J. Parasitol., 98(1), 2012, pp. 000–000

FLUCTUATIONS IN DENSITIES OF THE INVASIVE GILL PARASITE *CENTROCESTUS FORMOSANUS* (TREMATODA: HETEROPHYIDAE) IN THE COMAL RIVER, COMAL COUNTY, TEXAS, U.S.A.

Matthew S. Johnson*, Anne Bolick†, Mara Alexander, David Huffman‡, Ed Oborny§, and Allen Monroe‖
U.S. Fish and Wildlife Service, San Marcos National Fish Hatchery and Technology Center, 500 East McCarty Lane, San Marcos, Texas 78666.
e-mail: mara_alexander@fws.gov

1

ABSTRACT: *Centrocestus formosanus* (Trematoda: Heterophyidae) is an invasive fish parasite in the Comal River, Texas, and is considered a threat to the federally endangered fountain darter, *Etheostoma fonticola*. Monitoring densities of *C. formosanus* cercariae is crucial to determining levels of infection pressure. We sampled 3 sites in the Comal River during 2 sampling periods, the first during 2006–2007, and again during 2009–2010. Two of the sites were located in the upstream reach of Landa Lake, sites HS and LA, and the third site was located downstream of Landa Lake in the old channel of the river. Cercariae densities were highest at the downstream most site (EA), followed by sites LA and HS, during both sampling periods, but a significant decline in cercariae density was observed between the first and second sampling periods. Several abiotic factors were monitored, including total stream discharge, wading discharge, temperature, and dissolved oxygen, but no river-wide trends were observed. Therefore, we speculate that these factors do not adequately explain the observed long-term decline in cercariae density. We propose that the decline is simply a reflection of a typical pattern followed by most invasive species as they gradually become integrated into the local community following an initial explosive growth in population size. Although cercariae densities may be abating, fountain darters in the Comal River are still threatened by the parasite, and conservation efforts must focus on reducing levels of infection pressure from the parasite whenever possible.

Centrocestus formosanus (Nishigori, 1924) is a digenetic trematode that was originally described in Taiwan but has become widely distributed throughout Asia and warm-water areas of the world (Mitchell et al., 2000). Scholz and Zalgado-Maldonado (2000) believe the trematode was likely introduced into Mexico in 1979 but was not confirmed until 1985. The parasite then spread to the United States possibly in the early 1980s (Blazer and Gratzek, 1985; Mitchell et al., 2000, 2002). In 1996 metacercariae of the invasive trematode were observed infecting the gills of the endangered fountain darter, *Etheostoma fonticola* (Jordan and Gilbert, 1886), and several other species of fish in the Comal River in Comal County, Texas (Mitchell et al., 2000). The authors observed considerable gill damage caused by the encystment of up to 1,500 metacercariae per fish.

The definitive host for *C. formosanus* in central Texas appears to be the green heron, *Butorides virescens* (Linnaeus, 1758), in which adult trematodes colonize the colon (Kuhlman, 2007). Other piscivorous bird species in Central Texas, including Yellow-crowned Night Heron (*Nyctanassa violacea*), Belted Kingfisher (*Megaceryle alcyon*), and Cormorant (*Phalacrocoracidae* spp.), have been tested for parasite infestation, but no other definitive hosts have been identified (T. Brandt, pers. comm.) The adult trematodes are released via host feces. The first intermediate host is the red-rimmed melania, *Melanoides tuberculatus* (Müller, 1774) (Mitchell et al., 2005). The snail is infected either by directly consuming trematode eggs or via penetration by free-swimming miracidia that are released from the eggs (Lo and Lee, 1996). Once inside the snail, the miracidium transforms into a sporocyst and asexually produces rediae, which, in turn, produce cercariae (Schell, 1970). Cercariae exit the snail host and infect second intermediate hosts, 1 of which is the fountain darter. Cercariae penetrate the gill filaments, where they cause severe gill lesions and precipitate cartilage hyperplasia, resulting in severe gill lesions that reduce respiratory function (Balasuriya, 1988; Velez-Hernandez et al., 1988; Alcaraz et al., 1999; McDermott, 2000; Mitchell et al., 2000). Once the definitive host consumes the second intermediate host, the life cycle is complete.

Informal observations suggested that the density of *C. formosanus* cercariae in the water column decreases as stream discharge increases (T. Brandt, pers. comm). Upatham (1973) observed that *Schistosoma mansoni* (Sambon, 1907) numbers declined as stream discharge increased. If this same relationship exists between the *C. formosanus* cercariae and discharge in the Comal River, there are concerns that during low flow periods, increased levels of infection pressure would exacerbate the other stresses of low stream discharge on the fountain darter. Therefore, the purpose of the present study was to monitor the abundance of *C. formosanus* cercariae and determine which stream characteristics are driving fluctuations in cercarial abundance in the Comal River.

MATERIALS AND METHODS

Study system

Five kilometers long, the Comal River in New Braunfels, Comal County, Texas, issues from the state's largest spring complex at the edge of the Edwards Plateau region of central Texas (Brune, 1981). The headsprings are now impounded by 2 dams to form Landa Lake, from which the spring water flows into the New Channel and the Old Channel (Fig. 1). These channels converge approximately 2.5 km downstream of Landa Lake and flow another 2.5 km before the Comal River merges with the Guadalupe River (USFWS, 1996).

We completed 2 sampling periods of parasite monitoring at 3 sites for this study. Both collection periods were initiated during droughts. Two sites, Houston Street (HS) and Liberty Avenue (LA), were located in the shallow upstream reach of Landa Lake, at NAD27 coordinates 29.720777N, 98.128097W, and 29.718766N, 98.130305W, respectively. Both of these sites are located within 300 m of 1 of the springs that feed the upper portion of Landa Lake. These sites were characterized by substrates

Received 26 April 2011; revised 15 August 2011; accepted 18 August 2011.

*Texas A&M Institute of Renewable Natural Resources, 2632 Broadway Suite 301S, San Antonio, Texas 78215.
†U.S. Fish and Wildlife Service, California-Nevada Fish Health Center, 24411 Coleman Fish Hatchery Road, Anderson, California 96007.
‡Texas State University, Department of Biology, Aquatic Station, San Marcos, Texas, 78666.
§BIO-WEST, Inc., 1812 Central Commerce Court, Round Rock, Texas 78664.
‖North Carolina Aquarium at Pine Knoll Shores, 1 Roosevelt Blvd., Pine Knoll Shores, North Carolina 28512.

DOI: 10.1645/GE-2841.1

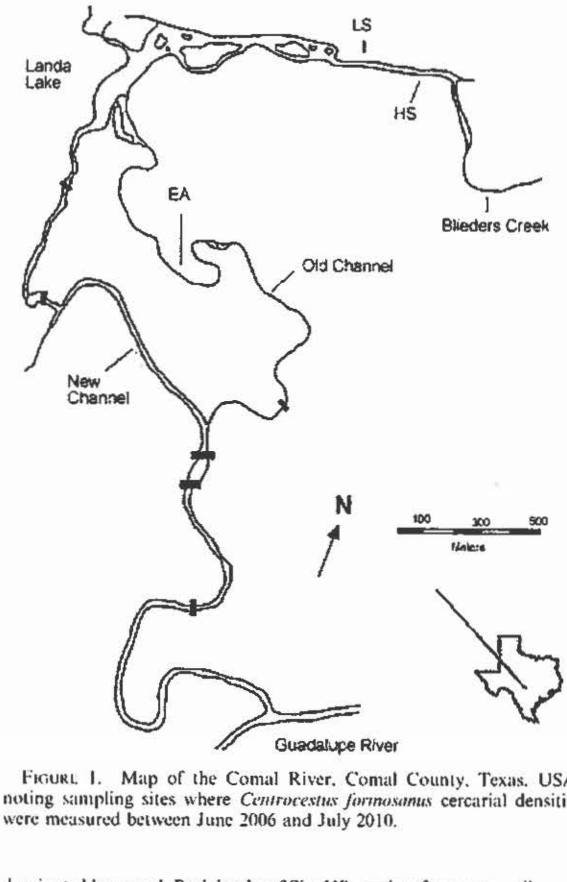

FIGURE 1. Map of the Comal River, Comal County, Texas, USA, noting sampling sites where *Centrocestus formosanus* cercarial densities were measured between June 2006 and July 2010.

dominated by gravel. Both banks of Site HS consist of concrete walkways. One bank of Site LA is also a concrete walkway, while the other bank is lined with large cobble and small boulder material. The invasive elephant-ear (*Colocasia spp.*) inhabits this bank. The third site, Elizabeth Avenue (EA), is located downstream in the Old Channel, approximately 500 m from the spillway of Landa Lake (29.710133N, 98.128703W) (NAD27). Flow in the Old Channel is regulated through a series of gated culverts and is considered 1 of the most stable areas in the river because most moderate flood pulses are directed through the New Channel. Sites HS and EA have been previously studied for the prevalence of the invasive trematode in the endangered fountain darter (Mitchell et al., 2000). Piscivorous birds, including Green Heron, Yellow-crowned Night Heron, Belted Kingfisher, and Cormorant, were observed at times at all sites during the study.

Collection of cercariae

At each of the 3 sites, we established a transect across the river perpendicular to flow and then established 6, equally spaced collection points along each transect. At each of the 6 points, we took 2, 5-L samples of water, 1 approximately 10 cm from the bottom and 1 at 60% depth from the surface. Each water sample was pumped through a flexible acrylic tube (6.4 mm internal diameter) into a 10-L bucket via a battery-operated submersible pump (Attwood aerator pump, Model A500, Lowell, Michigan), which was positioned at the desired depth on an adjustable 1.5-m rod before pumping was initiated. Sites were not sampled in a consistent order. Water samples were generally collected between 8:00 and 13:00, although some sampling events occurred outside of this time frame.

Immediately following collection, we added 5 ml of formalin to each water sample to fix the cercariae. We then filtered the sample using an apparatus described in Theron (1969) and Prentice (1984), but using modifications developed by Cantu (2003). Each sample was passed through 3 successive nylon mesh filters with pore sizes of 220, 86, and 30 µm, respectively (Fisher Scientific, Pittsburgh, Pennsylvania). The 220- and 86-µm filters were used to filter out larger debris. Cercariae freely passed through the 220- and 86-µm pre-filters and collected on the final 30-µm filter. A new 30-µm filter was used for each water sample. After each sample was filtered, we placed the 30-µm filter in a Petri dish and covered it with 3 ml of a 10% formalin solution. We then stained the cercariae on the filters in a 10% Rose Bengal solution. We then took all filters to the laboratory for analysis. Formalin wastewater was collected in 18-L jugs and transported to the laboratory. There all waste water was detoxified using DeToX Formaldehyde neutralizer (Scientific Device Laboratory, Des Plaines, Illinois) (0.05 oz. DeToX neutralizer/L formalin wastewater) and discarded.

Cercariae counts

In the laboratory, we placed each 30-µm filter on a 60 mm × 60 mm grid in a 95-mm diameter Petri dish. Excess Rose Bengal was cleared from the filter by gradually rinsing with tap water. We inspected the entire filter using a dissecting microscope (100×) and counted all *C. formosanus* cercariae present. Another invasive parasite, *Haplorchis pumolio* (Looss, 1896), is also present in lower densities within the Comal River but was not counted for this study. Once we counted all *C. formosanus*, we placed each filter in a 10% NaOH solution to dissolve remaining biological material. We then rinsed each filter and placed it in a 10% bleach solution to remove residual stain. The filters were then dried and available for use during the next sampling event. We calculated the number of cercariae/L by dividing the total number of cercariae counted on a filter by the total volume of the water sample (5 L).

Stream characteristics

Total stream discharge: We obtained total stream discharge for the Comal River using daily data from the United State Geological Survey (USGS) real-time water database (USGS, 2001). We used the Comal River gauge at New Braunfels, Texas, USGS 08169000. The gauge is located approximately 200 m downstream from the confluence of the Old Channel and New Channel (29.4221N, 98.0720W) (NAD27).

Stream wading discharge and temperature: Immediately following cercariae collection, we measured depth and stream velocity at 20 equidistant points along the site transect using methods adapted from Buchanan and Somers (1969). If the depth at a transect point was ≤ 0.76 m, we determined a point mean velocity value by collecting a single velocity measurement at 60% depth from the water surface. If the depth at that transect point was > 0.76 m, we calculated a mean velocity value by averaging 2 discharge measurements that we collected at 20 and 80% depth. We calculated the total wading discharge using the following formula:

$$\sum (d \times w \times v),$$

where d = depth (m), w = width between collection points (m), and v = point mean velocity (cms). We then measured stream temperature and dissolved oxygen at each site using a dissolved oxygen meter (Model 58, YSI, Yellow Springs, Ohio).

Data analysis

Cercariae abundance (*C. formosanus* cercariae/L) at each site was regressed against time to determine if the density of *C. formosanus* cercariae drifting in the water column changed between the 2 sampling periods of this study. We used the Kolmogorov-Smirnov Normality Test to screen cercariae density data for normality and determined that the data were normally distributed [$p(\chi_{5(1),2,106} \geq 6.33) = 0.08$]. Two-way analysis of variation was employed to evaluate differences in cercariae density among sites and between sites, and also to evaluate any interactions between site and sampling period. A Tukey/Kramer post hoc analysis was then used to determine where these significant differences existed, if anywhere. All statistical analyses used an α of 0.01 and were conducted using STATVIEW (version 5.0; SAS Institute Inc., Cary, North Carolina).

RESULTS

Collections

The first sampling period occurred between June 2006 and June 2007, during which samples were collected every 2 wk (Table I). A second period of sampling occurred between July 2009 and July 2010 during which samples were collected every 2 wk for the first 3 mo and then monthly thereafter (Table II). The sampling frequency was reduced to monthly because by October 2009 drought conditions had subsided and regional precipitation had returned to near normal. Additionally, observed total stream discharge also returned to near normal during this time (USGS, 2001).

Cercariae densities

The farthest upstream site (HS) generally had the lowest densities of cercariae of the 3 surveyed sites. During the first sampling period, the cercarial density ranged from 0.45 to 3.63 cercariae/L ($\bar{X} \pm s_{\bar{X}}$: 1.37 ± 0.20; Table I). Cercariae densities collected during the second sampling period at Site HS ranged from 0.08 to 0.87 (0.43 ± 0.07; Table II). During the first sampling period, cercariae densities of samples collected at Site LA ranged from 1.44 to 9.33 (4.65 ± 0.45). Densities of samples collected at Site LA during the second sampling period ranged from 0.08 to 2.65 (1.38 ± 0.17). Site EA, the furthest downstream site in the Comal River, generally had the highest densities of *C. formosanus* cercariae. Cercariae densities at Site EA during the first sampling period ranged from 1.37 to 14.13 (7.06 ± 0.74). Densities of cercariae from Site EA during the second sampling period ranged from 1.82 to 4.23 (2.75 ± 0.19). Simple linear regression confirmed that correlation between cercariae density and date was significant at all 3 sites (HS: $r^2 = 0.239$, $P = 0.0018$; LA: $r^2 = 0.505$, $P < 0.0001$; EA: $r^2 = 0.495$, $P < 0.0001$; Fig. 2).

A 2-way ANOVA found a main effect of site on observed cercariae densities [$P(F_{\alpha(1),2,106} \geq 40.37) < 0.0001$], indicating that significant differences existed in mean cercariae densities observed at each of the 3 sampled sites. There also was a main effect of sampling period [$P(F_{\alpha(1),1,106} \geq 62.22) < 0.0001$]. Finally, there was an interaction between site and period [$P(F_{\alpha(1),2,106} \geq 7.97) <$
$\boxed{3}$ 0.0006] (Table III). Tukey/Kramer multiple comparisons analysis showed that significant differences in mean cercariae density existed among all sites sampled during this study and between the 2 sampling periods. A mean difference of 2.27 existed between Site HS and Site LA, which surpassed a critical difference of 1.04. The mean difference of 4.23 that existed between Site HS and Site EA also exceeded the critical difference of 1.04, making the difference between Sites HS and EA significant. A mean difference of 1.96 existed between Site LA and Site EA. This difference also exceeded the critical difference of 1.04, making this final comparison between sites significant. The mean difference between the 2 sampling periods was 2.28. This difference exceeded the critical difference of 0.72 and showed that a significant difference existed in cercariae density between the 2 sampling periods.

Stream characteristics

Total stream discharge: The total discharge of the Comal River during the first sampling period ranged from 5.78 to 12.49 m^3/sec (7.55 ± 0.19) on days when samples were taken during the first sampling period, and from 4.42 to 11.38 (7.07 ± 0.52) when samples were taken during the second sampling period.

Wading discharge: The wading discharge of Site HS ranged from −0.05 to 0.82 m^3/sec (0.31 ± 0.05) during the first sampling period, and from 0.06 to 0.87 (0.48 ± 0.08) during the second sampling period. The wading discharge of Site LA ranged from −0.17 to 1.20 (0.31 ± 0.05) during the first sampling period, and from 0.02 to 1.27 (0.57 ± 0.11) during the second. Wading discharge of Site EA ranged from 0.97 to 1.62 (1.40 ± 0.03) during the first sampling period, and from 1.16 to 2.73 (1.50 ± 0.11) during the second sampling period.

Temperature: The stream temperature at the 3 sites remained generally constant throughout the first sampling period. The stream temperature at Site HS during the first sampling period ranged from 22.5 to 26.1 C (24.1 ± 0.21) during the first sampling period, and from 23.2 to 25.9 (23.92 ± 0.21) during the second sampling period. The stream temperature at Site LA during the first sampling period ranged from 21.0 to 24.5 (22.79 ± 0.24), and from 22.5 to 25.8 (23.63 ± 0.20) during the second sampling period. The stream temperature at Site EA during the first sampling period ranged from 21.5 to 25.5 (23.25 ± 0.26), and from 21.3 to 25.9 (23.56 ± 0.34) during the second sampling period.

Dissolved oxygen: During the first sampling period, dissolved oxygen ranged from 5.5 to 10.4 mg/L (6.62 ± 0.19) at Site HS. At Site LA, dissolved oxygen ranged from 6.1 to 9.0 (7.16 ± 0.19), and dissolved oxygen ranged from 5.7 to 10.4 (8.21 ± 0.19) at Site EA. During the second sampling period, dissolved oxygen ranged from 3.1 to 8.6 at Site HS (5.71 ± 0.30), from 3.5 to 9.5 at Site LA (6.35 ± 0.29), and from 5.9 to 9.88 at Site EA (7.88 ± 0.30).

DISCUSSION

None of the physical factors that we measured during this study (wading discharge, total stream discharge, temperature, and dissolved oxygen) appear to have been correlated with the decline in cercariae density observed between June 2006 and July 2010. Because spring-fed systems naturally have relatively stable temperature and dissolved oxygen, it is unlikely that these factors were the cause of the decline in cercariae density, despite some differences between the sampling periods. These differences in abiotic factors were likely caused by the drought conditions that occurred during the second sampling period, but do not appear to have been drastic enough to affect cercariae densities at the sampled sites. Additionally, we do not believe that differences in sampling times were the cause of the observed declines. Despite the evidence that the time of day affects cercariae densities in the water column (M. Johnson, unpublished data), it is highly unlikely that differences in sampling time would manifest as long-term declines. Any differences in cercariae density associated with these sampling differences would be observed on a smaller timescale. We considered other abiotic factors, such as flooding events, and biotic factors, such as invasive species population dynamics, that could potentially cause this trend in cercariae density.

Flooding events, such as those that occurred on 9 June 2010, appear to cause localized declines in cercariae density. We observed such a decline at Site LA during the sampling event following the 9 June 2010 flood. Cercariae density dropped from 1.37 cercariae/L to 0.08 cercariae/L. However, this reduction in

TABLE I. *Centrocestus formosanus* cercariae collected at 3 sites in the Comal River, Texas, June 2006–June 2007. N = number of filters used during the particular sampling event; Range = *C. formosanus* cercariae/5 L sample; total = total cercariae collected during the particular sampling event; mean = *C. formosanus* cercariae/L.

	Houston Street (HS)				Liberty Avenue (LA)				Elizabeth Avenue (EA)			
		Cercarial counts				Cercarial counts				Cercarial counts		
Inclusive dates	N	Range	Total	Mean	N	Range	Total	Mean	N	Range	Total	Mean
22 June 2006–22 June 2006	12	2–8	49	0.82	11	10–21	169	3.07	12	22–44	382	6.37
13 July 2006–14 July 2006	12	1–4	27	0.45	11	8–16	134	2.44	12	26–55	500	8.33
28 July 2006–29 July 2006	12	0–7	35	0.58	11	6–49	244	4.44	12	19–97	536	8.93
4 Aug 2006–5 Aug 2006	12	3–9	57	0.95	11	9–39	201	3.65	12	34–85	739	12.32
18 Aug 2006–19 Aug 2006	12	2–9	60	1.00	11	13–47	261	4.75	12	21–68	589	9.82
1 Sept 2006–3 Sept 2006	12	0–5	29	0.48	11	12–42	274	4.98	12	19–93	663	11.05
15 Sept 2006–16 Sept 2006	12	2–13	72	1.20	11	6–65	407	7.40	12	42–99	848	14.13
29 Sept 2006–30 Sept 2006	12	5–13	93	1.55	11	10–38	222	4.04	12	44–80	693	11.55
20 Oct 2006–21 Oct 2006	12	3–31	131	2.18	11	8–303	513	9.33	12	28–62	530	8.83
3 Nov 2006–4 Nov 2006	12	0–6	36	0.60	11	7–41	193	1.40	12	10–48	352	5.87
17 Nov 2006–18 Nov 2006	12	1–24	114	1.90	11	9–66	326	5.93	12	20–46	362	6.03
1 Dec 2006–2 Dec 2006	12	5–35	153	2.55	11	11–73	402	7.31	12	16–71	429	7.15
15 Dec 2006–16 Dec 2006	12	1–10	61	1.02	11	7–44	227	4.13	12	31–63	519	8.65
31 Dec 2006–1 Jan 2007	12	0–7	41	0.68	11	5–42	167	3.04	12	15–40	311	5.18
26 Jan 2007–27 Jan 2007	12	4–36	190	3.17	11	3–72	244	4.44	12	16–44	361	6.02
17 Feb 2007–17 Feb 2007	12	0–6	38	0.63	11	13–109	381	6.93	12	21–65	480	8.00
3 Mar 2007–4 Mar 2007	12	4–46	218	3.63	11	7–78	287	5.22	12	10–35	217	3.62
16 Mar 2007–17 Mar 2007	12	0–7	32	0.53	11	4–20	116	2.11	12	7–23	188	3.13
31 Mar 2007–1 Apr 2007	12	2–28	134	2.23	11	4–77	245	4.45	12	5–16	129	2.15
14 Apr 2007–15 Apr 2007	12	2–30	128	2.13	11	2–43	443	8.05	12	5–34	190	3.17
27 Apr 2007–28 Apr 2007	12	1–9	58	0.97	11	3–24	133	2.42	12	1–28	213	3.55
18 May 2007–20 May 2007	12	2–12	57	0.95	11	6–32	157	2.85	12	4–10	82	1.37
				1.37 ± 0.20				1.38 ± 0.17				7.06 ± 0.74

cercariae density did not last long. *Melanoides tuberculatus* typically are restricted to lentic habitats (Tolley-Jordan and Owen, 2008). Researchers have hypothesized that *M. tuberculatus* are found in these habitats because dislodgement prevents them from forming stable populations in lotic habitats (Giovanelli et al., 2005; Tolley-Jordan and Owen, 2008). In lotic habitats, the snails are more likely to inhabit substrata that they are able to burrow into more easily (Tolley-Jordan and Owen, 2008). Once the river returns to its average flow, snails protected in these lentic habitats will re-colonize lotic habitats scoured during high water events, making any declines localized and short-lived. On the second sampling event following the 9 June 2010 flood, cercariae densities had begun to return to preflood levels. This is likely because the snails protected from scouring had begun to return to the surface and recolonize the scoured substratum.

Sites HS and EA, however, did not experience a severe decline in cercariae density following the flood. This lack of decline can potentially be explained in 2 ways: first, these declines in cercariae density could be very localized. Alternatively, it is possible that the decline observed at Site LA following the 9 June 2010 flood is merely the result of sampling error. Wide deviations in observed cercariae density were not uncommon during this study, and it is reasonably possible that the low cercariae density observed during this sampling event is caused by sampling error and coincidentally occurred following the flood event.

Although the Comal River experienced several flooding events during this study, we believe that it is unlikely that they caused the overall decline of cercariae density. Individual sites had temporary declines in cercariae density of varying intensities after floods, but densities would rebound relatively quickly following the event. This rebound in cercariae densities following flooding events, combined with the stable nature of the other abiotic factors monitored in this study, lead us to speculate that the fluctuations in cercariae density were likely caused by some biotic factor.

Through this study, we may have observed the decline in population following a period of exponential growth, which Sakai et al. (2001) indicate as common for invasive species after they spread into new habitats. Following its introduction to Texas in 1960s, the snail could have potentially experienced this period of exponential population growth as it spread into suitable habitats throughout the Comal River. By the time the parasite was introduced in the 1990s, it had an existing snail population to infect, which aided in its invasion of the system. *Centrocestus formosanus* potentially then displayed the same population growth pattern observed in other invasive species, leading to high levels of *C. formosanus* cercariae. When researchers first observed the parasite infecting the gill tissues of the fountain darter (Mitchell et al., 2000), we believe it is possible that *C. formosanus* was either reaching or at its peak, and the parasite has been in slow decline since this time. Unfortunately, there are no parasite monitoring data available from 1996 to 2006, so we are unable to verify these potential changes in population growth during this period. Additionally, although *M. tuberculatus* were observed at each site throughout the study, snail density data was not collected as changes in physical habitat characteristics were the focus of this study. Further study would be required to determine if fluctuations in snail densities are causing declines in parasite density in the water column.

Table II. *Centrocestus formosanus* cercariae collected at 3 sites in the Comal River, Texas, July 2009–July 2010. N = number of filters used during the particular sampling event; range = *C. formosanus* cercariae/5 L sample; total = total cercariae collected during the particular sampling event; mean = *C. formosanus* cercariae/L.

	Houston Street (HS)				Liberty Avenue (LA)				Elizabeth Avenue (EA)			
		Cercariae counts				Cercariae counts				Cercariae counts		
Date	N	Range	Total	Mean	N	Range	Total	Mean	N	Range	Total	Mean
6 July 2009	—	—	—	—	12	0–20	92	1.53	—	—	—	—
7 July 2009	12	0–13	38	0.63	—	—	—	—	12	7–27	207	3.45
21 July 2009	—	—	—	—	12	4–21	127	2.12	12	6–22	155	2.58
3 Aug 2009	12	0–17	52	0.87	—	—	—	—	12	4–22	183	3.05
4 Aug 2009	—	—	—	—	12	3–26	159	2.65	—	—	—	—
17 Aug 2009	12	0–14	52	0.87	—	—	—	—	12	11–34	254	4.23
18 Aug 2009	—	—	—	—	12	2–13	82	1.37	—	—	—	—
31 Aug 2009	12	0–13	51	0.85	—	—	—	—	12	5–18	153	2.55
1 Sept 2009	—	—	—	—	12	3–16	80	1.33	—	—	—	—
14 Sept 2009	12	0–11	23	0.38	—	—	—	—	12	7–20	143	2.38
15 Sept 2009	—	—	—	—	12	1–16	101	1.68	—	—	—	—
28 Sept 2009	12	0–4	25	0.42	—	—	—	—	12	6–26	208	3.47
29 Sept 2009	—	—	—	—	12	7–29	153	2.55	—	—	—	—
4 Nov 2009	12	0–5	19	0.32	12	2–15	77	1.28	—	—	—	—
6 Nov 2009	—	—	—	—	—	—	—	—	12	6–12	111	1.85
14 Dec 2009	12	0–4	5	0.08	12	0–8	43	0.72	12	3–28	185	3.08
27 Jan 2010	12	0–2	14	0.23	12	0–14	88	1.47	12	4–19	118	1.97
25 Feb 2010	12	0–3	27	0.45	12	1–7	40	0.67	12	4–17	123	2.05
25 Mar 2010	12	0–4	19	0.32	12	0–19	80	1.33	12	3–13	109	1.82
21 Apr 2010	12	0–4	21	0.35	12	3–12	87	1.45	12	12–26	244	4.07
26 May 2010	12	0–3	18	0.30	12	2–14	82	1.37	14	7–24	209	2.99
22 June 2010	12	0–3	13	0.22	12	0–2	5	0.08	12	4–25	147	2.45
23 July 2010	12	0–3	12	0.20	12	0–4	24	0.40	12	4–15	125	2.08
				0.08 ± 0.87				0.08 ± 2.65				2.75 ± 0.19

Centrocestus formosanus cause serious damage to the gill arches of *E. fonticola* (Mitchell et al., 2000; Mitchell et al., 2002). The trematode causes an unusual inflammatory response in infected gill arches that lead to cartilaginous encapsulation of the cercariae. Increasing densities of cercariae encapsulations can lead to the destruction of the normal gill architecture and inhibit respiratory functions (Mitchell et al., 2000). The inhibited respiratory function can lead to increased mortality of heavily infected individuals.

Theoretically, a decline in cercariae density will lead to decreased infection pressure on fountain darters in the Comal River and a lower threat to the population's survival. However, we are unable to determine the exact implications of this decline without fully understanding its cause. If the decline in density is the result of a natural invasion process, the parasite will exist in the ecosystem at moderate to low densities and not pose a grave threat to the fish population.

Ultimately, maintaining low levels of infection pressure is vital to the continued existence of the fountain darter in the Comal River. Unfortunately, recreational activities, behavioral characteristics of *M. tuberculatus*, and the Comal River's proximity to other invaded rivers make the elimination of the parasite practically impossible. At best, managers can hope to maintain the parasite at levels that do not threaten the continued survival of the fountain darter. Understanding the causes of this decline can allow managers to predict what will happen to cercariae densities in the future and allow them to implement policies that will help them maintain low parasite densities. Unfortunately, uncertainty regarding future water usage will complicate the development of *C. formosanus* and *M. tuberculatus* management plans.

Decreased stream discharge is likely to become more frequent as droughts and demand for water from the Edwards Aquifer increase in the future. These changes in stream discharge could potentially affect this decline in cercariae density as snail

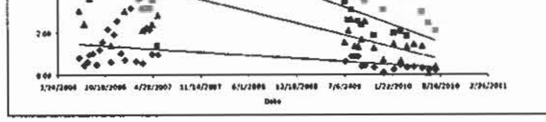

Figure 2. Plots of cercarial densities from 5 L water samples collected at 3 sites (HS, LA, and EA) on the Comal River, Texas, between June 2006 and July 2010. Correlation between cercarial density (*Centrocestus formosanus* cercariae) and date: HS: $y = 28.929\text{-}8.492E\text{-}9x$, $r^2 = 0.239$; $P = 0.0018$; LA: $y = 113.68\text{-}3.358E\text{-}8x$, $r^2 = 0.505$ $P < 0.0001$; and EA: $y = 166.327\text{-}4.899E\text{-}8x$, $r^2 = 0.495$, $P < 0.0001$.

populations adapt to changing microhabitats in the system. Microhabitats with *M. tuberculatus* could disappear as stream flows change. Simultaneously, new microhabitats could form and become inhabited by new populations of the invasive snail. Depending on the ratio of microhabitat creation versus the level of microhabitat loss, overall abundances of *M. tuberculatus* could increase or decrease in the Comal River. These changes in snail abundance would likely affect the number of *C. formosanus* cercariae found in the system. Future monitoring efforts should document these potential changes in localized snail density and overall snail abundances.

In addition to potential changes in snail densities, very low flows and zero flow conditions would reduce the removal rate of parasites released into the water column of Landa Lake, causing the numbers of parasites in the lake to increase. Increasing densities of cercariae in the water column would increase infection pressure on the fountain darter and, in addition to the compounding abiotic factors of increased water temperature and decreased
4 DO, could potentially cause major impacts to the fountain darter population.

ACKNOWLEDGMENTS

This project was partially funded by the Edwards Aquifer Authority, BIO-WEST, Inc., and the U.S. Fish and Wildlife Service. We thank San Marcos NFHTC staff, including D. Araujo, V. Cantu, and P. Grant, for laboratory and field assistance. Tom Brandt and J. Fries provided comments to improve the quality of the manuscript. The views in this article are those of the authors and do not necessarily reflect those of the U.S. Fish and Wildlife Service.

LITERATURE CITED

ALCARAZ, G., G. P. DE LEON, P. L. GARCIA, V. LEON-REGAGNON, AND C. VANEGASE. 1999. Respiratory responses of grass carp *Ctenopharyngodon idella* (Cyprinidae) to parasitic infection by *Centrocestus formosanus* (Digenea). Southwestern Naturalist 44: 222–226.

BALASURIYA, L. K. S. W. 1988. A study on the metacercarial cysts of a *Centrocestus* species (Digenea: Heterophyidae) occurring on the gills of cultured cyprinid fishes in Sri Lanka. Journal of Inland Fisheries 4: 3–10.

BLAZER, V. S. AND J. B. GRATZEK. 1985. Cartilage proliferation in response to metacercarial infections of fish gills. Journal of Comparative Pathology 95: 273–280.

BRUNE, G. 1981. Springs of Texas, vol. I. Branch-Smith, Fort Worth, Texas, 590 p.

BUCHANAN, T. J., AND W. P. SOMERS. 1969. Discharge measurements at gauging stations. *In* Techniques of water-resources investigations. U.S. Geological Survey, Washington D.C., book 3, chapter A8, 65 p.

CANTU, V. 2003. Spatial and temporal variation in *Centrocestus formosanus* cercarial abundance and metacercarial infection in endangered fountain darters (*Etheostoma fonticola*) in the Comal River, Texas. M.S. Thesis. Texas State University, San Marcos, Texas, 59 p.

GIOVANELLI, A., C. DA SILVA, G. LEAL, AND D. BAPTISTA. 2005. Habitat preference of freshwater snails in relation to environmental factors and the presence of the competitor snail *Melanoides tubercuatus* (Müller, 1774). Memorias do Instituto Oswaldo Cruz 100: 169–176.

KUHLMAN, T. A. 2007. The predominant definitive avian hosts of the invasive Asian trematode, *Centrocestus formosanus*, in the headwaters of the Comal, San Antonio, and the San Marcos rivers of central Texas. M.S. Thesis. Texas State University, San Marcos, Texas, 45 p.

LO, C. T., AND K. M. LEE. 1996. Patter of emergence and the effects of temperature and light on the emergence and survival of heterophyic cercariae (*Centrocestus formosanus* and *Haplorchis pumilio*). Journal of Parasitology 82: 347–350.

MCDERMOTT, K. 2000. Distribution and infection relationships of an undescribed digenetic trematode, its exotic intermediate host, and endangered fishes in springs of west Texas. M.S. Thesis. Southwest Texas State University, San Marcos, Texas, 64 p.

MITCHELL, A. J., A. E. GOODWIN, M. J. SALMON, AND T. M. BRANDT. 2002. Experimental infection of an exotic heterophyid trematode, *Centrocestus formosanus*, in four aquaculture fishes. North American Journal of Aquaculture 64: 55–59.

———, R. M. OVERSTREET, A. E. GOODWIN, AND T. M. BRANDT. 2005. Spread of an exotic fish-gill trematode: A far-reaching and complex problem. Fisheries 30: 11–16.

———, M. J. SALMON, D. G. HUFFMAN, A. E. GOODWIN, AND T. M. BRANDT. 2000. Prevalence and pathogenicity of a heterophyid trematode infecting the gills of an endangered fish, the fountain darter, in two central Texas spring-fed rivers. Journal of Aquatic Animal Health 12: 283–289.

PRENTICE, M. A. 1984. A field-evolved differential filtration method for recovery of schistosome cercariae. Annals of Tropical Medicine and Parasitology 78: 117–127.

SAIKI, A. K., F. W. ALLENDORF, J. S. HOLT, D. M. LODGE, J. MOLOFSKY, K. A. WITH, S. BAUGHMAN, R. J. CABIN, J. E. COHEN, N. C. ELLSTRAND, ET AL. 2001. The population biology of invasive species. Annual Review of Ecological Systems 32: 305–332.

SCHELL, S. C. 1970. How to know the trematodes. W. M. C. Brown, Dubuque, Iowa, 355 p.

SCHOLZ, T., AND G. ZALGADO-MALDONADO. 2000. The introduction and dispersal of *Centrocestus formosanus* (Nishigori, 1924) (Digenea: Heterophyideae) in Mexico: A review. American Midland Naturalist 143: 185–200.

THERON, A. 1979. A differential filtration technique for the measurement of schistosome cercarial densities in standing waters. Bulletin of the World Health Organization 57: 971–975.

TOLLEY-JORDAN, L. R., AND J. M OWEN. 2008. Habitat influences snail community structure and trematode infection levels in a spring-fed river, Texas, USA. Hydrobiologia 600: 29–40.

UPATHAM, E. S. 1973. Location of *Biomphalaria glabrata* (Say 1818) by miracidia of *Schistosoma mansoni* (Sambon 1907) in natural standing and running waters on the West Indian Island of St. Lucia. International Journal for Parasitology 3: 289–297.

U.S. FISH AND WILDLIFE SERVICE (USFWS). 1996. San Marcos and Comal Springs and Associated Aquatic Ecosystems (revised) Recovery Plan. Albuquerque, New Mexico, 134 p.

U.S. GEOLOGICAL SURVEY (USGS). 2001. National Water Information System data available on the World Wide Web (Water Data for the Nation). Available at: http://nwis.waterdata.usgs.gov/tx/nwis/uv/?site_no=08169000&PARAmeter_cd=00065,00060. Accessed 20 September 2010.

VELEZ-HERNANDEZ, E. M., F. CONSTANTINO-CASAS, L. J. GARCIA-MARQUEZ, AND D. OSORIO-SARABIA. 1988. Gill lesions in common carp, *Cyprinus carpio L.*, in Mexico due to the metacercariae of *Centrocestus formosanus*. Journal of Fish Diseases 21: 229–232.