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Metacercarial Distribution of *Centrocestus formosanus* among Fish Hosts in the Guadalupe River Drainage of Texas

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ARTICLE

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Abstract
We examined the gills of wild fish collected from central Texas for *Centrocestus formosanus* metacercariae to determine whether this temperature-restricted parasite had invaded the thermally dynamic Guadalupe River via an introduced population in its thermally stable tributary, the Comal River. We collected fish from three sites in the Guadalupe River near its confluence with the Comal River (upstream, at, and downstream) and one site in the Comal River. *Centrocestus formosanus* infected 14 of the 25 species examined (56.0%) and 171 of the individual fish (27.1%). Several of the infected fish represent new host records for the parasite, and two are listed as species of special concern by the state of Texas. Mean metacercarial intensities varied from 8 to 616 among species, and the highest recorded intensity was greater than 800 in two Guadalupe roundnose minnow *Dionda nigrotaeniata*. Among the 24 species examined from the Guadalupe River, 11 (45.8%) were infected with *C. formosanus*. Thorough surveys at the study sites yielded no living specimens of the first obligate intermediate snail host (red-rim melania *Melanoides tuberculatus*), which must be present to perpetuate the parasite. Thus, the infections were probably due to drifting cercariae that had been shed into the water column upstream of the study area in the Comal River. We therefore investigated spatial patterns in cercarial acquisition using caged fish to determine whether drifting cercariae were present in the water column at the study sites. Of 57 uninfected blacktail shiners *Cyprinella venusta* exposed to Guadalupe River water downstream from and at the confluence, 52 (91.2%) became infected with *C. formosanus* metacercariae at a mean rate of 4 metacercariae/d. This finding extends the known geographic range of this invasive exotic parasite and is the first report of the life cycle being advanced in the fish assemblage of a thermally variable temperate stream in the USA.

The heterophyid trematode *Centrocestus formosanus* (Nishigori 1924) is an exotic parasite native to Asia that has been introduced into warmwater systems around the world. It is a digenean that requires three separate hosts in order to complete its complex lifecycle (for full review see Scholz and Salgado-Maldonado 2000; Mitchell et al. 2005). It utilizes the gills of numerous fishes as second intermediate hosts (Chen 1942; Blazer and Gratzek 1985; Salgado-Maldonado et al. 1995; Scholz and Salgado-Maldonado 2000; Mitchell et al. 2002; Mitchell et al. 2005) and locally employs the green heron *Butorides virescens* as the definitive host (Kuhlman 2007). However, it has also been reported to use many other avian and mammalian definitive hosts as well (Chen 1942; Yamaguti 1975; Balasuriya 1988). This parasite has an innate dependence on the operculate thiarid snail, red-rim melania *Melanoides tuberculatus*, which it utilizes as its first intermediate host (Salgado-Maldonado et al. 1995). In North America, this snail is geographically restricted by water temperature (Mitchell and Brandt 2005) and thus has restricted the spread of the parasite. Both the parasite and snail are now firmly established in the upper and middle reaches of the Comal

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River, Texas, where the parasite has negatively impacted the local fish fauna (Mitchell et al. 2000).

Although *C. formosanus* appears strictly dependent on the red-rim melanias in North America, host specificity at the second intermediate fish host level is more relaxed. Metacercariae of the parasite have been reported in many species of fish (Nishigori 1924; Vazquez-Colet and Africa 1938, 1939, 1940; Chen 1948; Mitchell et al. 2000; Mitchell et al. 2002). Some species develop serious gill pathologies, including edema, hemorrhaging, loss of respiratory epithelium, fusion of primary lamellae, destruction of secondary lamellae, and hyperplastic distortion of gill cartilage. Combinations of these symptoms have been shown to severely weaken and even kill heavily infected fish (Blazer and Gratzek 1985; Vélez-Hernández et al. 1998; Alcaraz et al. 1999; Mitchell et al. 2000, 2002, 2005; McDonald et al. 2006; McDonald et al. 2007), and have reportedly caused mass mortalities (Yanohara and Kagei 1983; Tung et al. 1989; Mitchell and Brandt 2009).

While this parasite has been reported to utilize two other snail hosts worldwide, these snails are not found in the continental United States (Mitchell et al. 2005), and to date the geographic spread of the parasite has been restricted to the geographic range of the red-rim melania (Scholz and Salgado-Maldonado 2000; Mitchell et al. 2005; Mitchell et al. 2007). Although the snail is otherwise very hardy and is known for surprising resistance to long periods of desiccation (Dudgeon 1982), low dissolved oxygen levels (Neck 1985), and extreme salinity (Roessler et al. 1977), it is apparently susceptible to low (<18°C) water temperatures. In the USA, the snail inhabits water bodies with seasonal temperatures ranging from 18–32°C (Mitchell and Brandt 2005) and has been reported from at least 15 states (Rader et al. 2003; Mitchell et al. 2005; Mitchell et al. 2007; Tolley-Jordan and Owen 2008). The snail was first reported in Texas in 1964 (Murray 1964) and has since formed dense populations in the Comal River (Cauble 1998; Mitchell et al. 2000) in part because the thermally stable springs afford an optimal water temperature year around (USFWS 1995; Crowe and Sharp 1997).

The Comal River is formed by the issuance of water from the Edwards Aquifer through Comal Springs, the largest spring system in Texas (Crowe and Sharp 1997). Flow is very stable (USFWS 1996); consequently, water temperature varies only slightly from 23°C year-round (Crowe and Sharp 1997), which is well within the natural and experimentally established thermal limits of the red-rim melania (Mitchell and Brandt 2005; Mitchell et al. 2007). As a result, dense populations of the snail have been reported from the headsprings (Cauble 1998) and downstream into the middle reaches of the river (Mitchell et al. 2000). It is likely that snail populations probably exist throughout the lower reaches as well, but this has not been documented.

By comparison, the water temperature of the Guadalupe River above its confluence with the Comal River is much colder and is influenced by discharge from Canyon Reservoir approximately 40 km upstream. The overall effect of the hypolimnetic release through the dam has been to stabilize and lower water temperature (Hannan and Young 1974; Young et al. 1976; Edwards 1978), which has been sufficient to cause a reduction of diversity in the benthic macroinvertebrate and fish communities downstream (Young et al. 1976; Edwards 1978). As the river traverses toward the confluence, reservoir influence subsides and its temperature regime once again begins to follow ambient air temperature (Groeger and Bass 2005), which has been sufficient to prevent snail colonization in other local area streams. Whether due to atmospheric or dam influence, cold water temperatures probably prevent the snail from becoming established in the Guadalupe River upstream from the confluence with the Comal River, ultimately preventing establishment of the parasite.

Whether or not the snail and parasite can become established downstream from the confluence is uncertain. For a 10-year period prior to this study, the Comal River contributed, on average, approximately half of the volume of water to the Guadalupe River below the confluence (data from U.S. Geological Survey gauge station 08168500; Crowe and Sharp 1997). This high contribution of constant-temperature water certainly has a stabilizing effect on the temperature of the main-stem segment downstream, acting to mitigate seasonal temperature extremes. This effect might periodically be pronounced because the discharge rate of the Guadalupe River is highly variable (Groeger and Bass 2005). At times of low discharge from Canyon Reservoir dam, the volume of water in the main stem downstream from the confluence is dominated by discharge from the Comal River. The water temperature may therefore be more stable, warmer in winter months, and subsequently suitable for snail colonization.

Because the Comal River at times contributes such a high volume of thermally constant water to the Guadalupe River, we hypothesized that its stabilizing effects on the temperature regime may mitigate temperatures downstream of their confluence enough that the snail could become established there and lead to metacercarial infections in the local fish assemblage. The objectives of this study were to survey the lower Comal and Guadalupe rivers adjacent to their confluence to (1) determine if red-rim melanias had became established there, (2) determine if infections with *C. formosanus* metacercariae occur within the local fish assemblage, and (3) characterize intensity and abundance of any metacercarial infections. At the onset of field surveys, we immediately found evidence suggesting that cercariae may have been drifting downstream in the water column after having been released from snails in the upper and middle reaches of the Comal River. We therefore added an additional objective, to (4) experimentally determine if viable, infective cercariae were present in the water column in the study area.

METHODS

A total of seven sites (Figure 1) were utilized in this study, including one in the Comal River (CR site) approximately 810 m upstream from its confluence with the Guadalupe River. The six

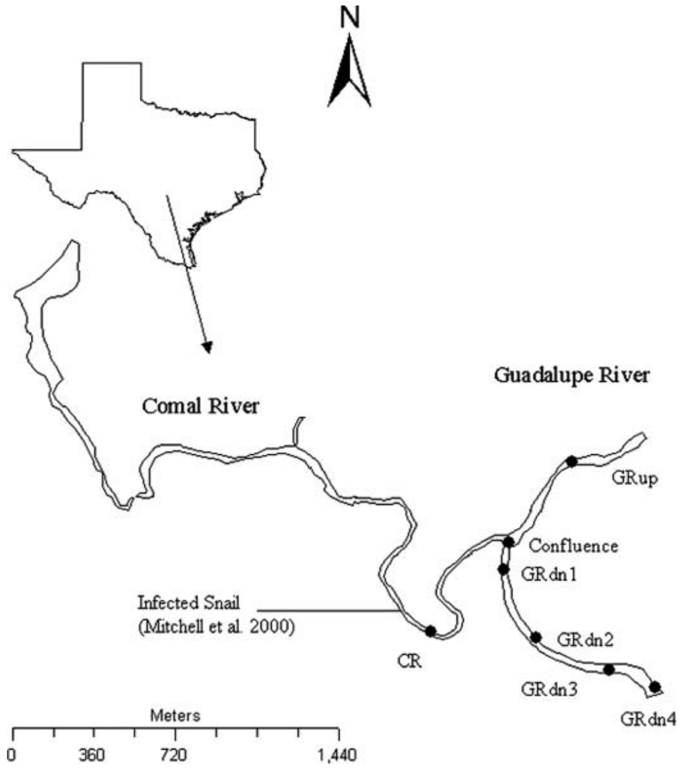

FIGURE 1. Locations of the sites of snail surveys, fish collections, and caged fish from the Comal and Guadalupe rivers, Texas, as well as the most-downstream documented site of infected snails in the Comal River from Mitchell et al. (2000).

remaining sites were in the Guadalupe River. One site was 560 m upstream from the confluence (GRup), another at the confluence (Confluence), and four at increasing distances downstream from the confluence (GRdn1 at 70 m, GRdn2 at 420 m, GRdn3 at 720 m, and GRdn4 at 1,100 m). The GRup site was purposefully selected upstream from a riffle in the river, which acted as a physical barrier to retrograde circulation patterns caused by the confluence of the two rivers. This was done to safeguard against detection of infections caused by drifting cercariae originating in the Comal River and carried upstream by retrograde circulations rather than those originating from snails residing at that study site.

Fish and snail surveys.—Wild fish were collected from four sites (CR site, GRup, Confluence, and GRdn3) using electrofishing, seines, angling, and gill nets in April and August 2001 and February and March 2002. Most fish were preserved in a 10% solution of formalin prior to examination, except for a few fish from the 2001 collections that were examined fresh. Fish were identified to species following Hubbs et al. (1991, 2008), and the left anterior gill arch was removed and examined with a dissecting microscope to identify and enumerate all metacercariae, both mature and immature. Gill examinations and metacercarial identifications followed that of Mitchell et al. (2005). Selected cysts were studied to determine the stage of development. The enclosed metacercaria was rated as "mature" only if an X-shaped excretory vesicle was visible within the cyst (Chen 1942, 1948; Yamaguti 1975). All others were considered immature.

Metacercarial intensity estimates for each fish were based on the counts of metacercarial cysts on the left anterior gill arch only. Madhavi (1986) reported that the metacercariae of *C. formosanus* infecting blue panchax *Aplocheilus panchax* are distributed approximately equally between the fish's left and right sides, and that the right and left anterior gill arches combined contained 26.1% (or 13.05% per arch) of the fish's total parasite load. We therefore counted a single anterior gill arch and then multiplied the count by eight for a total intensity estimate (Shaw et al. 2005). Parasite population parameters were estimated following Bush et al. (1997). Prevalence was defined as the percentage of a specified group of hosts infected with one or more parasites. Intensity was the number of parasites in a single infected host. Mean intensity was calculated as the average number of parasites per infected host in a specified group of hosts, excluding uninfected individuals.

The four fish collection sites were surveyed for snails approximately 1 week following each fish collection. All available habitat types were sampled by hand and by scraping the substrate with a D-frame dip net. In areas where the substrate was soft, a sample of substrate was washed through a fine mesh sieve to isolate snails.

Cercarial drift study.—Containment cages to house fish were designed with an approximate volume of 0.025 m^3 and were constructed from 6.4-mm galvanized wire hardware cloth. The blacktail shiner *Cyprinella venusta* was selected as the experimental host species because prior observations by the authors had shown susceptibility to infection. All experimental fish were seined from the Blanco River, Texas, an intermittent stream in the Guadalupe River drainage from which *C. formosanus* has never been reported. Subsamples of approximately 10% of the experimental fish were screened for infection prior to use (all fish were negative for the parasite). Fish ranged from 50 to 90 mm in total length (TL), and were placed in cages at densities of 10–15 fish per cage. Three trials were conducted for the following length of time: trial 1–3 d, trial 2–5 d, and trial 3–5 d. Trials 1 and 2 each involved placing one cage at each of the four fish collection sites. In trial 3, three additional sites were added downstream from the confluence, resulting in one cage at each of the seven sites (Figure 1). At the end of each trial, the fish were retrieved from cages, placed on ice, and transported to the laboratory. All four gill arches from the fish's left side were removed and examined for metacercariae as previously described.

RESULTS

Fish and Snail Surveys

Centrocestus formosanus was the only species of trematode found infecting the gills of the fish in this study (for complete review of identification, see Mitchell et al. 2005). We examined

TABLE 1. Number (*N*) and range of total length (TL) for each species of fish collected, and prevalence (P) and mean intensity (MI) of metacercarial infections of *C. formosanus* from the Comal and Guadalupe rivers, Texas.

			Guadalupe River			Comal River		
Family	Species	TL (cm)	*N*	P	MI	*N*	P	MI
Cyprinidae	Central stoneroller *Campostoma anomalum*	7.2–24.0	15	0				
	Red shiner *Cyprinella lutrensis*[a]	6.0	1	100.0	616.0[b]			
	Blacktail shiner *C. venusta*[a]	2.7–9.5	73	58.9	119.1			
	Guadalupe roundnose minnow *Dionda nigrotaeniata*[a,c]	5.2–6.5	1	100.0	120.0[b]	2	100.0	800.0
	Golden shiner *Notemigonus crysoleucas*	8.0–8.1	2	0				
	Texas shiner *Notropis amabilis*	3.0–6.9	99	62.6	179.6			
	Mimic shiner *N. vollucellus*[a]	3.2–6.4	44	61.4	204.7			
Catostomidae	Gray redhorse *Moxostoma congestum*	21.3[d]	16	0				
Characidae	Mexican tetra *Astyanax mexicanus*	5.6–11.7	8	75.0	46.7			
Ictaluridae	Yellow bullhead *Ameiurus natalis*	12.8				1	100.0	176.0[b]
Salmonidae	Rainbow trout *Oncorhynchus mykiss*	23.5	1	0				
Poeciliidae	Largespring gambusia *Gambusia geiseri*[a]	2.7–4.2	8	0		7	14.3	8.0
	Gambusia spp.	0.34–0.71	3	0				
Centrarchidae	Rock bass *Ambloplites rupestris*[a]	4.0–7.5	3	0		6	33.3	112.0
	Redbreast sunfish *Lepomis auritus*	5.2–21.5	90	0				
	Green sunfish *L. cyanellus*	6.4–13.0	9	0				
	Bluegill *L. macrochirus*[a]	3.6–16.6	16	25.0	64.0	3	33.3	16.0
	Longear sunfish *L. megalotis*	5.5–13.1	37	0				
	Redspotted sunfish *L. miniatus*	5.0–14.8	54	0		14	0	
	Lepomis spp.	2.9–7.3				15	13.3	16.0
	Smallmouth bass *Micropterus dolomieu*[a]	7.8–15.3	11	36.4	60.0			
	Largemouth bass *M. salmoides*	3.8–48.5	32	18.8	50.7	9	44.4	66.0
	Guadalupe bass *M. treculii*[a]	5.6–10.3	6	16.7	160.0			
Percidae	Orangethroat darter *Etheostoma spectabile*	4.5–4.7	3	0				
	Texas logperch *Percina carbonaria*[a]	6.6–13.0	4	75.0	168.0			
Cichlidae	Rio Grande cichlid *Cichlasoma cyanoguttatum*	3.3–20.1	15	0		13	0	
	Blue tilapia *Oreochromis aureus*	4.1–7.0	8	0		1	0	

[a]Represents the first record of infection by *C. formosanus* for the species.
[b]Intensity rather than mean intensity because only a single fish was examined.
[c]Counting in excess of 100 cysts impossible because of excessive mucus buildup.
[d]Only one fish measured.

a total of 630 fish, comprising 25 species and nine families, including three juvenile *Gambusia* and 15 *Lepomis* individuals that could not be conclusively identified and are not treated as distinct species (Table 1). Overall, 171 of the 630 individuals (27.1%) and 14 of the 25 species (56.0%) were infected with *C. formosanus*. Among the 14 positive species from the two rivers, prevalence per species varied from 6.7% to 100.0% (median = 60.1%), and mean intensity per species varied from 8 to 616 (mean = 181). Additionally, the preponderance of all metacercarial cysts (90.6%) collected was concentrated in the cyprinids, which constituted only 37.6% of the total number of fish collected. There were seven species of cyprinids collected, of which five were infected and each had a high prevalence (>58.0%) of metacercarial infections. We collected 237 individuals, and well over half (57.4%) were infected.

Among the fish from the Comal River site, 13 of the 71 individuals (18.3%) and six of the nine species (66.7%) were infected. Prevalence among the six positive species varied from 14.3% to 100.0% (median = 38.9%), and mean intensity per species varied from 8 to 800 (mean = 200); the highest intensity, greater than 800, was observed in Guadalupe roundnose minnow.

Among the fish from the Guadalupe River sites, 158 of the 559 fish (28.3%) and 11 of the 24 species (45.8%) were infected. Prevalence per species varied from 17.0% to 100.0% (median = 61.4%), and the mean intensity varied among infected species from 47 to 616 (mean = 163). The highest observed intensity of 656 was in a Texas shiner. Infected fish were collected from multiple species and individuals at all three of the Guadalupe River sites. Prevalence among species was highest at GRdn3,

TABLE 2. Prevalence (P), mean intensity (MI), and mean acquisition rate (AR; ranges in parentheses) of *C. formosanus* on the gills of blacktail shiners placed in cages at various distances (negative numbers are distances upstream) from the confluence of the Comal and Guadalupe rivers, Texas. The number (*N*) of live fish at the end of the trial period is also listed.

		Trial 1[a]				Trial 2[a]				Trial 3[b]			
Site	Distance (m)	*N*	P	MI	AR	*N*	P	MI	AR	*N*	P	MI	AR
Grup	–560	8	0	0	0	13	0	0	0	6	0	0	0
CR	–810	6	100	17 (10–40)	6 (4–13)	8	100	39 (32–48)	8 (6–10)	7	100	17 (6–22)	3 (1–5)
Confluence	0	5	100	14 (6–24)	2 (2–8)	7	100	35 (26–50)	7 (5–10)	4	100	24 (8–50)	5 (2–10)
GRdn1	70									8	88	17 (0–30)	4 (0–5)
GRdn2	420									14	93	11 (0–22)	2 (0–4)
GRdn3	720	6	50	5 (0–8)	2 (0–3)	0[c]				10	100	15 (6–28)	3 (1–6)
GRdn4	1,100									3	100	11 (4–24)	2 (1–5)

[a]Three-day exposure period.
[b]Five-day exposure period.
[c]All fish had died by the end of the trial.

where 7 of the 14 species were infected (50.0%), and lowest at the Confluence site, where 7 of the 19 species were infected (36.8%). At the site upstream from the confluence (GRup), 8 of the 20 species were infected (40.0%).

Mature cysts with X-shaped excretory vesicles were observed in blacktail shiner, Texas shiner, mimic shiner, Mexican tetra, bluegill, and largemouth bass. This indicates that these species have the potential to advance the life cycle of the parasite. Our snail surveys yielded no living red-rim melanias at any of the sites. Shells of dead individuals were collected from all sites and sampling periods, except at the GRup site upstream from the confluence where no shells were found.

Cercarial Drift Study

Fish mortality was observed in cages at all sites and trials. In trial 2, site GRdn3 had no surviving fish at the end of the trial period and was thus excluded from the calculations. No metacercariae were found in fish during any trial at the GRup site (Table 2) indicating that there were no viably infective cercariae in the water column in the Guadalupe River upstream from the confluence. This site too was excluded from subsequent calculations. At all remaining stations and trials (those exposed to Comal River water), total prevalence of *C. formosanus* metacercariae among the 78 caged blacktail shiners combined was 94%. The prevalence of infection in fish was high (>87.0%) at all stations (CR, Confluence, and GRdn1–GRdn4), except for GRdn3 in trial 1 (50.0%). Mean intensity per cage ranged from 5 to 40 and prevalence from 50.0% to 100.0%. When examined by river kilometer, mean intensity dropped sharply at the confluence as the Comal River water was diluted by water from the Guadalupe River, but then remained relatively constant in the reach downstream from the confluence (Figure 2). Mean intensity of *C. formosanus* in the caged fish in the CR site and Confluence site were similar within trials but varied substantially between trials. The sequentially performed trial 1 and 2 varied from one another more than trial 1 differed from trial 3. The rate at which fish acquired metacercerciae (acquisition rate) was, however, relatively consistent between trials as well as longitudinally downstream (Figure 2). The overall mean acquisition rate was 4 metacercariae/d. Some individual acquisition rates were extremely high, one fish acquiring parasites at a rate of 13/d (trial 1, Comal site).

DISCUSSION

The results of this study confirm that *C. formosanus* is infecting the fish community in the lower Comal River and the segment of the Guadalupe River adjacent to their confluence. *Centrocestus formosanus* is widely recognized as having low host specificity at the second intermediate fish host level (Yamaguti 1975; Balasuriya 1988; Salgado-Maldonado et al. 1995; Scholz and Salgado-Maldonado 2000; Mitchell et al. 2005), and where it becomes established, a substantial portion of the fish community is infected (Mitchell et al. 2000; Salgado-Maldonado

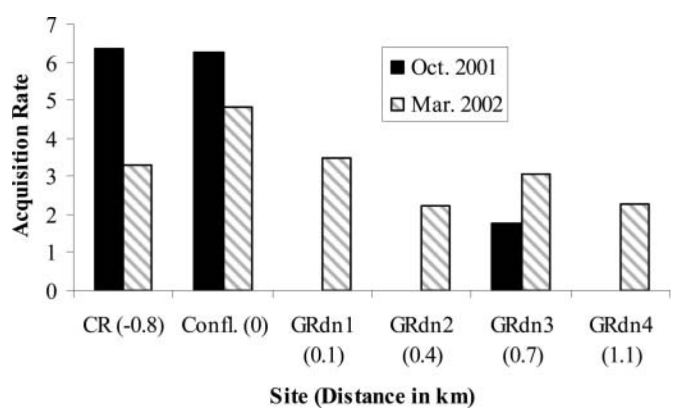

FIGURE 2. Mean acquisition rates (metacercariae/d) in trials 1 and 2 combined (October 2001) and trial 3 (March 2002) of *C. formosanus* in blacktail shiners placed in containment cages at varying distances (m) from the confluence of the Comal and Guadalupe rivers, Texas. Negative distances represent linear distances upstream.

et al. 2004a, 2004b). Our results corroborate this observation as we found *C. formosanus* cysts on 56.0% of the species we collected. It is difficult to assess from the literature if this proportion is typical of infection success of this parasite in newly invaded systems because seldom are uninfected species reported. However, based on our observation and data gleaned from various sources, including other ecological surveys involving *C. formosanus* (Salgado-Maldonado et al. 2004a, 2004b), it appears that about half of the fish species do become infected when exposed to cercariae. However, only a small subset of the fish species known to occur near the confluence (Hubbs et al. 1953; Edwards 1978) was collected in our study. Consequently, the actual number of species infected with *C. formosanus* in the confluence community may, in fact, be higher than our estimate.

This high prevalence of infection among species is particularly concerning because of the often destructive nature of metacercarial infections (Blazer and Gratzek 1985), coupled with a high degree of endemism and imperilment in the freshwater fish fauna across Texas (Hubbs et al. 2008). Hubbs et al. (2008) reports that of the 259 extant fish species in Texas, over 40.0% are already of conservation concern and in need of protection. Any additional expansion of the geographic range of this parasite could further impact these and other species such as has been reported for the endangered fountain darter *Etheostoma fonticola* (Mitchell et al. 2000). Our study also documents several new fish taxa as hosts for the parasite (Table 1). Included among the infected species are four Texas endemics: Guadalupe roundnose minnow, Guadalupe bass, largespring gambusia, and Texas logperch. The first two are listed as species of special concern by the state of Texas (Hubbs et al. 2008) because of, among other things, range restrictions and habitat loss. Whether or not infections will have adverse effects on these species is uncertain and requires further study. Some adverse effects seem likely, though, especially with the high parasite intensities observed such as in *D. nigrotaeniata*.

Despite the occurrence of dense populations of red-rim melanias upstream in the Comal River (Mitchell et al. 2002), which presumably provide ample opportunity for recruitment, surveys were negative for the snails at the Guadalupe River sites (GRup, Confluence, and GRdn3) as well as at our site in the lower Comal River (CR Site), where the optimal temperature is maintained throughout the year (Crowe and Sharp 1997). Why snails were absent below the confluence, and especially at the Comal River site, is unclear, but the temperature regime appears intermittently conducive to their survival. Mean daily temperatures downstream from the confluence for an 8-year period surrounding our study (A. W. Groeger, Texas State University, personal communication) had intervals of up to 8 months when water temperature remained suitable for red-rim melania survival (above 18°C: Mitchell and Brandt 2005; Mitchell et al. 2007). During a pilot survey just prior to the initiation of this study, immature snails were collected in the Comal River at the confluence but were never detected since. However, a recent survey (2009) conducted by one of the authors (but independent of our study) did return numerous red-rim melanias that were actively shedding cercariae of the parasite in the Guadalupe River downstream from the confluence (D. G. Huffman, unpublished). These sporadic accounts suggest that there is intermittency in snail presence in the study area as temperatures allow.

By all previous accounts, the geographic distribution of *C. formosanus* in North America is limited to the geographic distribution of its first intermediate snail host (Scholz and Salgado-Maldonado 2000; Mitchell et al. 2005, 2007). We speculated that the metacercariae found in wild fish collected from the Guadalupe River must have been the result of one, or a combination of, the following causes: (1) infected snails that we failed to detect at or between study sites; (2) fish movement, where fish acquired metacercarial infections in the upper and middle reaches of the Comal River, and subsequently moved downstream to our study sites; or (3) cercariae drifting several kilometers downstream into the Guadalupe River after being shed from snails in the upper and middle reaches of the Comal River, and subsequently infecting fish.

We recognize the possibility that undetected snails could have contributed to the infections we observed but discount this as the sole source or even a major contributor because of the physical size of our sample sites and the rigor with which our surveys were conducted. We did, however, find dead red-rim melania shells at the CR, Confluence, and GRdn3 sites but none in the Guadalupe River upstream from the confluence (GRup site), indicating that the shells had drifted, either while still alive or after they had died, downstream from the upper Comal River into our sample sites. If some living snails had drifted downstream and survived long enough to shed cercariae, we would probably have discovered them during our surveys, but none were found.

It has been shown that some fish utilize spring-fed tributaries as a thermal refuge during winter months (Peterson and Rabeni 1996). However, Peterson and Rabeni (1996) hypothesized that this is generally limited to fish immediately adjacent to the confluence. Consequently, fish movement would likewise only contribute minimally to the infections we observed. We therefore concluded that most infections must have been acquired locally through actively infective cercariae drifting into the Guadalupe River from inflows of the Comal River. Based on flow rates from Crowe and Sharpe (1997), we calculated that it would take less than 1 h for cercariae to drift the entire length of the study area. It seems very likely that drifting cercariae would be able to infect fish well beyond our most-downstream study site since they can survive free-living in the water column for as long as 130 h at 20°C (Lo and Lee 1996). The fact that caged fish at the site in the Comal River (CR Site) and downstream from the confluence (GRdn1–4) acquired infections also support this rationale. Furthermore, caged fish exposed for as long as 5 d upstream at the GRup site were all negative, indicating that there were no free cercariae in the water column at this site. We did, however, collect 33 infected individuals at that site, suggesting that fish were moving around within the system.

Data from the cercarial drift study strongly indicate a longitudinal decrease in free cercariae in the water column, supporting the idea that most of the cercariae originated in the headwaters of the Comal River. We saw a general decline in cercarial acquisition rates from our most-upstream site in the Comal River to our farthest downstream site in the Guadalupe River. Without the addition of cercariae into the water column from snails along the course of the river, absolute cercarial concentrations probably decrease spatially as a result of natural mortality and loss from forces such as riffle turbulence (Lozano 2005). We also saw a decline in acquisition rates at the confluence, which we attribute to the addition of cercarial-free water from the Guadalupe River acting to dilute cercariae in the water column. Cantu (2003) also reported a general declining trend in acquisition rates of metacercariae in fountain darter in cages placed from the headwaters downstream into the middle reach of the Comal River. He found that caged fish in Landa Lake at the headwaters, where very dense populations of infected snails are known to occur (Mitchell et al. 2000), acquired cercariae at rates as high as 50 cysts/d and saw intensities as high as 1,600 cysts in a single fish. Farther downstream at the Elizabeth Street Bridge, which is just upstream from our Comal site (CS), acquisition rates dropped to 2 cysts/d. The general declining trend in Cantu's (2003) data, combined with the same trend that we saw downstream, indicate that there are decreasing numbers of cercariae in the water column as the distance downstream from the dense populations of infected snails in the headwaters increases.

We hypothesized that the stabilizing effects of the Comal River tributary on the temperature regime of the Guadalupe River main stem allow red-rim melanias to become established below their confluence, leading to infections of *C. formosanus* in the resident-fish community. Our results indicate that the parasite is actively infecting fish in the Guadalupe River main stem even though its first intermediate snail host may only be present intermittently. Nevertheless, as long as infected snails remain in the Comal River headwaters, fish downstream will probably continue to acquire infections. In our study area, the spatial scale of successful cercarial infectivity has been greatly extended from a few meters to at least several kilometers between snail and fish because of rapid downstream transport. While the large-scale geographic distribution of the parasite in North America remains a function of the warmwater requirements of red-rim melanias, localized effects on fish communities may be more encompassing than originally thought. Because cercarial transport by drift extends the parasites infective range beyond the confines of thermally stable water, such as spring runs, fish that may have been previously considered thermally segregated from infected snails may be at risk of infection. In light of this finding, management and conservation efforts concerning this parasite may benefit by expanding beyond the immediate physical range of the snail as more species and individuals may be impacted.

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