout the study.

After each trial, fish were euthanized using a lethal dose of tricaine methane sulphonate (Finquel®, Argent Chemical Laboratories, Redmond, Washington) and placed individually in vials containing 10% formalin. Metacercariae infestation was estimated by counting the number of metacercariae in gill arches from the left side and multiplying by two (Madhavi 1986). For the uninfected fish, gill arches on both sides of fish were examined to confirm absence of metacercariae. Fish also were dissected and gonads were inspected to confirm proper pairings of breeding adults. For three experimental units (Trial 2, Treatment: 24°C, N = 1; Trial 3, Treatment: 24-26°C, N = 2), pairings consisted of two males. These experimental units were deleted from statistical analyses.

Total egg and larval production were compared among temperature and parasite treatments with a two-factor analysis of variance (α = 0.05; SAS Institute, Inc., Cary, North Carolina). Dependent variables were $\log_{10}(N + 1)$-transformed to meet assumptions of homogeneity and normality. Percent hatch (number of larvae per total number of eggs) was compared among temperature treatments using a single factor analysis of variance (α = 0.05).

RESULTS

Actual mean temperatures (± SD) across replications for each treatment were 24.2°C (0.34), 24.4°C (0.4) to 26.0°C (0.4), 26.2°C (0.6) to 27.8°C (0.6), and 28.1°C (0.4) to 29.7°C (0.6). Dissolved oxygen concentration averaged 6.1 mg/l (SD = 1.06; range = 4.8 to 8.2), pH ranged from 7.9 to 8.2, and total gas saturation ranged from 84 to 95%. Ranges observed in water quality parameters were similar among treatments and

trials and not deemed a contributing factor in affecting fountain darter reproduction among treatments or across blocks.

Average number of metacercariae of infected fountain darters (± SD) was 510 (134), ranging from 244 to 948 metacercariae per fish. Infected and uninfected fountain darters did not differ in the number of eggs ($P = 0.78$) or larvae produced ($P = 0.11$) over a 21-d period. Consequently, numbers for eggs and larvae from infected and uninfected fish were combined within each temperature treatment for each trial. These combined totals were used to test for differences among temperature treatments.

Total egg production differed ($F_{3, 57} = 17.9$, $P < 0.01$) among temperature regimes (Fig. 1). Egg production was greatest at constant 24°C and decreased ($P < 0.05$) by 42% (averaged across blocks) at fluctuating temperature 24 to 26°C; 65% at fluctuating temperature 26 to 28°C; and 99.6% at fluctuating temperature 28 to 30°C. Total egg production did not differ ($P > 0.05$) between fluctuating temperatures 24 to 26°C and 26 to 28°C, but both differed from total egg production at fluctuating temperature 28 to 30°C.

Larval production also differed ($F_{3,57} = 18.6$, $P < 0.01$) among temperature regimes (Fig. 1). Larval production was greatest at 24°C and decreased ($P < 0.05$) by 63% at fluctuating temperature 24 to 26°C; 99.9% at fluctuating temperatures 26 to 28°C; and 100% at fluctuating temperature 28 to 30°C. Percent of larvae hatched differed ($F_{2,6} = 5.64$, $P < 0.05$) among temperatures. Percent hatch (mean ± SD) was greatest (34.9 ± 21.7) at 24°C and lower ($P < 0.05$) at fluctuating temperature 24 to 26°C (11.2 ± 5.7) and at fluctuating temperature 26 to 28°C (0.1 ± 0.2). Larvae were not produced at fluctuating temperature 28 to 30°C.

DISCUSSION

Egg production and larval production were similar between fish infected with *C. formosanus* cercariae and those that were not infected. Parasite infestation typically lowers reproductive output in fishes because energy is diverted to maintenance of the parasites instead of used for fish reproduction (Meakins 1974, Kenedy 1975, Roberts 1978, Wootton 1990). Lowering of reproductive output did not occur in this study possibly because of the fountain darter's strong host response to the parasite. The fountain darter encysts and kills the parasite shortly after it attaches to the gill filament cartilage (Mitchell et al. 2000). After the initial response to the parasite, fountain darters might no longer expend energy fighting or feeding the parasite; thus, egg production is not affected.

The year-round exposure and accumulation of this parasite in darters in the wild might have an eventual effect on the darter population. During a companion study to this one (McDonald et al. 2006), we learned that the number of metacercariae to cause fish death differed among age groups of darters. Mean number of metacercariae (± SE) per fish causing death was 60 (± 18.6) for larval darters (9 to 12 mm in total length), 353 (± 28.8) for juveniles (16 to 20 mm), and 1,131 (± 101.0) for adults (36 to 41 mm). In the wild, this size selective mortality might reduce recruitment by eliminating the smaller darters. Also in the wild, the darters are constantly expending energy encysting the parasite and repairing tissue damage from initial attachment. Older and larger fountain darters will accumulate metacercariae through time (Mitchell et al. 2000), making them susceptible to higher mortality. It has been shown that mate size plays an important role in the numbers of eggs released by females (Kolm 2002), and fish age is positively correlated with success and abundance of future generations (Berkeley et al. 2004). Collectively, the gill parasite might play a greater role in the wild than demonstrated in this study.

Bonner et al. (1998) found that fountain darter egg production was not significantly different when water temperatures were held constant at 14, 17, 20, 23, or

25°C, but were significantly reduced at constant 27°C; constant 26°C was not tested. In the same study, larvae production and percent hatch were not significantly different between constant 14°C through 23°C but were reduced at 25°C; constant 24°C was not tested. Our study showed that egg and larval production and percent hatch were greatest at a constant 24°C and significantly decreased at higher fluctuating temperatures including treatment 24 to 26°C. These results suggest that the upper limit for optimum egg production is lower (< 26°C instead of < 27°C) than previously reported. The upper limit for optimum larvae production and percent hatch might be higher than previously reported (up to 24°C instead of 23°C), although we did not directly test this.

The negative effect of temperatures > 24°C on fountain darter reproduction might be a factor influencing the existence of fountain darters, but water temperature might not be the most important factor. Fountain darters are only found in the upper San Marcos River and Comal River where conditions are highly influenced by spring discharge. In addition to stable water temperatures (21 – 25°C), flows are fairly constant, water clarity is high (< 10 NTU), and submersed vegetation (i.e., *Zizania, Potamogeton, Sagittaria, Ludwiga*, and filamentous algae) is abundant (Lemke 1989, Crowe and Sharp 1997, Groeger et al. 1997). In downstream areas where fountain darters have access but are not found, river conditions become more influenced by ambient air temperature and surface runoff. Water temperature (12 – 28°C), water clarity (< 1000 NTU), and flow are more variable, and submersed vegetation is less abundant (Groeger et al. 1997, Saunders et al. 2001).

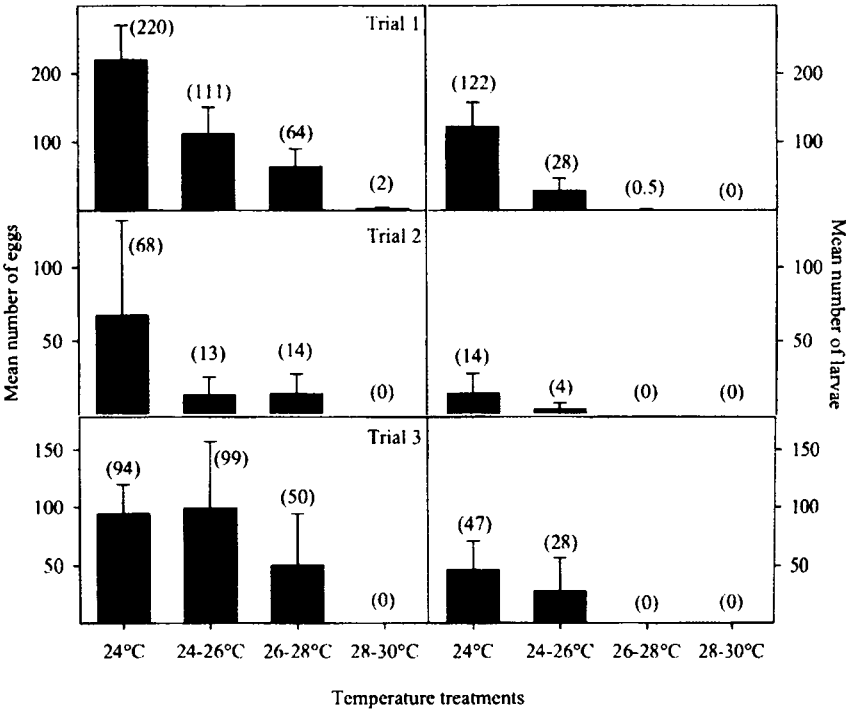

Figure 1. Mean number of eggs and larvae produced by *Etheostoma fonticola* during three, 21-d trials among four temperature treatments. Each temperature regime and trial consisted of six breeding paris of fish, except the 24°C Trial 2 ($N = 5$ breeding pairs) and the 24 to 26°C Trial 3 ($N = 4$ breeding pairs). Treatment means are in parentheses. Data were log (N+1)-transformed for statistical analyses.

The fountain darter produces fry between water temperatures of 14°C (Bonner et al. 1998) and 26°C (this study) under laboratory conditions and physiologically should be able to exist in the lower San Marcos River and the Comal River where temperature is between 12 and 28°C (Brandt et al. 1993, Bonner et al. 1998), but they do not exist there. Until we understand why the fountain darter has such a restricted range, water temperature and the associated physical and chemical conditions within the upper San Marcos River and the Comal River should continue to be controlled by spring discharge and not ambient surface conditions.

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