DOWNSTREAM SPREAD OF THE DIGENETIC TREMATODE, *CENTROCESTUS FORMOSANUS*, INTO THE GUADALUPE RIVER, TEXAS

THESIS

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Abstract.—An exotic heterophyid trematode, *Centrocestus formosanus*, was recently determined to have adverse effects on fishes in the Comal River, a tributary of the Guadalupe River. This prompted an investigation to determine if the parasite and its obligate first intermediate host, *Melanoides tuberculata*, a gastropod snail, was established in the Guadalupe River. Of the 22 species and 529 fish collected, 50 % of the species (n = 11) and 29 % (n = 152) of the individuals were infected between 28 May 2001 and 09 March 2002. The highest single intensity observed was 656 metacercarial cysts in *Notropis amabilis*. Among the infected species, prevalence ranged from 16-100 %, mean intensities ranged from 46-616 cysts per fish, and relative densities ranged from 9-616 cysts per fish. In addition *M. tuberculata* were not collected at any site during the course of this one-year study. This prompted a series of fish-cage studies in order to isolate the source of cercariae that infected Guadalupe River fishes. Mean intensity of cysts in blacktail shiners *Cyprinella venusta* showed a consistent decrease in the

Guadalupe River as cages were placed increasingly further downstream from the Comal River and Guadalupe River confluence. However, no infection was observed in caged fish in the Guadalupe River upstream from the confluence. The results of the cage studies and the lack of snails in the Guadalupe River indicate that fish were infected with cercariae released from snails upstream in the Comal River.

INTRODUCTION

Centrocestus formosanus (family Heterophyidae), an exotic parasitic trematode, was recently determined to have adverse effects on the endangered fountain darter *Etheostoma fonticola* in the Comal River (Comal County, Texas; Mitchell et al. 2000). This parasite is harmful to native fish populations (Mitchell et al. 2000) by encysting in gill tissue (Yamaguti 1975) causing respiratory problems. *Centrocestus formosanus* has been reported to parasitize more than 40 species of fish (Salgado-Maldonado et al. 1995). Infections with this parasite can be life threatening to fish hosts. Balasuriya (1988) states that it is a major cause of low survival of cultured cyprinids in Sri Lanka. He reported survival rates as low as 8 % from infected culture ponds. He found that infected fish demonstrated abnormal behavior including lethargy and signs of respiratory difficulties and when fish reached this stage, even mild stress caused mortality.

Respiratory difficulties in fish infected with this parasite are probably due to extensive damage to gill tissue. Velez-Hernandez et al. (1998) observed moderate to severe hyperplasia of cartilage of the primary lamellae, epithelial hyperplasia of lamellae, and gill hyperemia and congestion. Fountain darters in the Comal River were found to have severely swollen branchial tissue, as well as shortened, thickened, and distorted gill filaments and cartilage lesions and proliferation (Mitchell et al. 2000).

Centrocestus formosanus requires three hosts to complete its lifecycle: a first intermediate snail host, a second intermediate fish host, and an avian or mammalian definitive host. It has been reported to have a very low specificity for fish intermediate hosts and avian and mammalian definitive hosts (Chen 1942; Scholz and Salgado-

Maldonado 2000). It has not however, been reported to use any other snail hosts in the United States other than *Melanoides tuberculata* (family Thiaridae) and has not been reported from any location in Texas where this snail was not present. It has been reported, however to use *Stenomelania newcombi* as first intermediate host in Hawaii (Martin 1958) and *Thiara* sp. in Okinawa (Yanohara 1985).

The spread of *C. formosanus* in Texas is linked to *M. tuberculata*, an Asian snail that was first reported in Texas in the San Antonio River, Bexar County, in 1964 (Murray 1964). In 1990 Knott and Murray (1991) discovered a trematode infecting *M. tuberculata* in the San Antonio Zoo but were unable to identify the parasite using existing keys. Since that report, the same trematode, *C. formosanus*, has been found in *M. tuberculata* and many fishes in five additional locations in central and west Texas: Comal and San Marcos rivers (Mitchell et al. 2000), San Felipe Creek, San Solomon Springs, and Phantom Lake Springs (McDermott 2000).

Mitchell et al. (2002) states that the spread of this parasite is possibly restricted by the temperature preferendum of *M. tuberculata*, which is of tropical origin (Livshits and Fishelson 1983). According to Mitchell et al. (2000) the snail only has been found in association with warm-water springs, a power plant reservoir, and in a pond and two lakes in Hidalgo County in southern Texas. Scholz and Salgado-Maldonado (2000) partially attribute this parasite's rapid range expansion in Mexico to the wide geographical distribution of *M. tuberculata* in Mexico. Since *M. tuberculata* does not have a wide distribution in central and northern Texas, wide-range spread of the parasite in Texas does not seem likely.

It is important to examine the Guadalupe River for the presence of this parasite because it has the potential to cause considerable damage to native fish populations. Close proximity of established populations of the parasite (Mitchell et al. 2000) and its first intermediate host (Cauble 1998), and an abundance of potential fish and avian hosts all indicate the Guadalupe River is at risk for the establishment of this parasite. Further, if the range of this parasite has been expanded into the Guadalupe River, it has the possibility of increasing the number of birds becoming infected. Since birds are the best means by which this parasite can disperse, there is the possibility of an increase in the parasite's range.

The objectives of this study are to: 1) determine if *M. tuberculata* is present in the Guadalupe River around its confluence with the Comal River and 2) to determine if *C. formosanus* is present in fish or snails in the Guadalupe River, and if so 3) to determine what species of fish it is infecting, and the intensities, prevalence, mean intensities, and relative densities of infections.

METHODS AND MATERIALS

Fish

Fishes were collected quarterly from three sites in the Guadalupe River and one site in the Comal River on 28 May and 25 August 2001 and 01 February and 09 March 2002 (Figure 1). Flood conditions during November and December 2001 caused the postponement of the third fish collection until 01 February. Samples were collected by electro-fishing, seining, hook and line, and gillnetting. To minimize damage to the endangered fountain darter, sampling at the Comal site was restricted to seining only. Water temperature data was collected at each site.

Collected fish were placed on ice and transported to the laboratory for examination. During May and August, a few fresh fish were examined to confirm the presence of the parasite. Most of the May and August fishes and all of the February and March fishes were preserved in 10 % formalin prior to examination. Each fish was identified to species, measured (total length in mm), and the left anterior gill arch was removed. The gill arch was placed on a wet mount slide and examined for metacercarial cysts with a dissecting microscope.

The left gill arch was chosen since cysts are evenly distributed between the right and left sides. The distribution of cysts between the first, second, third and fourth gill arches are non-uniform (26.1 %, 31.0 %, 26.3 %, and 16.4 % respectively) (Madhavi 1986). A weighted estimate using these percentages revealed that using the first gill arch to estimate the total number of cysts per fish gives less error (4.2 % over-estimation) than the other three. We therefore removed only the left anterior gill arch, enumerated metacercariae, and multiplied by eight to extrapolate the total number of cysts per fish.

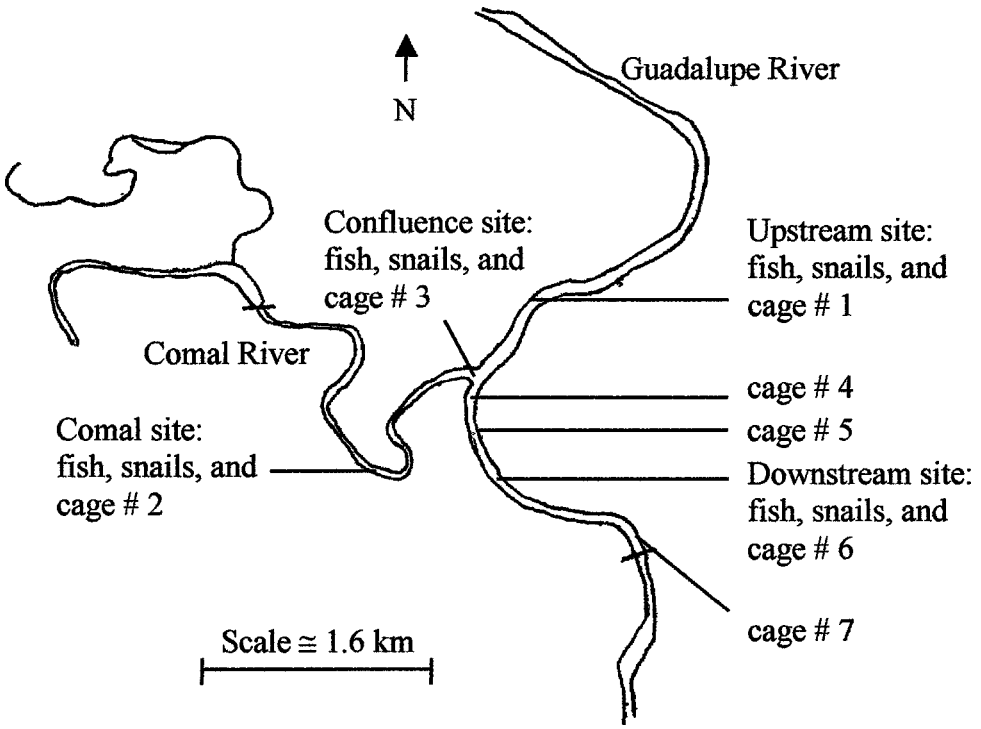

Figure 1. Sites on the Guadalupe and Comal rivers, Comal County, Texas where fish and snails were collected as well as locations of caged fish.

All parasite loads are reported here in extrapolated numbers and not corrected for the percent deviation.

Prevalence, intensity , mean intensity, and relative density were calculated for each species as defined by Margolis et al. (1982). For clarification, definitions of each of these terms are repeated below:

Prevalence = ((Number of infected hosts)/(Number of hosts examined)) X 100%

Intensity = Number of individual parasites in each infected host

Mean intensity = (Total number of parasites in a sample of hosts)/
(Number of infected hosts in that sample)

Relative density = (Total number of parasites in a sample of hosts) /
(Total number of host (infected + uninfected) in that sample)

Metacercarial Development

Once cercariae attach to the gills of a fish they go through a series of developmental stages. Development time of *C. formosanus* has been shown to vary between fish species (McDermott 2000). According to Yamaguti (1975) encystment is completed within 7-8 d in goldfish *Carassius auratus*. Newly encysted metacercaria retain a pair of very distinct eyespots that begin to gradually fade until the parasite reaches infective maturity at 18-20 d (Yamaguti 1975). McDermott (2000) however found that the X-gland had not formed in some species in excess of 28 d. Metacercarial development was catagorized as mature only if the X-shaped excretory vesicle was present. All others were classified as immature.

Snails

Quarterly surveys for *M. tuberculata* were also conducted at the same sites where fish samples were collected approximately one week following fish sampling. Samples were taken using a D-frame dip net by scraping the substrate in all available habitat types. Snails were transported back to the lab alive in river water and examined for either the presence of redial or cercarial stages of the parasite as described by Mitchell et al. (2000).

Fish-Cage Studies

Three fish cage trials were conducted; one 3 d study beginning on 07 October 2001 and two 5 d studies beginning 10 October 2001 and 24 March 2002 (trials A, B, and C respectively). During trials A and B, one cage was placed at each of the four fish

collection sites (cages # 1, # 2, # 3 and # 6). During trial C, cages were placed at three additional sites downstream from the confluence (cages # 4, # 5, and # 7; Figure1).

One wire mesh cage made of 25 mm hardware cloth with a volume of 0.027 m^3 was placed at each site. Ten to fifteen blacktail shiners *Cyprinella venusta* were placed in each cage. The fish had been obtained from multiple sites in the Blanco River in San Marcos, (Hays County), Texas. Approximately 10 % of the fish were examined to ensure that none were infected.

Once each trial was complete, all fish from each cage (except cage # 7, trial C) were placed in plastic bags, put on ice, and transported back to the lab. Fish from cage # 7, trial C were transported to the lab alive and incubated in a raceway for an additional 16 d before examination. Total length of each fish was measured and all gill arches from the fish's left side were removed and examined for metacercariae. The total number of cercariae from all arches on the fishes's left side were then enumerated and multiplied by two to extrapolate the total number of metacercariae per fish. Metacercariae were also classified as to their stage of development.

RESULTS AND DISCUSSION

Fish

A total of 529 fish were collected from the Guadalupe River comprising 7 families and 22 species. Four (57 %) of the families and 11 (50 %) of the species were infected with *C. formosanus* (Table 1). The highest intensity in a single fish was 656 cysts in a *Notropis amabilis*. Prevalence ranged from 0-100 % among the species examined. All of the 11 species that were infected had a prevalence greater than 16 % and six were in excess of 50 %. Mean intensities ranged from 47-616 cyst per fish and relative densities ranged from 0-616 cysts per fish, with the highest value for both observed in *Cyprinella lutrensis*.

The most complete record of natural infections with this parasite in fish from North America has been compiled by Scholz and Salgado-Maldonado (2000). They have given the ranges of intensities as well as mean intensities for 24 species of infected fish from Mexico. Although we collected none of the same species, their account serves as the best available guide to which we can compare our findings. Of the four families we collected for which they have data, the mean intensities that we observed were generally lower for two families and higher for two families.

Scholz and Salgado-Maldonado (2000) listed seven *Cichlasoma* spp. (family Cichlidae) with mean intensities ranging from 1-1974. Interestingly, none of the 37 individuals from two species of cichlids that we collected (one of which was a *Cichlasoma* species), were infected. Likewise, of 14 individuals from the one species of poeciliid, that we collected only one was infected (8 cyst), while they reported a range of mean intensities of 1-1277. Conversely the lowest mean intensity that we observed for

Table 1.—Highest observed intensity (HI), prevalence (P;%), mean intensity (MI), and relative density (RD) of *Centrocestus formosanus* for each species of fish as well as collection site (U-upstream, C-confluence, and D-downstream) from the Guadalupe River, Comal County, Texas during 2001-2002.

Family Species	Site	Number Collected	Number Infected	HI	P	MI	RD
Centrarchidae							
Ambloplites rupestris	U C	3	0				
Lepomis auritus	U C D	90	0				
Lepomis cyanellus	C D	9	0				
Lepomis macrochirus	U C D	16	4	184	25.0	64.0	16.0
Lepomis megalotis	U C D	55	0				
Lepomis punctatus	U C D	36	0				
Micropterus dolomieu	U C D	11	4	88	36.4	60.0	21.8
Micropterus salmoides	U C D	32	6	160	18.8	50.7	9.5
Micropterus treculi	U	6	1	160	16.7	160.0	26.7
Characidae	U						
Astyanax mexicanus	U C	8	6	136	75.0	46.7	35.0
Cichlidae							
Cichlasoma cyanoguttatum	U C D	15	0				
Tilapia aurea		8	0				
Cyprinidae							
Campostoma anomalum	U C D	14	0				
Cyprinella lutrensis	U	1	1	616	100.0	616.0	616.0
Cyprinella venusta	U C D	69	43	528	62.3	119.1	74.2
Dionda episcopa	U	3	1	120	33.3	120.0	40.0
Notemigonus crysoleucas	C	2	0				
Notropis amabilis	U C D	96	57	656	59.4	189.5	112.5
Notropis volucellus	U C D	43	26	504	60.5	212.3	128.4
Percidae							
Percina caproides	U C D	4	3	400	75.0	168.0	126.0
Poecilidae							
Gambusia geiseri	C	7	0				
Salmonidae							
Oncorhynchus mykiss	U	1	0				

the family Cyprinidae was higher than the highest mean intensity that they reported (119-616 as opposed to 2-58). The same was also true for the one species of characid that we collected, however it was only slightly higher.

An additional 71 fish were collected from the Comal River. Two species, *Ameiurus natalis* and *Ictalurus punctatus*, were collected here that were not collected in the

Guadalupe River. *Ictalurus punctatus* was not infected but *A. natalis* was lightly infected (176 cysts). The ranges of prevalence, mean intensity and relative abundance were 0-100 %, 8-800, and 0-800 respectively (Table 2) which were relatively close to that observed in the fishes from the Guadalupe River.

We did not attempt to compare mean intensities by month or by site to determine whether there were any seasonal patterns of infection. It was not possible to make these types of comparisons because although we collected a total of 24 species, no single species was present at all sites during all months and because sample sizes were never the same. Since it is clear that there are differences in susceptibility of various fish species to *C. formosanus* (McDermott 2000; Scholz and Salgado-Maldonado 2000), pooling the data would have likely yielded unreliable results. However, we did consistently find a higher prevalence for each of the four sampling periods at the confluence (mean 32.8 % for all sampling periods) than was found at either the upstream, downstream, or Comal sites (means of 18.4 %, 23.3 %, and 7.2 % respectively). We attributed the elevated rate of infection to downstream drift of cercariae that had been released from infected snails upstream in the Comal River. It is likely that this would have also been observed at the Comal site if sample sizes had been larger. Our use of only seines as well as deep, swift water prevented collecting high numbers of fish from the Comal site.

Metacercarial Development

Live metacercariae were observed when fresh specimens were examined during May and August 2001. In the May sample, cysts from three species of fish that had been stored in an icy-slush in excess of 24 hours, began to actively move upon warming from

Table 2.—Highest observed intensity (HI), prevalence (P;%), mean intensity (MI), and relative density (RD) of *Centrocestus formosanus* for each species of fish collected from the Comal River, Comal County, Texas during 2001-2002.

Family	Species	Number Collected	Number Infected	HI	P	MI	RD
Centrarchidae	*Ambloplites rupestris*	6	2	160	33.3	112.0	37.3
	Lepomis macrochirus	3	1	40	33.3	40.0	13.3
	Lepomis punctatus	14	0				
	Lepomis spp.	15	2	24	13.3	16.0	2.1
	Micropterus salmoides	8	4	120	50.0	66.0	33.0
Cichlidae	*Cichlasoma cyanoguttatum*	13	0				
	Tilapia aurea	1	0				
Cyprinidae	*Dionda episcopa* [a]	2	2	800	100.0	800.0	800.0
Ictaluridae	*Ameiurus natalis*	1	1	176	100.0	176.0	176.0
	Ictalurus punctatus	1	0				
Poecilidae	*Gambusia geiseri*	7	1	8	14.3	8.0	1.1

[a]Counting in excess of 100 cysts/arch impossible due to excessive mucus build-up.

the microscope light. In all collection periods, both mature cysts with well developed X-shaped excretory glands, as well as immature cysts that still retained eyespots, were observed on the same fish. Balasuriya (1988) found that the presence of various developmental stages of metacercarial cysts in the same fish indicates continuous infection. Mature cysts were observed on eight species: *Astyanax mexicanus, C. venusta, Dionda episcopa, N. amabilis, N. volucellus, Lepomis macrochirus, Micropterus dolomieu, and M. salmoides*. Cysts were only present in small specimens (< 20 cm) of both *M. dolomieu* and *M. salmoides* but not all small individuals were infected.

Snails

A pilot survey for *M. tuberculata* had been conducted in both rivers one month prior to the initiation of this study. Only *M. tuberculata* < 15 mm were collected at the Comal site. Although no attempt was made at that time to quantify their abundance, they were numerous. All snails examined were found to be uninfected. However no snails were

collected at that site thereafter, and there were no indications as to why they had disappeared. Dead *M. tuberculata* shells of varying sizes were collected from the Comal, the confluence, and the downstream sites but no live snails were ever collected. No *M. tuberculata* shells were collected at the upstream site.

All of the empty shells that were recovered from the confluence and downstream sites were all presumed to have washed down from upstream in the Comal River. All of these shells, as well as those from the Comal site, were obviously old because they were badly worn and packed with mud. The furthermost downstream record of this snail living in the Comal River is from Mitchell et al. (2000) at their Garden Street site. Their Garden Street site overlapped this study's Comal site. *Melanoides tuberculata* was collected by Mitchell et al. (2000) each of the 12 times snails were sampled at the site, and none were infected. They did however report infected fountain darters at this site on all sampling events.

Cage Studies

Survival of fish was generally low (mean 54.2 %, range 0-93.3 %). No data was collected from cage # 6 on trial B because all fish had died and were too decomposed to examine. Excluding cage #1 in all three trials, 100 % prevalence was observed in all but three cages; cage # 6, trial A (50 %) and cages # 4 and # 5, trial C (88 % and 93 %). No fish became infected at the upstream site (cage # 1) while fish in or downstream of the Comal became infected. The range of mean intensity of metacercariae in caged fish was

5-39 and generally decreased as cage distance downstream from the Comal site increased (Figure 2).

Since no *M. tuberculata* were collected after the pilot survey, it is suspected that cercariae were being released upstream in the Comal River and drifting downstream. We were surprised to find that all three of the remaining fish in cage # 7 (trial C), the farthest downstream cage, became infected. We had supposed that only a few, if any, fish in this cage would become infected because in trial A, only 3 of 6 fish farther upstream (cage # 6) had only been lightly infected.

None of the cysts that were examined on caged fish had matured enough to loose their characteristic eyespots. The three fish from cage # 7 that had been transported back alive and maintained in the raceway for an additional 16 d, had metacercariae that had developed an easily distinguishable X-shaped excretory gland. These three fish were examined fresh and, just as with the wild infected fish that were examined fresh, the cysts were alive and actively moving.

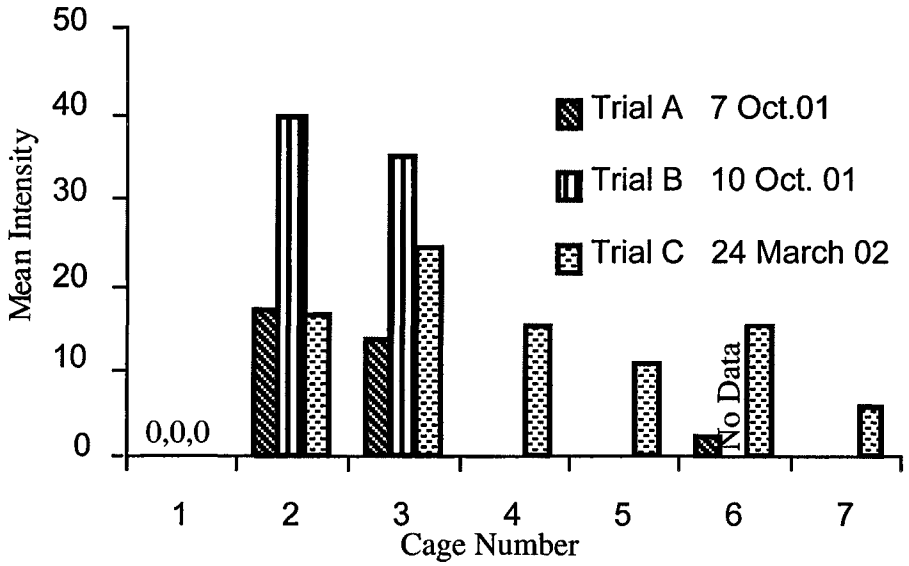

Figure 2. Mean intensities of *Centrocestus formosanus* in *Cyprinella venusta* from three (trial A) and five day (trials B and C) fish cage trials conducted on the Guadalupe River, Comal County, Texas in October 2001 and March 2002.

CONCLUSION

It is evident that some of the fishes in this stretch of the Guadalupe River are being infected by *C. formosanus*. We believe however that the source of infection is from cercariae drifting downstream from the Comal River rather than from an infected population of *M. tuberculata* in the Guadalupe River. There were several indications that the source of infection was in the Comal River, the most significant being a lack of living *M. tuberculata* in our surveys.

The downstream extent of infected snails in the Comal River is unknown, and without an extensive survey, it is impossible to know how far these cercariae would have drifted. Mitchell et al. (2000) did report infected snails as far downstream as Elizabeth Street, located approximately 335 m upstream from this study's Comal site. We estimated, using stream flow velocities from Crowe and Sharp (1997), that it would take these cercariae approximately 6 h to drift that distance. Lo and Lee (1996) reported that cercaria of *C. formosanus* could live in the water column up to 160 h (6.7 d) at 15 °C and that no cercaria died in less than 50 h (2.1 d) between 15-25 °C. Given that the Comal Springs is a constant 23 °C (U.S. Fish and Wildlife Service 1995), it is entirely possible for cercariae to drift that far and still be in adequate condition to be infective. A higher prevalence of infection at the confluence as well as the decrease in infection in caged fish downstream also indicates that the Comal River was the source of infection.

The effects this parasite may have on this fish assemblage is uncertain because there are very few data on pathogenicity of these metacercaria on fish hosts (McDermott 2000; Scholz and Salgado-Maldonado 2000). Although the intensities and prevalences that

were observed in this study seem to be consistent with the ranges that other researchers have observed on various species, it cannot be assumed that the parasite's effects on these fish will be negligible. The most pronounced effect this parasite will likely have on these fish is during times of extreme environmental conditions, such as extremely low flow when temperatures often rise and dissolved oxygen levels drop. Multiple researchers have reported elevated mortality rates of infected fish when exposed to additional stress such as capture and handling (Balasuriya 1988; Mitchell et al. 2000; Scholz and Salgado-Maldonado 2000). We suppose that added environmental stress could possibly have the same effect.

Infected fish in the Guadalupe River serve to increase the possibility of spread of this parasite by increasing the possibility of infecting wading birds that feed on fish. Although no official attempt to quantify possible definitive hosts on the Guadalupe River was made, it should be noted that Great Blue Herons *Ardea herodius*, Yellow-crowned Night-Herons *Nyctanossa violacea*, and Double-crested Cormorants *Phalocrocorax auritus* were observed on several occasions feeding in the river. As to whether or not the metacercaria that we observed in infected fish could have infected a definitive host is unknown. We suspect that the species we collected with mature cysts could, because, although the examination of cysts went no farther than gross examination, it was evident on two occasions that mature metacercariae were alive and presumably healthy.

Whether or not *C. formosanus* will have any far reaching effects on the fish assemblage in the Guadalupe River below the confluence is uncertain. It is clear that more research on the tolerances of these species to this parasite under various ecological conditions, as well as continued monitoring of infection levels is needed in order to

obtain a better understanding of what effects this parasite will have on this fish assemblage.

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