EFFECTS OF FLUCTUATING TEMPERATURE AND AN INTRODUCED TREMATODE ON REPRODUCTION AND MORTALITY OF *ETHEOSTOMA FONTICOLA*

THESIS

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CHAPTER 1

EFFECTS OF FLUCTUATING TEMPERATURES AND TREMATODE INFECTION ON THE REPRODUCTION OF THE FOUNTAIN DARTER (*ETHEOSTOMA FONTICOLA*)

ABSTRACT

Studies were conducted to assess the effects of fluctuating temperatures and gill parasitism on egg and larval production of the endangered fountain darter *Etheostoma fonticola*. Fountain darters with and without *Centrocestus formosanus* were exposed 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 difference was detected between total egg ($P = 0.78$) and larval production ($P = 0.11$) between infected and healthy fountain darters so egg and larvae production were combined to test differences among temperature treatments. Total egg production was greatest at 24°C and significantly decreased ($P < 0.05$) by 42% at temperature regime 24 to 26°C, 65% at temperature regime 26 to 28°C, and 99.6% at temperature regime 28 to 30°C. Larval production was greatest at 24°C and significantly decreased ($P < 0.05$) by 63% at temperature regime 24 to 26°C, 99.9% at temperature regime 26 to 28°C, and 100% at temperature regime 28 to 30°C. Temperatures that fluctuated between optimum and sub-optimum levels also reduced the number of eggs

and larvae produced by fountain darters. Results of this study, combined with others, refined temperature requirements of the fountain darter reproduction with water temperatures $\geq$ 26°C reducing egg production and water temperatures $\geq$ 25°C reducing larval production.

INTRODUCTION

The federally endangered fountain darter *Etheostoma fonticola* is a year-round spawning oviparous fish that is endemic to the near thermally constant headwater reaches of the San Marcos and Comal rivers (Brandt et al. 1993; Schenck and Whiteside 1976). Flows and constant temperatures in these headwater reaches are dependent on spring discharges of the Edwards Aquifer (U.S. Fish and Wildlife Service 1996; Saunders et al. 2001). At normal discharge rates (4.81 m^3 sec^{-1} for the San Marcos Springs and 8.21 m^3 sec^{-1} for the Comal Springs), main channel water temperatures in the upper reaches of the San Marcos and Comal rivers are approximately 21 and 24°C; respectively (Linam et al. 1993; Groeger et al. 1997; Saunders et al. 2001). In waters peripheral to the main channel, water temperatures normally fluctuate ± 2°C over a 24-h period (E. Oborny, BioWest, Inc. personal communication). However, water temperatures in the main channel can also fluctuate > 2°C through ambient heating if flows diminish because of low precipitation and/or groundwater withdrawals (Ogden et al. 1985; Hubbs 1995; Saunders et al. 2001). If so, these higher temperatures have the potential to lower reproductive output of the fountain darter (Brandt et al. 1993; Bonner et al. 1998). Under laboratory conditions, water temperatures ≥ 27°C decrease fountain darter egg production and water temperatures ≥ 25°C decrease larval production (Bonner et al. 1998).

Spring discharges and river flows are monitored to ensure among other concerns that water temperatures near the springs and in the upper reaches of the San Marcos and Comal rivers are sufficient to maintain water temperatures < 27°C (P. Connor, U.S. Fish and Wildlife Service, personal communication). Hence, groundwater withdrawals are regulated to maintain minimum flows > 2.8 m^3 sec^{-1} in the San Marcos River and > 5.7

$m^3 sec^{-1}$ in the Comal River (U.S. Fish and Wildlife Service 1996). Maximum water temperatures and minimum flows are based on the premise that fountain darters need water temperatures < 27°C in a 24-h period for successful spawning. One purpose of this study was to assess egg and larval production at different temperature regimes that remained within optimum levels (< 27°C) and that fluctuated between optimum and sub-optimum (> 27°C; representing a modified flow regime) within a 24-h period. If fountain darters only need a portion of the diel cycle to be at optimum temperatures for egg and larval production, then more water could be potentially available for groundwater withdrawals.

A secondary 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 were observed with *Centrocestus formosanus* metacercariae encysting in the gill cartilage and lamellae (Mitchell et al. 2000). Upon further investigation, 3% of the fountain darters in the San Marcos River and 100% of the fountain darters in the Comal River had *C. formosanus* in their gills. *Centrocestus formosanus* is a digenetic trematode native to southeast Asia (Faust and Nishigori 1926). Its first intermediate host is a freshwater operculate snail, *Melanoides tuberculata*, which is also native to south-east Asia (Cheng 1964) and introduced into San Antonio, Texas, U.S.A., as early as 1964 probably due to the aquarium trade (Murray 1964; Roessler et al. 1977). *Centrocestus formosanus* cercariae are shed from the snail, numbers usually increasing as water temperatures increase (Lo and Lee 1996). Cercariae of *C. formosanus* have been found in 6.1% (N = 2,279) of *M. tuberculata* (mean length 24.9 mm) taken from the Comal River during 1997 and 1998, but in only 1 of the 2,241 snails (mean length 33.2 mm)

examined from the San Marcos River during that same time period (Mitchell et al. 2000). The cercariae 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 (Yamaguti 1975; Roberts 1978; Blazer and Gratzek 1985; Balasuriya 1988; Salmon 2000). 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, the trematode's definitive host (Cheng 1964; Ribelin and Migaki 1975).

Fish mortality can be high in aquaculture ponds infected with *C. formosanus* (Yanohara and Kagei 1983; Subasinghe 1992; Paperna 1996; Mohan et al. 1999; Zeng and Liao 2001). However, mortality in wild populations is suspected, but undocumented (Mitchell 2000). If *C. formosanus* inhibits respiration in the fountain darter (Salmon 2000), this may limit distribution of the fountain darter to areas with high dissolved oxygen and increase mortality in areas with low dissolved oxygen. Parasite infestations often cause fish to expend energy through physiological defense responses, thus lowering reproductive output (Meakins 1974; Wootton 1990). For the fountain darter, a decrease in reproductive capacity may alter substantially population abundance in these spring-fed systems.

Objectives of this study were to determine differences in the number of eggs and larvae produced at four temperature treatments (constant 24°C, fluctuating between 24 and 26°C, 26 and 28°C, and 28 and 30°C within 24 hours) over a 21 d period and to determine if fish infected with *C. formosanus* will have lower egg production at the four temperature treatments than those that are not infected with *C. formosanus*.

MATERIALS AND METHODS

Fountain darters were collected initially from a raceway at the National Fish Hatchery and Technology Center (NFHTC), San Marcos, Texas. These darters were descendents of some that incidentally were stocked in the raceway during a previous aquatic vegetation study. In addition, wild fountain darters were collected from the San Marcos River to supplement the needs of this study. 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 because each exceed the minimum length for sexually maturity (> 26 mm; Brandt et al. 1993). Fish were inspected visually for gill inflammation prior to experimental use and only fish without gill inflammation were kept.

We used a randomized block experimental design to test for differences in number of total eggs and larvae produced at four temperature treatments: constant 24°C, fluctuating 24 to 26°C, fluctuating 26 to 28°C and fluctuating 28 to 30°C. Temperatures for fluctuating treatments cycled 10 h at each lower target temperature and 10 h at each higher target temperature within a 24 h period to mimic a natural diel temperature cycle. Between fluctuations, temperatures were increased or decreased slowly over a two-hour 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 artificially infected with cercariae (mean = 500) by methods described by Lo and Lee (1996) at a level similar to that observed in wild fish from the Comal River (Salmon 2000). Infections were done prior to each trial; 7 d for trial 1, 12 d for trial 2, and 7 d for trial 3. Half of the fish remained without parasites.

Infected and uninfected fish were distributed among 12, 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 water from the fiberglass tanks through the aquaria at an expected 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 a Delta System Controller (DiVCON, 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, three infected and three uninfected pairs were redistributed randomly among six 9-L, flow-through glass aquaria so that each aquarium had one male/female pair of either infected or uninfected pairs. For a 21 d period, eggs were removed from aquarium sides and spawning substrate every three d, enumerated, and classified as healthy or with fungus. Healthy eggs were placed in 9-L flow-through larval rearing aquaria next to each breeding pair aquarium and labeled as egg storage. From the egg storage aquaria, larval fish were removed daily and enumerated. Larvae were also counted 5 d after the completion of each 21 d trial to allow time for all larvae to hatch.

These procedures were repeated three times and new fish were used each time. During all replications, fish were fed black worms (Aqualife, Friant, California) to satiation. Dead darters (N = 13 among three trials) were removed at least daily 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.

After each trial, all 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. Number of metacercariae was determined for infected fish by counting the number of metacercariae in gill arches from the left side and multiplying by two (Madhavi 1986). The gill arches on both the right and left sides of uninfected fish were examined for metacercariae. Total egg and larval production were compared among temperature and parasite treatments with a two-factor analysis of variance ($\alpha = 0.05$). Percent hatch was compared among temperature treatments using a single factor analysis of variance ($\alpha = 0.05$).

RESULTS

Actual mean temperatures (± SD) across replications for each treatment were (24.2°C ± 0.34) for the target constant temperature: 24°C, ((24.4°C ± 0.4) to (26.0°C ± 0.4)) for target fluctuating temperature: 24 to 26°C, ((26.2°C ± 0.6) to (27.8°C ± 0.6)) for target fluctuating temperature: 26 to 28°C, and ((28.1°C ± 0.4) to (29.7°C ± 0.6)) for target fluctuating temperature 28 to 30°C. 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.

The average number of metacercariae of infected fountain darters (± SD, range) was 510 (± 134, 244 to 948). 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 among all three trials. Thus, numbers for eggs and larvae 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 ($F_{11,57} = 8.4$, $P < 0.01$) among temperature regimes (APPENDIX A). Egg production was greatest at 24°C and significantly decreased as temperatures increased ($P < 0.05$): by 42% (within block average) at temperature regime 24 to 26°C; 65% at temperature regime 26 to 28°C; and 99.6% at temperature regime 28 to 30°C. Total egg production was not significantly different ($P > 0.05$) between

temperature regimes 24 to 26°C and 26 to 28°C, but differed from those at temperature regime 28 to 30°C.

Larval production differed ($F_{11,58} = 7.34$, $P < 0.01$) among temperature regimes (APPENDIX A). Larval production was greatest at 24°C and significantly decreased as temperatures increased ($P < 0.05$): by 63% at temperature regime 24 to 26°C; 99.9% at temperature regime 26 to 28°C; and 100% at temperature regime 28 to 30°C.

Percent of larvae hatched differed significantly among temperatures ($F_{2,6} = 5.64$, $P < 0.05$). Percent hatch (mean ± SD) was greatest (34.9 ± 21.7) at 24°C and substantially lower at 24 to 26°C temperature regime (11.2 ± 5.7) and at 26 to 28°C temperature regime (0.1 ± 0.2). Larvae were not produced at temperature regime 28 to 30°C.

DISCUSSION

Egg and larval production was similar between fish infected with *C. formosanus* cercariae and those that were not infected. Parasite infestation typically lowers reproductive output in fishes because greater energy is diverted to maintenance instead of reproduction (Meakins 1974; Kenedy 1975; Roberts 1978; Wootton 1990). However, lower reproductive output attributed to parasite infestation was not detected in the fountain darter in this study. One feasible explanation is that the duration of study trials was too short. Perhaps an extended assessment of egg and larval production would improve the ability to detect effects of parasite infestation if differences exist. Alternatively, gill parasites at levels tested here 500 cysts may not have been sufficient to induce reproductive stress in the fountain darter for any period of time. At similar levels of infestation, Salmon (2000) found that fountain darters exhibited loss of equilibrium, listlessness, or rising for air immediately after infection, but then slowly recovered through the first 14 days post-infected. Thus, infestation level of 500 cysts per fish may only produce stress initially but not over longer periods of time to effect reproduction.

Among treatments, egg and larval production was greatest at constant 24°C and significantly decreased at higher fluctuating temperatures. For egg production, this was unexpected. At constant temperatures, fountain darter egg production was the highest at 23° and 25°C, and decreased at 27°C (Bonner et al. 1998). Based on this range, I expected egg production to be similar between the constant 24°C and the fluctuating 24 to 26°C treatment. However, egg production was lower at fluctuating temperature treatment 24°C to 26°C. Combining these results with that of Bonner et al. (1998),

fountain darter egg production significantly decreases when water temperatures are $\geq 26°C$.

For larval production, Bonner et al. (1998) found that the number of larvae decreased when temperatures equaled or exceeded 25°C. My results were consistent with theirs in that fewer larvae were produced when water temperatures exceeded 25°C (24 to 26°C temperature treatment) within a 24-h period although for about 12 h water temperatures were $< 25°C$. Similarly, percent hatch decreased as temperatures exceeded 25°C here and in Bonner et al. (1998). Thus, fewer larvae are produced and proportionally fewer larvae hatch when water temperatures exceed 25°C under laboratory conditions.

Experimentally and empirically, reproduction and presence of fountain darters are dependent upon temperature. My results showed that temperatures $\geq 26°C$ reduced the number of eggs produced and temperatures $\geq 25°C$ (combined with the results of Bonner et al. 1998) reduced the number of larvae produced and hatched. Empirically, fountain darters are found in reaches of the San Marcos River (Kelsey 1997) and the Comal River (Hubbs 2001) that are mostly constant. In the San Marcos River, fountain darters are less abundant in areas where temperatures fluctuate greater than 24°C, about 5 km downstream from spring discharges (Schenck and Whiteside 1976; U.S. Fish and Wildlife Service 1996; Kelsey 1997; Groeger et al. 1997; Saunders et al. 2001). Temperature is a typical reproductive terminating cue for numerous fishes (Hubbs and Strawn 1957; Marsh 1977; Sandstrom et al. 1997; Koger et al. 1999). Few North American species other than spring head inhabiting species such as fountain darters (Brandt et al. 1993), Devils Hole pupfish (*Cyprinidon diabolis*), Comanche Springs

pupfish (*Cyprinidon elegans*), Leon Springs pupfish (*Cyprinidon bovines*) spawn year-round (Lee et al. 1980). Inhibition of spawning can affect dynamics and long-term viability of the fountain darter population if constant water temperatures are altered (Linam et al. 1993; Crowe and Sharp 1996). Thus, diminishing flows that cause higher water temperatures (> 26°C) in San Marcos and Comal rivers would negatively impact reproductive success of the fountain darter.

Although temperature seems to be an important factor for fountain darter viability, other factors are not precluded from impacting and limiting fountain darter distribution. Dams in the San Marcos River and Comal River may affect the distribution of the fountain darter (Schenck and Whiteside 1976; Linam et al. 1993). Dams affect fish populations and assemblage structures in both upstream and downstream directions by altering water chemistry, habitats, flow regimes, and fragmenting fish populations (Bain et al. 1988; Winston et al. 1991; Wilde and Ostrand 1999; Bonner and Wilde 2000). In the San Marcos River, four impoundments create greater areas of pool habitats that are inhabited by non-indigenous and exotic fish species (Saunders et. al 2001). These non-native fishes possibly could compete with, or predate on, various life stages of the fountain darter, thus reducing abundance or occurrence.

Constant water temperatures in the headwaters of San Marcos and Comal rivers also may inhibit the occurrence and abundance of possibly competitor species such as invertivore cyprinids. Currently, cyprinids comprise < 1% of the fish assemblage in the upper 5 km of the San Marcos River, but comprise > 80% farther downstream in the San Marcos River where temperatures fluctuate according to ambient temperatures (Groeger et al. 1997; Kelsey 1997). This suggests that invertivore cyprinids avoid areas of the San

Marcos River with year-round constant water temperatures of 21°C. Thus, constant temperature in upper reaches of these systems may both directly and indirectly benefit persistence and abundance of the fountain darter.

APPENDIX A

Total mean numbers of fountain darter (*Etheostoma fonticola*) eggs and larvae produced by breeding pairs over three 21 d trials among four temperature treatments. Replication was conducted through time (block). The letter "N" denotes the number of breeding pairs.

			Number of eggs		Number of larvae	
Temperature regime (°C)	Block	N	Mean	SD	Mean	SD
24	1	6	220.7	121.05	122.3	87.22
	2	5	67.8	143.81	14.4	30.55
	3	6	94.3	62.73	46.7	59.52
24-26	1	6	111.7	96.43	28.0	45.37
	2	6	13.3	29.29	3.8	9.39
	3	5	79.4	109.73	22.4	50.09
26-28	1	6	64.3	61.82	0.5	1.22
	2	6	14.2	33.25	0.0	
	3	6	50.5	107.14	0.0	0.00
28-30	1	6	2.3	3.14	0.0	0.00
	2	6	0.0	0.00	0.0	0.00
	3	6	0.0	0.00	0.0	0.00

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CHAPTER 2

SUSCEPTIBILITY OF FOUNTAIN DARTER AGE GROUPS TO MORTALITY BY AN EXOTIC TREMATODE

ABSTRACT

We determined survival and infestation rates for larvae, juveniles, and adult fountain darters infected with an exotic trematode, *Centrocestus formosanus*. Generated survival curves estimated that 50% of the larval fish would die at 116 minutes whereas 50% of the juvenile fish would die at 330 minutes when exposed to 512,200 cercariae per L. Less than 25% of the adult fish died within the 8 h trial period. Number of cercariae that induced mortality was directly related ($P < 0.001$) to fish length although length alone did not explain the quicker death observed in smaller fish. Differential effects of trematode infestation among size groups suggested that larvae and juveniles were more susceptible to *C. formosanus* mortality than adults.

INTRODUCTION

The exotic digenetic trematode, *Centrocestus formosanus*, was reported in the San Antonio River basin of Bexar Co., Texas in 1990 (Knott and Murray 1991). In Texas, the trematode has been documented in 27 fishes (Mitchell et al. 2000, McDermott 2000, Fleming 2002). Among these, six are listed as threatened or endangered federally or by the state. Fish serve as the second intermediate host in the life cycle of *C. formosanus* (Chen 1948; Scholz and Salgado-Maldonado 1999). The trematode encysts in the gill lamellae of its host and at high levels can produce extensive gill damage, respiratory difficulties, and death (Blazer and Gratzek 1985; Lo and Lee 1996; Velez-Hernandez et al. 1998; Alcaraz et al. 1999). Various piscivorous wading birds are presumed to be the definitive host and the exotic red-rimmed melania snail *Melanoides tuberculata* is the first intermediate host (Lo and Lee 1996; Yamaguti 1975). Currently, the snail is present throughout the headwaters of the San Antonio, Comal, San Marcos rivers, San Solomon, Phantom, Diamond Y, and San Felipe springs; and San Felipe Creek (Murray 1964; Mitchell et al. 2000; McDermott 2000). Thus, *C. formosanus* has the potential to increase its distribution to other areas of the state.

The fountain darter *Etheostoma fonticola* is an endangered species, endemic to Comal and nearby San Marcos rivers (U.S. Fish and Wildlife Service 1996). The trematode was found in fountain darters collected from the Comal River in August 1994 (Mitchell et al. 2000). Their study found that 100% of 194 fountain darters collected from the Comal River were infected with *C. formosanus*; they also suspected that 9% of the examined fish were infected with life threatening levels.

There are several reports of mortalities of juvenile fish associated with trematode infections (Sommerville 1982; Balasuryia 1988; Paperna 1996; Mohan et al. 1999). Infestation of *C. formosanus* can produce mortality in fountain darters (Salmon 2000). Thus, the objective of this study is to determine if different fountain darter life stages (larvae, juveniles, adults) are differentially susceptible to death when exposed to similar concentrations of *C. formosanus*. If differential susceptibility to death does exist among fountain darter life stages, larval and juvenile fish may die before reaching sexual maturity, thus reducing recruitment and negatively affecting the fountain darter population size.

MATERIALS AND METHODS

Hatchery-reared fountain darters (n = 54) were obtained from the National Fish Hatchery and Technology Center (NFHTC) in San Marcos, Texas. Of the 54 darters, 18 were larvae (5 - 13 mm; total length [TL]), 18 were larvae/juveniles (16 – 21 mm TL; juveniles), and 18 were adults (26 – 30 mm TL). Fish were held in an aerated, 40-L glass aquarium with 35-L of water at 23°C and 12:12 (light: dark) photoperiod and fed blackworms (Aqualife, Friant, California) until the start of the study.

Melanoides tuberculata were collected with dip nets from the Comal River and transported to the NFHTC. Snails were held in an aerated, 40-L glass aquarium for 30 d. Emergence of *C. formosanus* cercariae was induced from individual snails by placing them in 900-ml containers under incandescent light for 2 h (Lo and Lee 1996). Approximately, 500,000 cercariae were collected from the snails and were placed into each of the three 9-L aquaria with 900 ml of water. Three other 9-L aquaria contained 900 ml of water with no cercariae and were set up as controls.

Three larval, three juvenile, and three adult fountain darters were placed into each of the three aquaria containing cercariae. Three of each of the three life stages were also placed in three other aquaria that contained no cercariae as a control. The time that each group was placed into the aquarium was recorded. Fish remained in each aquarium for up to 8 h or until death. Loss of equilibrium and cessation of respiratory movements were used as the criterion indicating death (Baldwin et al. 1967). After a fish died, the time of death was recorded before the fish was removed and preserved in 10% formalin. After 8 h the remaining live fish were anesthetized in tricaine methane sulphonate

(Finquel®, Argent Chemical Laboratories, Redmond, Washington) and preserved in 10% formalin. The gill arches from the left side of each fish were removed and the encysted metacercariae were counted using a dissecting microscope (110X for larvae; 80X for juveniles; 50X for adults). Total number of cysts per fish was obtained by multiplying the number of cysts on the left gill arches by two (Madhavi 1986).

The survival distribution function (SAS, SAS Institute Inc., Cary, NC) was used to estimate the distribution of the survival times for each life stage and to test for differences in survival curves among life stages. Survival distribution function accounts for censored test specimens (those that did not expire before the termination of the experiment) and thus provides unbiased survival curves for censored and uncensored observations. Linear and polynomial least-squares regression (Neter et al. 1996) were used to model lethal infestation rates of metacercariae and the number of metacercariae per mm of fish length as a function of fish total length.

RESULTS AND DISCUSSION

Survival curves differed significantly (Log-Rank Test: $X^2_{(2)} = 17.2$, $P = 0.0002$, N = 27) among larval, juvenile, and adult fish (APPENDIX A). All larval fish died within the 8 h trial with the survival curve estimating 25% mortality at 83 minutes, 50% mortality at 116 minutes, and 75% mortality at 287 minutes. Approximately 65% of the juvenile fish died within the 8 h trial with the survival curve estimating 25% mortality at 251 minutes and 50% mortality at 330 minutes. Less than 25% of the adult fish died within the 8 h trial. No control fish died during the study.

Adult fish that did not expire during the 8 h trial contained fewer metacercariae than their size cohorts that did die (APPENDIX Ba). However, fish that did not expire during the 8 h trial did possess greater numbers of metacercariae than those in younger life stages. Thus, the number of metacercariae that induced mortality was dependent ($r^2 = 0.96$, $P < 0.0001$) upon fish life stage. Mean number of metacercariae (± SD) to cause mortality was 60.2 (± 55.7) for larvae, 353.3 (± 70.4) for juveniles, and 1131 (± 142.8) for adults.

Smaller fish succumbing to death with fewer metacercariae than larger fish is expected because they have less gill surfaces area than those of larger fish (Pauly 1981); consequently, fewer metacercariae can produce greater disruption of respiratory processes on smaller fish (Fischer and Kelso 1988). However, a direct relationship ($r^2 = 0.81$, $P < 0.0001$) existed between number of metacercariae per mm of fish length and the total length of fish (APPENDIX Bb). Mean number of cysts (± SD) per mm of total length to cause mortality was 5.4 (± 4.5) for larvae, 19.9 (± 4.6) for juveniles, and 28.6 (±

3.1) for adults. This length-standardized relationship suggests that larval fish per mm of length were more susceptible to encysted metacercariae than larger fish. Larger fish can survive greater loads of metacercariae than smaller fish independent of total length.

Two reasons seem plausible to explain this differential effect in metacercariae infestation among age groups. First, oxygen demand is higher for juvenile fish as compared to adult fish (Blazer and Gratzek 1985). Thus, greater gill surface area being affected combined with higher oxygen demand in smaller fish may have produced the differential effect observed here among size groups. Second, greater susceptibility to death may be attributed to a difference in the defense response among size groups. Some examples of host defense responses would be extensive hypertrophy and hyperplasia of the gill epithelium, mucus secretion, extensive hemorrhaging and edema, and the surrounding of parasites by macrophages and fibroblasts (Fustish and Millemann 1978; Blazer and Gratzek 1985; Lo and Lee 1996). It's possible that larval fish would respond slower to a defense response than large fish because defense responses in general are slower in smaller fish (Maule and Schreck 1991; de Jesus and Hirano 1992; Reddy and Leatherland 1998).

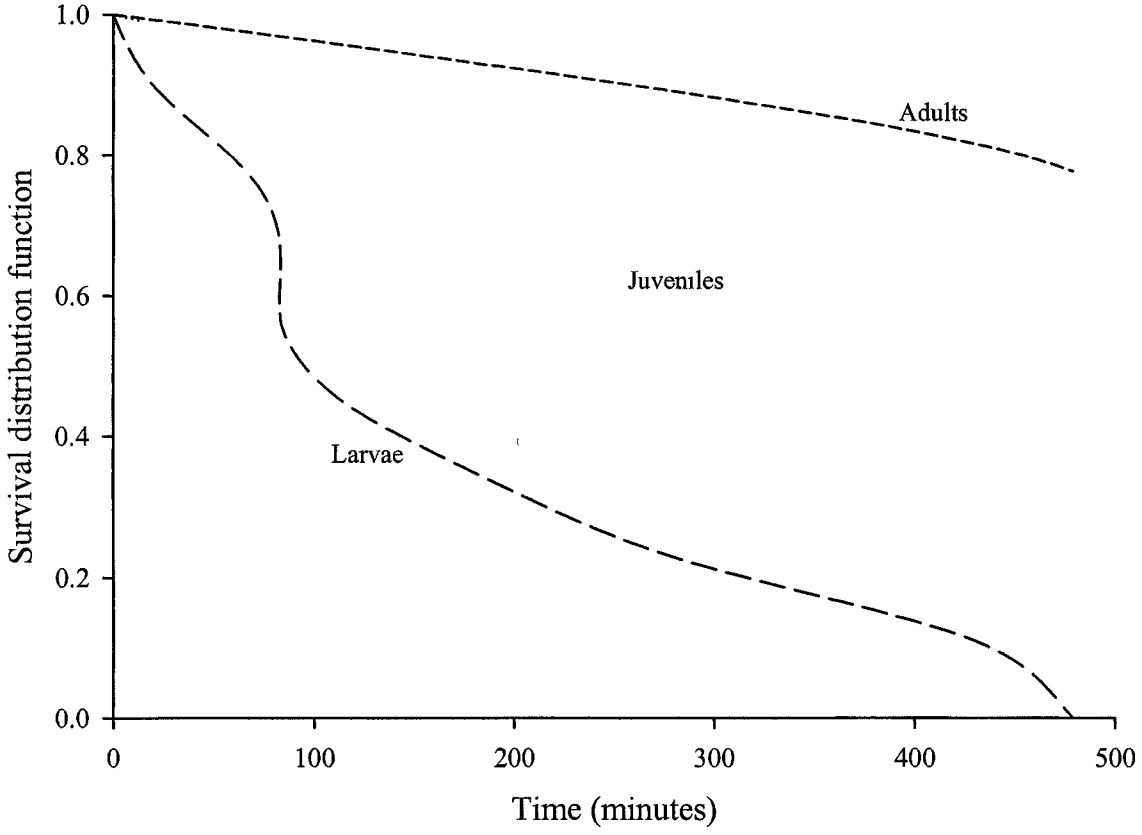
Smaller fish typically are more susceptible to death from parasite infestation than adults. With parasites, smaller fish differentially experience poorer condition, slower growth, and greater mortality as compared to adults (Baldwin et al. 1967; Lemly and Esch 1984; Fischer and Kelso 1990). Parasite-induced mortality in age-0 bluegills (*L. macrochirus*) is up to 20% in one natural population (Lemly and Esch 1984) and age-0 mortality caused by *C. formosanus* can exceed 90% in a dense population of cyprinids reared for commercial use (Balasuriya 1988). Thus, increased and abnormal form of

mortality caused by exotic parasites has the potential to alter population structure of natural fish populations, problematic especially for a fish with limited range and distribution such as the fountain darter.

Parasite loads used in this study to induce mortality of adult fish (> 1000 cysts per fish) were greater than the average numbers of metacercariae (mean number of metacercariae per fish: 130, 193, and 550) observed in fountain darters among three sections of the Comal River. One fish was found with 1,524 metacercariae (Mitchell 2000). The intent of this study was to kill the fish quickly (< 8 h) to elicit differences among size groups. In the Comal River the metacercariae undoubtedly were acquired over a much longer period of time. However, parasites loads of the number tested here could be expected if conditions change in the Comal River such as reduced flow that concentrate fish, cercariae, snail/definitive hosts, and cause higher waters temperatures, all of which are possible in an area where spring discharge is dependent on groundwater withdrawals for surrounding municipality and agriculture purposes (Ono et al. 1983). Thus, regulation of flow and its associated effect on water temperatures may help to limit problems associated with *C. formosanus* infestation for the fountain darter.

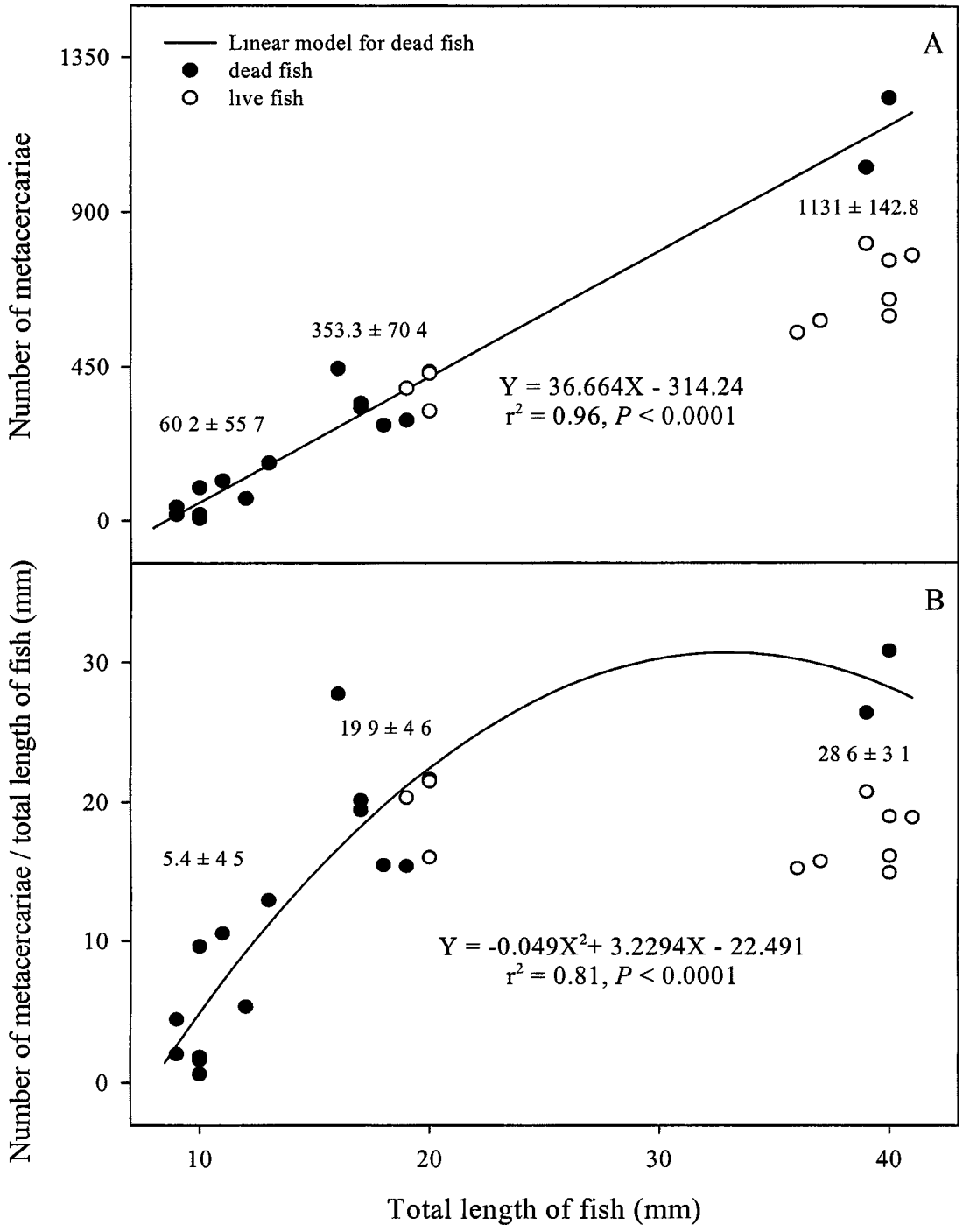
APPENDIX A

Estimated distribution of survival times for larval, juvenile, and adult fountain darters that were exposed to approximately 500,000 *Centrocestus formosanus* cercariae in 900 ml of water for up to 8 h.



APPENDIX B

Relationship between number of metacercariae (*Centrocestus formosanus*) (A) and number of metacercariae per mm of fish length (B) and fish length for dead fish (closed circle). Open circles represent fish that did not die within 8 h of exposure.



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