Size Susceptibility to Trematode-Induced Mortality in the Endangered Fountain Darter (*Etheostoma fonticola*)

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ABSTRACT

We determined infestation rates for dead and surviving larval, juvenile, and adult fountain darters (*Etheostoma fonticola)* exposed to an exotic trematode, *Centrocestus formosanus*. Estimates from survival curves indicated that 50% of the larval fish (total length 9 to 13 mm) would die within 116 minutes and 50% of the juvenile fish (16 to 20 mm) within 330 minutes when exposed to approximately 555,000 cercariae/L. Less than 25% of the adult fish (36 to 41 mm) had died in 8 h of exposure. Number of metacercariae that caused mortality was directly related ($P < 0.01$) to fish length, although length alone did not explain the accelerated rate of mortality observed in smaller fish. Varying degrees of trematode tolerance among size groups suggested that larval and juvenile fish were more susceptible to mortality induced by *C. formosanus* infection than adults. This result has implications for population health as greater mortality in smaller fountain darters could limit the number of fish reaching sexual maturity.

INTRODUCTION

Edwards Plateau and Trans Pecos springs provide unique and relatively stable abiotic environments in arid and semi-arid regions of central and west Texas, and support numerous endemic crustaceans, fishes, and amphibians. However, these spring environments also are susceptible to tropical fauna invasions because their thermally stable waters provide refuge for exotic biota during periods of cold temperatures. Examples of tropical fauna invasions in several Texas springs, which were initially introduced by aquarium releases, include mollusks (i.e., red-rim melania, *Melanoides tuberculatus*, and giant ramshorn snail, *Marisa cornuarietis*; Howells 1992) and fishes (i.e., suckermouth catfish, *Hypostomus plecostomus,* and blue tilapia, *Oreochromis aureus*; Hubbs et al. 1991). Introductions not only potentially affect the native and endemic fauna through competition or predation but also are sources of parasites such as trematodes (Scholz and Salgado-Maldonado 1999), nematodes, and cestodes (Vincent and Font 2003).

The exotic digenetic trematode *Centrocestus formosanus* was reported from snails in the San Antonio River basin of Bexar County, Texas in 1990 (H. D. Murray, Trinity University, pers. comm.) and from snails and fishes in Guadalupe River and Rio Grande

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drainages of Texas in 1999 (McDermott 2000). Within these drainages, *C. formosanus* was documented in 27 species of fishes, including six that are restricted to spring-influenced habitats and listed as species of conservation concern (Mitchell et al. 2000, McDermott 2000, Fleming 2002). Among fishes of conservation concern, the fountain darter (*Etheostoma fonticola)* is listed as a federally endangered species and is restricted to two spring runs within the Guadalupe River drainage, the Comal River and the upper San Marcos River. The trematode was found in eight of 15 fountain darters collected from the Comal River in 1994, and later found in all fountain darters (n=194) collected from the Comal River during 1997 and 1998 (Mitchell et al. 2000).

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 fish 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). Piscivorous wading birds are the definitive host along with possibly other birds or mammals (Mitchell et al. 2005), and the red-rim melania snail is the first intermediate host (Lo and Lee 1996, Yamaguti 1975). This snail was first discovered in Texas in the San Antonio River (Murray 1964) and later in the Guadalupe River and Rio Grande drainages (Mitchell et al. 2000, McDermott 2000).

High fish mortalities are attributed to trematode infestations in confined areas such as aquaculture ponds (Balasuryia 1988, Paperna 1996, Mohan et al. 1999). Likewise, an exotic trematode such as *C. formosanus* might produce similar levels of mortality in fishes confined to spring habitats, especially when spring discharge diminishes to low levels as exemplified by Comal Springs in the mid-1950s (Schenck and Whiteside 1976), and it is a continuing threat to many springs because of increased groundwater withdrawals. Laboratory studies by Salmon (2000) found that *C. formosanus* metacercariae infection intensities of 500 cysts per fish caused stress and mortality in adult fountain darters. Similar infection intensities were observed in fountain darters taken from the Comal River (Mitchell et al. 2000). To further assess *C. formosanus* effects on a spring-endemic fish, the objectives of our study were to determine the metacercarial concentration and exposure duration that could be expected to kill larval, juvenile, and adult fountain darters and to determine if larval and juvenile fish were more susceptible (absolute and standardized by length) to trematode-induced mortality than adults. Information on size and species susceptibility is needed for better understanding of the parasites effect on native fauna and to develop better controls and prevention of host and parasite introductions (Scholz and Salgado-Maldonado 1999).

MATERIALS AND METHODS

Red-rim melania snails were collected with dip nets from the Comal River and transported to the National Fish Hatchery and Technology Center (NFHTC) in San Marcos, Texas. Snails were held in aerated water in a glass aquarium for 30 d. Cercariae were induced to emerge by placing snails in 500 mL of water in small beakers, which were then warmed under incandescent light for 2 h (Lo and Lee 1996). All of the emerged cercariae were combined, vigorously mixed with additional water, and redistributed into three test aquaria, each with 900 mL of water. Average concentration in each test aquaria was 550,000 cercariae per L. Three other aerated aquaria served as controls and contained no cercariae.

Hatchery-reared fountain darters (n=54) were obtained from the NFHTC. Among these 54 darters, 18 were larvae (9 – 13 mm in total length [TL]), 18 were juveniles (16 – 20 mm in TL), and 18 were adults (36 – 41 mm in TL). Three larval, three juvenile, and three adult fountain darters were placed into each of the three aquaria, treatment and control alike. The fish were observed continuously for the next 8 h. Loss of equilibrium and cessation of respiratory movements were used as criteria indicating death (Baldwin et al. 1967). When a fish died, the time of death was recorded and the fish was removed and preserved in 10% formalin. After 8 h, the remaining live fish were anesthetized in tricaine methanesulphonate and preserved in 10% formalin. The gill arches from the left side of each fish were excised, and the encysted metacercariae on the arches were counted under magnification. Total number of cysts per fish was approximated by doubling the number of cysts on the left gill arch (Madhavi 1986).

Survival times were estimated for each life stage and differences in survival curves among life stages were compared using the Survival Distribution Function (Proc LIFETEST, SAS, SAS Institute Inc., Cary, NC). The Survival Distribution Function accounts for censored test specimens (e.g., 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 regressions (Neter et al. 1996) were used to model lethal infestation rates of metacercariae and relative number of metacercariae as a function of fish total length.

RESULTS AND DISCUSSION

Survival curves differed (Log-Rank Test: $\chi^2_2 = 17.2$, $P < 0.01$, n=27) among larval, juvenile, and adult fish (Fig. 1). All larval fish died within the 8 h trial with the survival curve estimating 75% survival at 83 minutes, 50% survival at 116 minutes, and 25% survival at 287 minutes. Approximately 35% of the juvenile fish lived within the 8 h trial with the survival curve estimating 75% survival at 251 minutes and 50% survival at 330 minutes. Less than 25% of the adult fish died within the 8 h trial. No control fish died during the study.

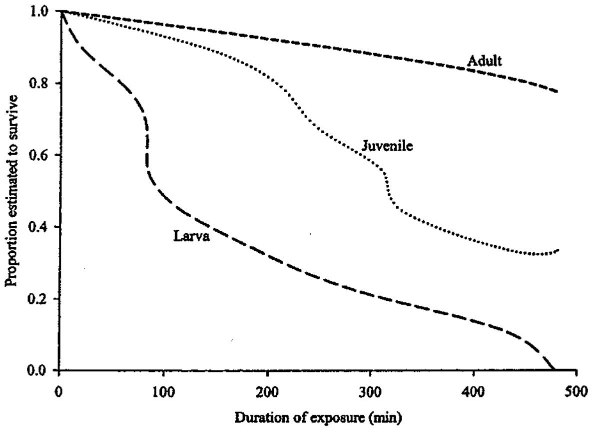

Figure 1. Estimated distribution of survival times for larval, juvenile, and adult fountain darters (*Etheostoma fonticola*) exposed to approximately 500,000 *Centrocestus formosanus* cercariae in 900 mL of water for up to 8 h.

Mean number of metacercariae (± SE) per fish causing death was 60.2 (± 18.6) for nine larvae, 353.3 (± 28.8) for six juveniles, and 1,131 (± 101.0) for two adults (Fig. 2). In comparison, mean number metacercariae per fish that survived the time trials was 378.7 (± 31.9) for three juveniles, and 672.9 (± 39.7) for seven adults. Surviving juveniles had about as many metacercariae as juveniles that died, whereas surviving adults had about 50% fewer metacercariae than adults that died. Overall, the number of metacercariae that caused mortality was positively correlated to fish length (r^2=0.96, n=18, $P < 0.01$).

It was expected that smaller fish would succumb to death with fewer metacercariae than larger fish because they have less gill surface area than larger fish (Pauly 1981); smaller fish require fewer metacercariae to cause greater disruption of respiratory process (Fischer and Kelso 1988). Indeed, we found that there was a strong correlation between metacercarial infestation intensity and fish length mainly because smaller fish were killed before they were allowed to reach infestation intensities similar to those of large fish. In addition, a direct relationship (r^2=0.81, n=18, $P < 0.01$) existed between number of metacercariae per mm of fish length and the total length of fish (Fig. 2). Mean number of cysts causing death (± SE) per mm of fish length was 5.4 (± 1.5) for larvae, 19.9 (± 1.9) for juveniles, and 28.6 (± 2.1) for adults. This length-standardized relationship suggests that larval fish were more susceptible (per mm) to encysted metacercariae than larger fish.

Two reasons seem plausible to explain this differential effect in metacercarial induced death among age groups. First, oxygen demand is higher for juvenile fish as compared to adult fish (Blazer and Gratzek 1985). Greater gill surface area being affected combined with higher oxygen demand in smaller fish might have produced the

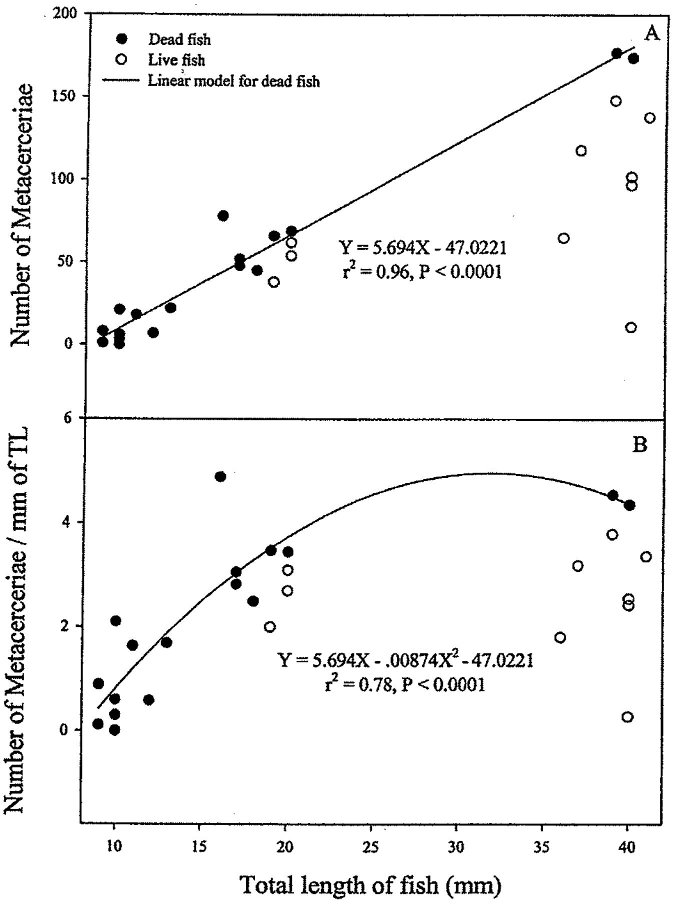

Figure 2. Relationship between number of metacercariae (*Centrocestus formosanus*) (A) and number of metacercariae per mm of *Etheostoma fonticola* length (B) and fish length for dead fish (closed circle). Open circles represent fish that did not die within 8 h of exposure.

differential effect observed here among size groups. Second, greater susceptibility to death may be attributed to differences in the host reaction among size groups. Examples of host defense reactions include 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 is possible that the larval defense response was slower than that of the larger fountain darters, a situation which has been observed in other fishes (Maule and Schreck 1991, de Jesus and Hirano 1992, Reddy and Leatherland 1998).

Parasite loads for live and dead adult fish in this study (mean = 774.6 with a range of 548 to 1,232 metacercariae per fish) were similar to the number of parasites in fountain darters collected in 1998 (951.2 with range of 542 to 1,524; Mitchell et al. 2000) in 2003 (780 with a range of 52 to 1,662; Cantu 2003) from the individual Comal River. However, metacercariae in these studies likely were acquired over a much longer span of time than those in our laboratory study. Higher parasite loads in fountain darters reported by Mitchell et al. (2000) followed a two-year period of below normal spring discharge that concentrated the cercariae, snails, and definitive hosts, all of which possibly explains higher parasite loads in 1998 compared to parasite loads during periods of normal spring discharge.

Wild fountain darters probably would never be exposed to 500,000 cercariae per L, all darters probably would have died before levels reached that high. Cantu (2003) was the first to measure *C. formosanus* cercarial concentrations in the Comal River and his highest concentration obtained was 45 *C. formosanus* per L. Cantu (2003) also placed hatchery-reared uninfected fountain darters in cages in the Comal River for 7 days. Number of metacercariae acquired by fountain darters ranged from 0 to 348 per fish; the fish containing the 348 metacercariae was accumulating metacercariae at the rate of 49.7 per day. It would have taken a minimum of 24.8 days in a cage in the river for the fish in our study to acquire the maximum load of 1,232 metacercariae.

Smaller fish typically are more susceptible to death from parasite infestation than adults. In addition, complications of parasite infestation typically are more pronounced in smaller fishes, resulting in poorer body condition, slower growth, and greater mortality as compared to adults (Baldwin et al. 1967, Lemly and Esch 1984, Fischer and Kelso 1990). For example, parasite-induced mortality in age-0 bluegill (*L. macrochirus*) approached 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). Consequently, increased mortality caused by exotic parasites has the potential to alter the structure of natural fish populations. This is especially problematic for a fish like the fountain darter that has a narrow geographic distribution, a short life span, and is limited to two spring-influenced habitats.

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