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Prevalence and Pathogenicity of a Heterophyid Trematode Infecting the Gills of an Endangered Fish, the Fountain Darter, in Two Central Texas Spring-Fed Rivers

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Abstract.—Gills of 194 fountain darters *Etheostoma fonticola* collected from the Comal River in Texas from May 1997 through May 1998 were found to be parasitized with 8–1,524 metacercarial cysts of a heterophyid trematode tentatively identified as *Centrocestus formosanus.* The intensity of infection varied among three sites on the Comal River. In contrast, of 130 darters from the nearby San Marcos River that were examined, only 4 (3%) were infected, and these had 1–2 cysts per fish. Of 2,279 *Melanoides tuberculata* snails from the Comal River that were examined, 139 (6.1%) were infected with the trematode. Only 1 snail in 2,241 from the San Marcos River that were examined was infected. The presence of metacercariae in darters was associated with flared opercula, shortened or thickened gill filaments, epithelial hyperplasia, and engorged lamellae. The normal cartilage support of the filaments was distorted and displaced, leading to severe deformities of filament structure. Gill damage was severe and possibly life threatening for the darters with more than 800 cysts per fish (9% of examined fish). We suspect that fountain darter deaths were caused by the parasite in the Comal River during this study.

The fountain darter *Etheostoma fonticola* is listed as endangered and is endemic to only two spring-fed rivers in central Texas, the Comal and the upper San Marcos rivers (U.S. Fish and Wildlife Service 1995). In response to the drought in central Texas in 1996, separate refugia for fountain darters were established at the National Fish Hatchery and Technology Center (NFHTC), San Marcos, Texas, and the Uvalde National Fish Hatchery, Uvalde, Texas. While collecting fountain darters for the refugia in October 1996, NFHTC staff members noticed that the fish from the Comal River had swollen gills extending past the opercula. Further examination revealed a heavy infection of trematode metacercariae encysted in the gill cartilage and lamellae. Subsequently, the parasite was found in several other fish from the Comal River.

The trematode has been tentatively identified as *Centrocestus formosanus* (R. M. Overstreet, Gulf Coast Research Laboratory, personal communication). *Centrocestus formosanus* infects the gills of a wide variety of fish species causing severe gill lesions, uses *Melanoides tuberculata* as the snail host and several aquatic birds as definitive hosts, and is found in many countries including Mexico (Yanohara 1985; Salgado-Maldonado et al. 1995). In the United States, the parasite was apparently observed first in aquarium fish from Florida fish farms in the early 1980s (Blazer and Gratzek 1985) and later in San Antonio, Bexar County, Texas, in 1990 (H. D. Murray, Trinity University, personal communication). Murray also thinks that the mature trematode

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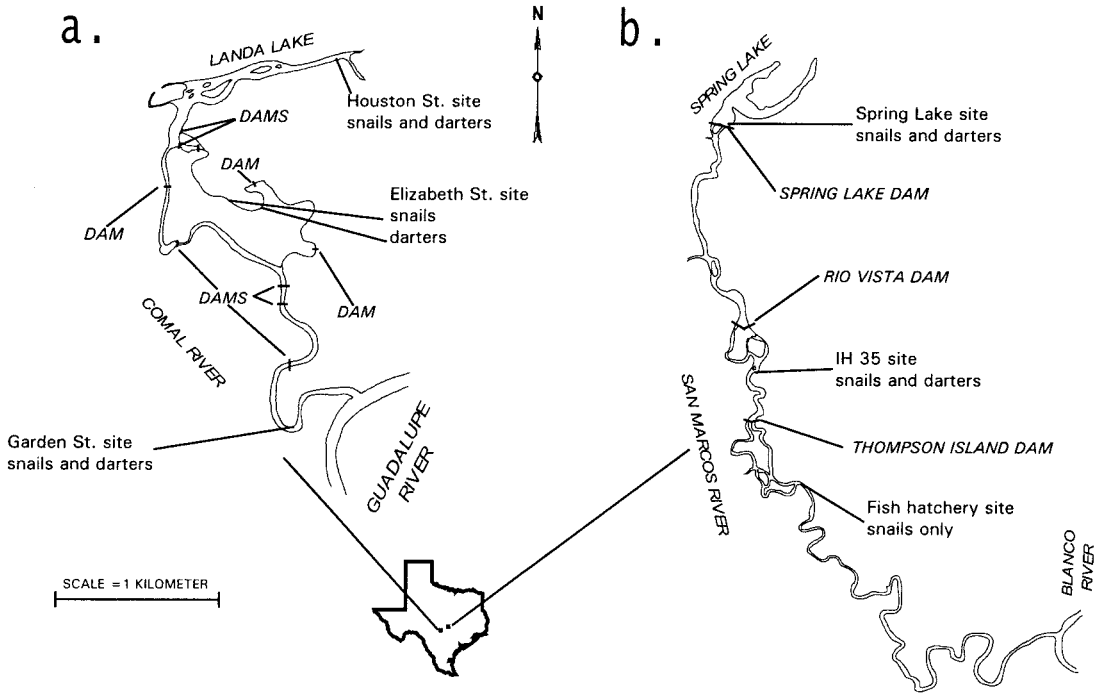

FIGURE 1.—Sites on (**a**) the Comal River in Comal County and (**b**) the San Marcos River in Hays County, Texas, where fountain darters and snails (*Melanoides tuberculata*) were collected; IH = interstate highway.

completes its life cycle in the yellow-crowned night heron *Nyctanassa violacea*. This migratory bird generally is present in central Texas from April through September (Travis Audubon Society 1994). *M. tuberculata*, an exotic freshwater thiarid snail, serves as the first intermediate host in the Comal River (preliminary work by one of the authors [unpublished]). Pointier et al. (1992) have reported that *M. tuberculata* is present in all intertropical continental countries and most Pacific islands. Livshits and Fishelson (1983) reported occurrences of the snail in tropical, subtropical, and temperate regions. In the United States, *M. tuberculata* has been found in Florida, Texas, and Louisiana (Dundee and Paine 1977). *M. tuberculata* is well established in both the Comal and San Marcos rivers.

This study was initiated to document the intensity and monthly prevalence of this gill trematode in the fountain darter in the Comal and San Marcos rivers and the gill pathology associated with this trematode. The monthly prevalence of the parasite in the intermediate snail host *M. tuberculata* also was documented.

Methods

Monthly snail collection.—An attempt was made to collect approximately 70 *M. tuberculata* monthly (to ensure that there would be 60 for examination) from each of three sites on the Comal and San Marcos rivers (Figure 1) from 22 May 1997 through 19 May 1998. The snails were collected by hand or small dip nets, and the collection effort was limited to 2 h per site per collection. The snails were placed in approximately 1 L of river water in 2-L Ziploc freezer bags. After returning to the NFHTC, oxygen was added to the bags, and the bags were resealed and stored at approximately 4°C until they could be examined. Sixty snails were examined per site per date. When fewer than 60 live snails were collected at a site, all of the snails collected were examined.

Each snail shell was measured for length (mm) and broken at the second suture between the second and third whorl from the aperture. The distal shell portion was removed, exposing the entire digestive gland. The gland was removed with forceps and rinsed with tap water to remove shell fragments. The entire gland was placed in a drop of tap water on a microscope slide and compressed under a coverslip. Glands from the larger snails were divided and placed on two slides. The wet mounts were examined for the presence of cercariae and rediae under 100× magnification. No attempt was made to enumerate the stages in in-

dividual snails. From October 1997 until the end of the study, the prevalence of male snails (presence of red male gonads; Heller and Farstay 1989) was recorded. The percentages of snails infected were determined for each collection site and date.

Monthly fish collection.—Five fountain darters between 19 and 35 mm in total length (TL) were collected with dip nets from each of three sites on the Comal River (Figure 1) and two sites on the San Marcos River within 30 h of the corresponding snail collections. Fountain darters were not collected at the fish hatchery site on the San Marcos River because of the limited number of these fish in this section of the river.

The darters were transported in Ziploc freezer bags (2-L-capacity bags; about 650 mL of river water) to the NFHTC. The water volume in each bag was reduced to about 400 mL, and oxygen was added to the bags before overnight shipment to the Harry K. Dupree Stuttgart National Aquaculture Research Center, Stuttgart, Arkansas, where the fish were examined. The total length (mm) and weight (g) of the fish were obtained before dissection. Gill arches were removed with microiris scissors and prepared as wet mounts for microscopic viewing. Most wet mount microscopy observations were made at 40–400×. Length and width measurements of 85 cysts, 75 with dead and 10 with live metacercariae, were made by the use of a calibrated ocular micrometer at 400×. Gross pathologic features of the gills were noted and recorded.

Madhavi (1986) found metacercarial cysts of *C. formosanus* evenly distributed between the right and left gills of 307 fish that were examined ($P < 0.05$). Therefore, all four arches on the right side of the fish were removed first, and the cysts were counted and classified as containing either dead or live metacercariae. The count from the right gill arches was doubled and used as an estimate of the total number of cysts per fish. If no cysts were found in the right gill arches, the left gill arches were examined, and the number of cysts found was recorded as the total number of cysts per fish.

The gills of four darters with flared opercular flaps (an indication of heavy trematode infection) from the Elizabeth Street site (Figure 1) and the gills of three fish with light to moderate infections (Houston Street site) were processed for histopathological analysis. Specimens were fixed in Bouin's fixative, transferred to 50% and then 70% ethanol, and then sectioned and stained with hemotoxylin and eosin.

Other gill parasites on the fountain darter were noted, and gills with parasites that were associated with obvious pathology were processed for histological examination as described above.

Results and Discussion

Snails

In all, 2,279 and 2,241 snails from the Comal and San Marcos rivers, respectively, were examined. On six occasions, we were unable to collect 60 snails at a site within 2 h (Table 1), suggesting that the population density of snails at the collection sites varied over the 13-month collection period. Additionally, in June 1997, we were unable to collect at two sites because of dangerous flood conditions.

Mean shell lengths (ranges) of the Comal River snails at the Houston, Elizabeth, and Garden Street sites were 29.5 (20–39), 26.5 (11–43), and 18.7 mm (12–26 mm), respectively, and at the Spring Lake, interstate highway-35, and fish hatchery sites on the San Marcos River, they were 41.5 (16–65), 27.7 (12–48), and 30.3 mm (14–53 mm), respectively. Minimum and maximum lengths of the infected snails were 17 and 38 mm. This observation is consistent with the findings of Yanohara (1985), who reported that *M. tuberculata* infected with *C. formosanus* were from 15 to 38 mm in length. Males were collected from all sites and ranged from 0.2% of the population at Spring Lake in the San Marcos River to 22.5% at Elizabeth Street in the Comal River.

There were 139 infected snails among the 2,279 (6.1%) from the Comal River that were examined. The mean and range of the monthly prevalence of the trematode in snails at the Houston, Elizabeth, and Garden street sites were 8.5% (3.3–16.7%), 9.4% (0–28.3%), and 0%, respectively. No monthly trends were evident for the prevalence of the trematode in the Comal River snails (Table 1). Only one snail was infected in the San Marcos River, and it was collected at the fish hatchery site.

Fountain Darters

All 130 fish from the San Marcos River (mean total length, TL = 28 mm; range, 21–35 mm) and 194 of 195 fish from the Comal River (mean TL = 27 mm; 19–35 mm) were examined. One fish died and decomposed during shipment. It was not examined. Other fish died before processing (4 fish from the San Marcos River and 17 fish from the Comal River). These 21 fish were in a condition that allowed the trematodes to be counted but did not allow the viability of the trematodes to be determined. Only 4 of the San Marcos River fountain

TABLE 1.—Prevalence (P, %) and mean intensity (I), trematodes per fish of a heterophyid trematode infecting fountain darters and the snail *Melanoides tuberculata* from the Comal and San Marcos rivers during 1997 and 1998.

	Comal River[a]									San Marcos River[b]						
	Houston Street			Elizabeth Street			Garden Street			Spring Lake			IH-35[c]			Hatchery
	Snail	Fish		Snail	Fish		Snail	Fish		Snail	Fish		Snail	Fish		Snail
Date	P	P	I	P	P	I	P	P	I	P	P	I	P	P	I	P
1997																
May	8.3[d]	100	196.4	10.0	100	71.2	0	100	48.8	0[d]	0	0	0[d]	0	0	0[d]
Jun	16.7	100	70.8	28.3	100	175.2	[e]	100	78.0	0	0	0	0[d]	0	0	[e]
Jul	13.3	100	217.2	5.0	100	503.6	0	100	161.2	0	0	0	0	0	0	0
Aug	10.0	100	146.8	18.3	100	472.0	0	100	98.4	0	0	0	0	40.0	0.4	0
Sep	5.0	100	347.2	5.0	100	517.2	0	100	169.2	0	0	0	0	0	0	0
Oct	5.0	100	91.6	6.7	100	461.6	0	100	108.8	0	0	0	0	0	0	0
Nov	5.0	100	54.4	1.7	100	652.0	0	100	219.8[f]	0	0	0	0[d]	0	0	1.7
Dec	8.3	100	258.4	0.0	100	796.8	0	100	169.2	0	0	0	0	0	0	0
1998																
Jan	13.3	100	383.6	5.0	100	917.6	0	100	128.0	0	0	0	0	0	0	0
Feb	6.7	100	163.6	8.3	100	984.8	0	100	188.0	0	0	0	0	0	0	0
Mar	5.0	100	200.4	6.7	100	621.2	0	100	205.2	0	0	0	0	0	0	0
Apr	3.3	100	186.0	15.0	100	666.8	0	100	75.6	0	0	0	0	0	0	0
May	10.0	100	154.4	11.7	100	479.2	0	100	78.4	0	0	0	0	40.0	0.8	0

[a] Five fish and 60 snails per site.
[b] Five fish and 60 snails per site; no fish were from the hatchery site.
[c] Interstate highway 35.
[d] Scarcity of snails prevented the collection of 60 snails within 2 h.
[e] Flooding of the river prohibited the collection of snails.
[f] Some fish died during shipment; only four fish were examined.

darters were infected with the trematode (1–2 cysts per fish), but all 194 fish from the Comal River that were examined were infected (8–1,524 cysts per fish and 0–251 cysts per gill arch). The ratio of live to dead metacercariae taken from 177 live darters from the Comal River was 1:274. Only 95 of 26,044 (0.4%) metacercariae observed in the right gill arches were alive. The mean sizes of 75 cysts with dead and 10 cysts with live metacercariae chosen for measurement were 95 (73–114) μm × 49 (36–66) μm and 153 (107–180) μm × 107 (78–131) μm, respectively.

Most dead metacercariae were incompletely developed (eye spots present and an X-shaped excretory vesicle absent; Yanohara 1985). The metacercariae apparently had died shortly after attachment to the gills (Figure 2A). Live metacercariae were found during each sampling period, suggesting that the fish were infected year-round. The fish appeared to accumulate metacercariae at a low rate because 22 of 35 fish (62.9%) infected with living metacercariae were infected with only one living metacercaria. Positive correlations between the increasing length of the fish and increasing numbers of cysts on gills at the Houston Street ($r = 0.58$, $P < 0.0001$) and Elizabeth Street ($r = 0.64$, $P < 0.0001$) sites support this assumption. Martin (1958) exposed three fish species (goldfish *Carassius auratus*, western mosquitofish *Gambusia affinis*, and green swordtail *Xiphophorus helleri*) to cercariae of *C. formosanus* in the laboratory and examined wild fish that had been exposed naturally to *C. formosanus* before capture. He concluded that heavily infected wild fish accumulated their infections over an extended period because a single pulse of cercariae resulting in a similar intensity of infection probably would kill the fish.

This trematode may have begun parasitizing the fountain darter only within the last few years. An examination of preserved specimens of the fountain darter used in a previous stomach analysis study (Bergin 1996) revealed that all five fish collected from the Comal River during February 1994 were not infected with the trematode, whereas three of five fish from a collection taken during August 1994 and all five fish from both December 1994 and March 1995 had light infections. All nine fountain darters collected from the Comal River on 24 October 1996, when the epizootic was first noticed, had 4–40 cysts on the right second gill arch.

A comparison of the monthly mean intensity per site revealed no consistent pattern in variation either monthly or among the Comal River sites over the study period (Table 1). We cannot form any definite conclusions regarding intensity patterns

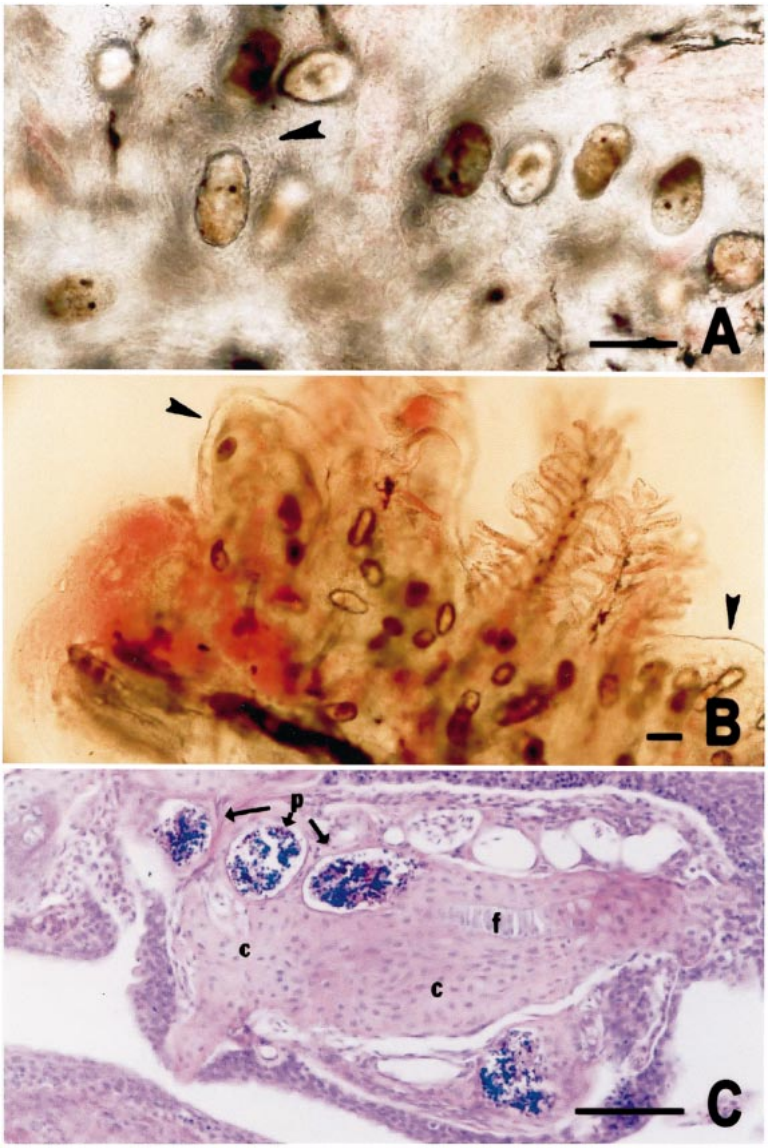

FIGURE 2.—Branchial tissues of the fountain darter infected with a heterophyid trematode. (**A**) Cartilage proliferation (arrowhead) around dead metacercariae as seen in a wet mount preparation. Note the eye spots present in some cysts. Scale bar = 100 μm. (**B**) A heavy infection of metacercarial cysts in the gills of a fountain darter. Distorted branchial tissue, hyperplastic tissue (arrowheads), and areas engorged with red blood cells are apparent. Scale bar = 100 μm. (**C**) Tissue section showing trematode cysts (p) adjacent to the filament cartilage (f) and surrounded by proliferated cartilage (c) several layers thick. Hematoxylin and eosin stain was used. Scale bar = 100 μm.

because only five fish were collected per site per month and no site characterization data (substrate-type, water velocity, water depth, vegetation- type, vegetation quantity, bird usage, etc.) were collected. The trematode is well established in the Comal River because all fountain darters examined were infected; however, mean intensity differences do exist between sites along the river. The overall mean intensities (trematodes per fish) were 192.9, 550.6, and 128.9 for the Houston, Elizabeth, and Garden Street sites, respectively. We can explain neither the difference in parasite intensity among fountain darters at the Comal River sites nor the dramatic difference in parasite prevalence between

fountain darters in the Comal River (100%) and the San Marcos River (3.1%).

Wet Mount Examination

Examination of wet-mounted gills of fountain darters with flared opercular flaps revealed swollen and deformed branchial tissues. A clear layer of tissue often was noted surrounding the cyst and appeared to be a host reaction (Figure 2A). Infected gill filaments were shortened, thickened, and often distorted. Epithelial hyperplasia and fusion of the filaments also were noted. Affected lamellae often had several areas that were engorged with red blood cells and massive cartilage lesions and apparent proliferation resulting in displacement of the epithelial tissue (Figure 2A, B).

Histopathological Examination

Histopathological results were consistent with the wet mount observations but offered a much more detailed picture. The trematodes were encysted in chondrocysts and affixed to the side of gill filament cartilage. The proliferated cartilage caused affected filaments to expand to several times the normal diameter, and this resulted in the complete disruption of the normal gill morphology. The filaments were thick, distorted, and apparently fused. Few typical lamellae were present, and epithelial hyperplasia was extensive (Figure 2C). These lesions are virtually identical to those previously described in tropical fish by Blazer and Gratzek (1985).

Cysts of the *Myxobolus* sp., a species that is morphologically similar to *M. angustus* (Desser and Paterson 1978), were found in 113 of 324 darters examined during the present study. The *Myxobolus* sp. caused extensive gill damage, including cartilage lesions. Although cysts of the unidentified myxosporidian were seen often, the intensity of infection was low. Also, the parasite was not observed often when the pathologic features described above were present.

On the basis of the results of this study, there is little question that the health of fountain darters in certain localities is compromised by the trematode tentatively identified as *C. formosanus.* Because of the massive number of trematodes found in the gills (up to 1,524 cysts per fish) and the extent of damage done to the gills, we suspect that deaths were caused both directly and indirectly by this trematode in the Comal River during the course of this study. Most of the fountain darters that died in shipment from Texas to Arkansas were heavily infected with the trematode, an indication that an additional stress (handling and captivity) caused these fish to die. Stressors such as low dissolved oxygen or other water quality problems that occur in the Comal River probably could result in the death of some of the most severely infected darters.

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