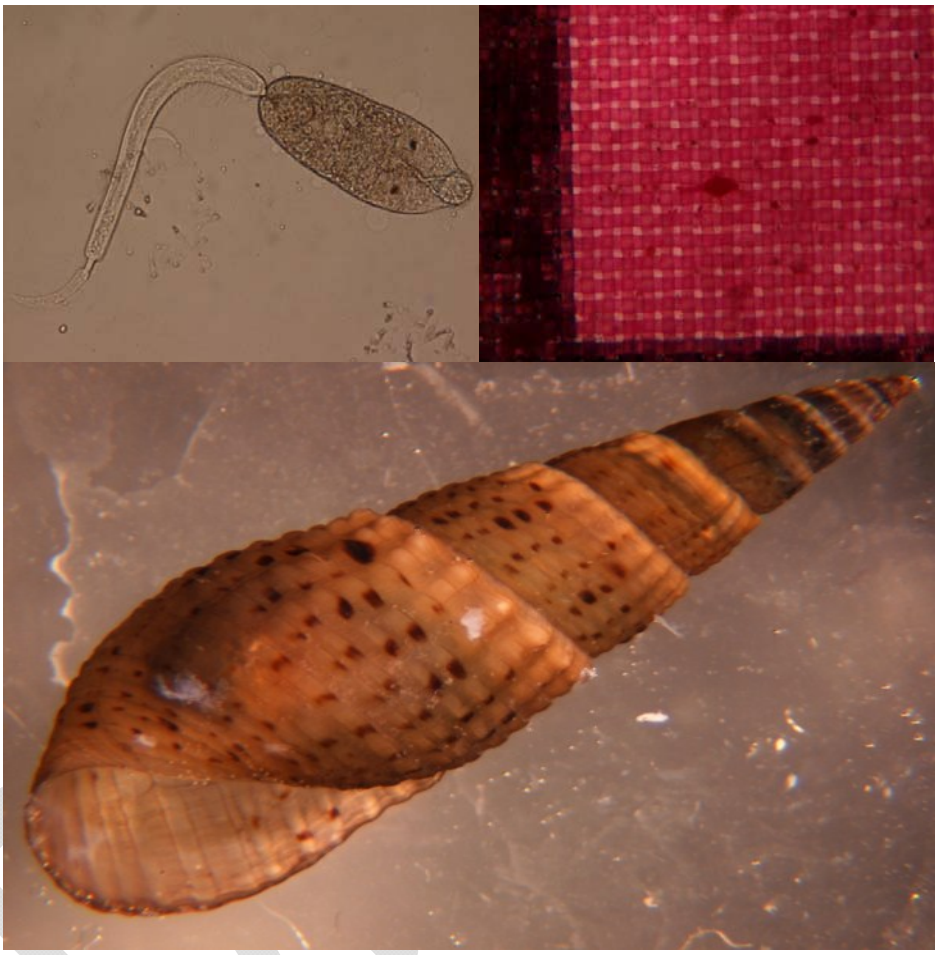
Effectiveness of Host Snail Removal in the Comal River, Texas and its Impact on Densities of the Gill Parasite *Centrocestus formosanus* (Trematoda: Heterophyidae)



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This report summarizes the effort by the United States Fish and Wildlife Service (USFWS) San Marcos National Fish Hatchery and Technology Center (SMNFHTC) and BIO-WEST, Inc. to determine the effectiveness of *Melanoides tuberculatus* removal on lowering drifting gill parasite (*Centrocestus formosanus* cercariae) numbers in the Comal River.

INTRODUCTION

Centrocestus formosanus (Nishigori 1924) is a digenetic trematode originally described in Taiwan that has become widely distributed throughout Asia and warm-watered areas of the world (Mitchell et al. 2000). The trematode was likely introduced into Mexico in 1979 but was not confirmed until 1985 (Scholz and Zalgado-Maldonado 2000) and possibly spread to the United States in the early 1980's (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), in the Comal River in Comal County, Texas (Mitchell et al. 2000). USFWS SMNFHTC biologists observed considerable gill damage caused by the encystment of up to 1,500 metacercariae per fish.

The life cycle of *C. formosanus* has three stages, including a definitive host, a first intermediate host, and a second intermediate host. The definitive host for *C. formosanus* in central Texas appears to be the Green Heron, *Butorides virescens* (Linnaeus 1758), where adult trematodes colonize the colon (Kuhlman 2007). The adult trematodes release eggs into the avian host's feces which, when released into a water body, can infect the first intermediate host, the invasive red-rimmed melania, *Melanoides tuberculatus* (Müller 1774) (Mitchell et al. 2005). The snail is infected either by directly consuming trematode eggs or by being penetrated by free-swimming miracidium that are produced from the eggs (Lo and Lee 1996). Once inside the snail, the miracidium transforms into a sporocyst and asexually produces rediae larvae, which produce cercariae (Schell 1970). Cercariae are released in the exhalant respiratory current and are free to infect the second intermediate host, one of which is the fountain darter. Cercariae penetrate the gill filaments and precipitate cartilage hyperplasia resulting in severe gill lesions, which subsequently reduces 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.

In 2009/2010, a study was conducted to 1) evaluate gill parasite concentrations in the water column following an extended drought, and 2) to compare to a similar study completed in 2006/2007 to evaluate gill parasite trends in the Comal system over time (USFWS in preparation). A significant decline in the gill parasite in the water column from July 2006 to July 2010 was observed in the Comal River (USFWS in preparation). Although cercarial densities may be abating, *C. formosanus* still poses a threat to fountain darters in the Comal River. Informal observations suggest that the density of *C. formosanus* cercariae in the water column may increase as stream discharge decreases and vice versa (T. Brandt, USFWS SMNFHTC, personal communication). Upatham (1973) observed that the ability of the bloodfluke, *Schistosoma mansoni* (Sambon 1907),

to infect the bloodfluke planor, *Biomphalaria glabrata* (Say 1818), decreased as stream discharge increased. If this same relationship exists between the *C. formosanus* cercariae and discharge in the Comal River, there are concerns that increased levels of infection pressure could exacerbate the other stresses of low spring discharge on the fountain darter, and could negatively impact fountain darters in the Comal River during low-flow periods.

Elimination of the gill parasite from the Comal River is unlikely. However, a practical approach to manage the parasite in the river might be to control the parasite's snail host, *M tuberculatus*. United States Fish and Wildlife Service and U.S. Environmental Protection Agency authorizations to use chemicals known to be lethal to the snail likely cannot be obtained for the Comal River due to potential impacts to endangered species. Therefore, alternative methods for decreasing abundances of *M. tuberculatus* and the associated parasite need to be evaluated. The goal of this study was to determine the effectiveness of *M. tuberculatus* removal by physical methods on lowering drifting gill parasite numbers in the Comal River.

MATERIALS AND METHODS

The Comal River in New Braunfels, Comal County, Texas, is an approximately 5 kilometer (km) long river issuing from the state's largest spring complex at the edge of the Edwards Plateau region of central Texas (Brune 1981). The headsprings are impounded by two dams to form Landa Lake, from which the spring water flows into two channels, the New Channel and the Old Channel. These channels converge approximately 2.5 km downstream of Landa Lake and flow another 2.5 km before the Comal River converges with the Guadalupe River (USFWS 1996).

A reconnaissance survey of the Comal Springs system was initially conducted to identify areas with high densities of *M. tuberculatus*. This survey focused on areas of high snail density (> 25 *M. tuberculatus*/square meter [m^2]) 5 m^2 or larger. Other considerations for site selection included avoiding potential outside influences which could affect the outcome of the study, such as flood-flows from tributaries or recreational activities. Additionally, the ability to make an accurate and repeatable cross-sectional measurement of gill parasites in the water column immediately below the location was also considered in the selection of study sites.

During the reconnaissance survey, 20 individual locations were selected. At each location, preliminary water samples were collected to evaluate the current level of drifting gill parasites in the water column. Locations with water column concentrations of gill parasites greater than 5 cercariae / liter (L) were considered for study sites while locations with less than 5 cercariae/L were discounted for this study.

Three high density snail areas (snail hotspots) were identified near Spring Island in Landa Lake which had adequate densities of drifting cercariae (Sites S1, S2, and S3) to test the effects of snail removal (Figure 1). One site (Site US) was placed upstream as a control to measure cercarial densities in the water column before approaching snail hotspots

(Figure 1). A fifth site (Site DS) was placed downstream to measure total cercarial densities after passing through all three snail hotspots (Figure 1).

Figure 1. Map of sites sampled near Spring Island in Landa Lake (Comal River, Texas), including three snail hotspots (Sites S1, S2, and S3), Upstream (Site US) and Downstream (Site DS) sites.

At Spring Island, the Comal River splits into two channels immediately below the area of snail hotspots (Figure 1). To determine the downstream site (Site DS) location, cercarial densities in each channel were compared to the upstream site (Site US). This preliminary sampling determined that a significant number of the cercariae released within the study area were passing down the river-right channel ($p < 0.05$), opposed to the river-left channel, which showed no significant change in cercarial density ($p > 0.05$) when compared to Site US.

Once sites were selected, three collection points were established at each of the five sites. Each point was marked with a flagged stake to ensure that subsequent samples would be collected from the same location. At each of the collection points, 5 L water samples were taken at 60% depth from the water's surface. Each water sample was pumped through a flexible acrylic tube (6.4 millimeter [mm] internal diameter) into a 10 L bucket via a battery-operated submersible pump (Attwood aerator pump, Model A500, Lowell, Michigan). The submersible pump was positioned at the desired depth on an adjustable 1.5 meter (m) rod before pumping was initiated. Immediately following collection, 5 milliliters (mL) of 10% formalin was added to each water sample to fix the cercariae. This procedure was performed at each of the five collection sites. Sampling was initiated at Site DS and continued at successive upstream sites, until Site US was reached. This sampling pattern was performed three times per day, at 9:00 AM, 11:00 AM, and 1:00 PM, for three successive days prior to snail removal.

Each water sample was then filtered using an apparatus described in Theron (1969) and Prentice (1984), but using modifications developed by Cantu (2003). Each sample was passed through three successive mesh filters with pore sizes of 220 µm, 86 µm, and 30 µm, respectively. The 220 µm and 86 µm filters were used to filter out larger debris. Cercariae freely passed through the 220 µm 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, the 30 µm filter was placed in a Petri dish and covered with 3 mL of 10% formalin. The cercariae were then stained on the filters with a 10% Rose Bengal solution. Filters were then transported to the SMNFHTC laboratory where the number of cercariae on each filter was counted with the aid of a dissecting microscope. Formalin wastewater was collected in 18 L jugs and transported to the laboratory. All wastewater was detoxified using DeToX Formaldehyde neutralizer (Scientific Device Laboratory, Inc., Des Plaines, IL) (0.05 oz. DeToX neutralizer/L formalin wastewater) and discarded.

Following three days of baseline water sampling, snails were removed by placing a 2 m x 1 m drop net over the snail hotspot area to be sampled and using a 1 m^2 dip net within the enclosed area to collect all exotic snails to the extent possible. Dip-netting consisted of pushing the dip net across the site bottom and extending down to 5 centimeters (cm) below the surface of the substrate. The area within each drop net was searched until no more *M. tuberculata* were collected. The drop net was then moved to an adjacent area and the procedure repeated until all of the snail hotspot had been covered. This process was continued until snails were removed from approximately 10 m^2 of substrate surrounding each hotspot. All live *M. tuberculatus* were collected, counted, and measured in order to quantify the total number and density of host snails removed from the hotspot.

Following exhaustive snail removal from 10 m^2 around all three hotspots, additional snail removal was conducted for a 30 minute period (3.0 person-hours total) at other locations within the general area. The area covered during this additional removal process was not quantified. As with the snails collected during the previous removal process, snails collected during the timed removal were counted and measured.

Finally, three days following snail removal, cercariae collection was repeated exactly as described above (three water samples per day at five sites for three consecutive days).

RESULTS

Pre-removal gill parasite sampling was conducted from November 9 to November 11, 2010. Prior to snail removal, the furthest upstream site, Site US, had the lowest densities of *C. formosanus* cercariae. Of the three snail hotspots sampled prior to snail removal, the highest densities of cercariae were observed at Site S1, followed by Sites S3 and S2. Site DS showed higher cercarial densities than Site US, but lower densities than Sites S1, S2, and S3. Table 1 shows the densities of cercariae at each site prior to snail removal.

Table 1. Number and densities of cercariae at each site prior to snail removal.

Site	Number of Samples	Total Cercariae	Range (Cercariae/L)	Mean (Cercariae/L)	Standard Deviation
DS	27	379	0.2 - 9.6	2.81	2.24
S1	26	1077	1.6 - 23.4	8.28	4.93
S2	27	572	1.0 - 12.0	4.24	3.18
S3	18	796	1.2 - 23.2	7.24	5.08
US	27	286	0.2 - 4.2	2.12	1.11

The number of samples at some sites was slightly lower due to the occurrence of excessive silt. Excessive silt can cloud filters and prevent accurate counting of cercariae in a sample. One sample from the 9:00 AM round of sampling at Site S1 on November 10, 2010 could not be analyzed because of excessive amounts of silt on the filter. Similarly, five samples collected on November 9, 2010 from Site S3 were also covered with excessive amounts of silt. Consequently, all samples collected from Site S3 on this date were removed from further analysis.

Snail removal was conducted as described in the methods section on November 12, 2010. Snail density was highest at Site S1 followed by S3 and S2. Table 2 shows the number of snails collected, densities, average length, and percentage of snails greater than or equal to 17 mm total length at each snail removal site. The parasite is usually only present in snails larger than 17 mm (Mitchell et al. 2000). The additional 30-minute effort resulted in collection of 859 snails with an average length of 33 mm, of which 93% were 17 mm or larger.

Table 2. Details of *M. tuberculatus* collected at Sites S1, S2, and S3.

Site	Number of Snails Collected	Density (snails/m^2)	Average Total Length (mm)	Percentage ≥ 17mm TL
S1	676	67.6	30	89%
S2	299	29.9	29	98%
S3	374	37.4	20	48%

Post-removal gill parasite sampling was conducted between November 15 and November 17, 2010. Following snail removal, Site US continued to have the lowest cercarial densities. Sites S1, S3, and S2 remained in the same order of declining cercarial densities among the three hotspots. Table 3 shows the densities of cercariae at each site post snail removal.

Table 3. Number and densities of cercariae at each site following snail removal.

Site	Number of samples	Total Cercariae	Range (Cercariae/L)	Mean (Cercariae/L)	Standard Deviation
DS	27	367	0.4 - 7.2	2.72	2.03
S1	27	650	0.8 - 11.8	4.82	2.73
S2	27	408	0.6 - 8.4	3.02	2.03
S3	27	647	1.0 - 15.0	4.79	2.89
US	27	272	0.0 - 4.6	2.02	1.33

Analysis of the pre- and post-snail removal densities (using a paired *t*-test) determined that differences in cercarial densities between the pre- and post-snail removal samples are significant for Sites S1, S2, and S3 ($p < 0.05$; Table 4). Differences in densities were not found to be significant for Sites US or DS ($p > 0.05$). Figure 2 shows a graphical representation of pre- and post-removal cercarial densities.

Table 4. Statistical comparisons of pre- and post-snail removal cercariae densities.

Site	Mean Density (Cercariae/L)		t	p
	Pre-removal	Post-removal		
DS	2.81	2.72	0.316	0.754
S1	8.28	4.82	3.563	0.002
S2	4.24	3.02	2.276	0.031
S3	7.24	4.79	3.463	0.003
US	2.12	2.02	0.435	0.667

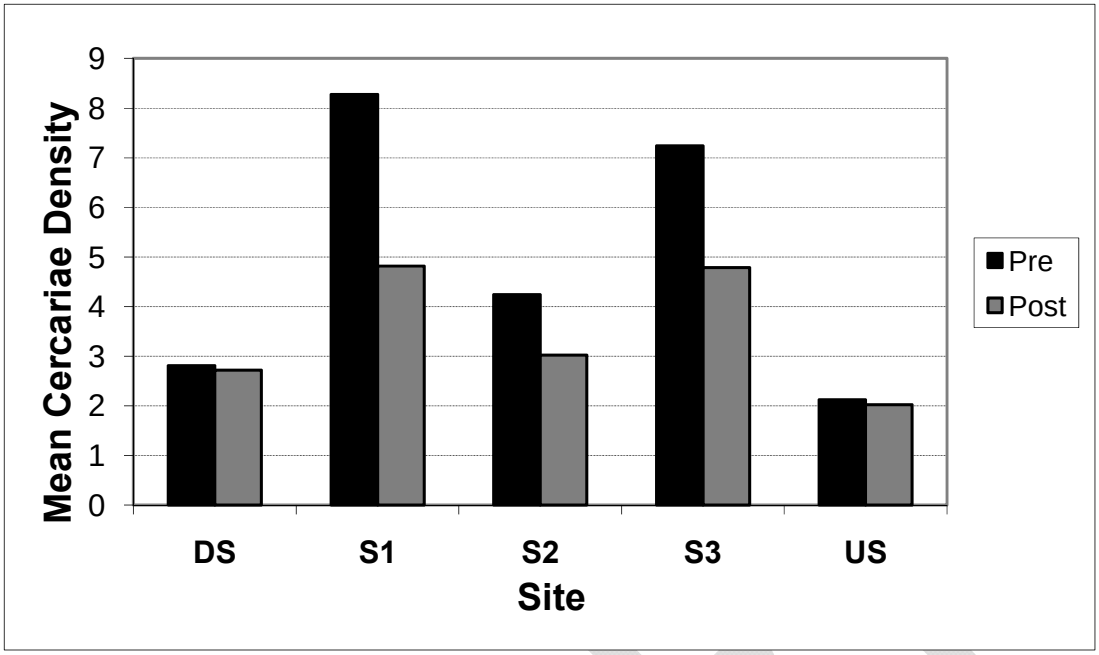

Figure 2. Mean pre- and post-removal cercariae density at each study site.

Cercarial densities varied greatly among the three sampling time periods at each collection site (Figure 3). One-way ANOVA with Fisher's LSD multiple comparison testing determined that cercarial densities declined significantly between the 9:00 AM sampling period and the 11:00 AM and 1:00 PM sampling periods for all five sites ($p < 0.05$). No significant differences were detected between cercarial densities collected during the 11:00 AM and 1:00 PM sampling periods ($p > 0.05$). Only pre-snail removal density data were used in these comparisons to avoid any biases in data that may have been caused by snail removal.

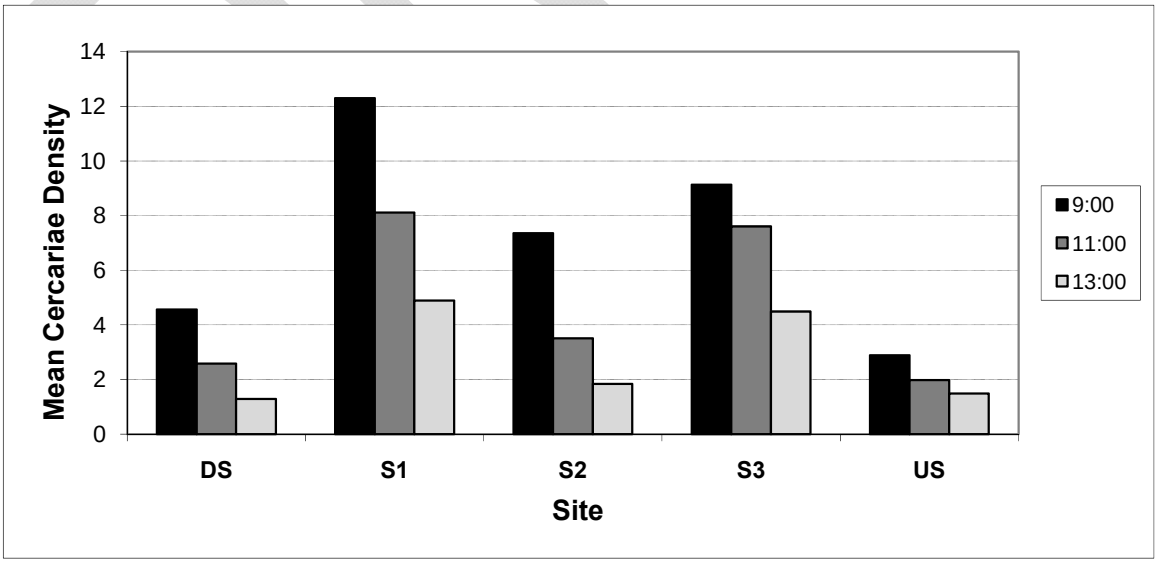

Figure 3. Pre-snail removal cercariae densities at each site across three sample times.

DISCUSSION

The results of this study support the hypothesis that removing *M. tuberculatus* from the Comal River will result in a decrease in *C. formosanus* cercariae in the water column. This was accomplished with a rather modest effort of approximately eight hours of labor by a team of six biologists. However, the effect was localized. Although significant differences in cercarial density were noted in the immediate vicinity of snail removal areas, the downstream site showed no difference, suggesting that a more intensive effort is required to affect snail densities at this location. This downstream location is influenced by cercarial contributions from snails residing outside the specific snail hotspots where intensive removal occurred.

Cercarial densities documented both pre- and post-removal were correlated with the densities of snails at each hotspot. For example, Site S1 had the highest density of snails, as well as the highest cercarial density (both pre- and post-removal), whereas Site S2 had the lowest snail density and the lowest cercarial density of the snail removal sites. Another factor which could have led to differences in cercarial densities between sites is the amount of upstream contribution. Site S1 is the most downstream of the snail removal sites and may have had higher cercarial densities due to contributions by the other hotspots upstream.

Differences in cercarial density between time periods are obviously influenced by time of day. This study found that cercarial concentrations were highest at 9:00 AM and declined at 11:00 AM and 1:00 PM. Cantu (2003) showed a similar trend, with *C. formosanus* cercarial abundance increasing sharply after dawn, peaking around 9:30 AM, and then declining until approximately 3:30 PM. This emphasizes the importance of examining time of day when comparing between samples.

During this study, snails were removed by dragging dip nets across the top layer of the substrate. Drop nets were only used as a means to quantify the area sampled. This dip net method seemed to be rather efficient, especially after biologists became familiar with the habitats where snails were most abundant (low-flow edges and depositional areas). However, this method is only applicable in areas that are wadeable.

This study did not analyze the long-term effects of snail removal on cercarial density. Effects were apparent up to five days after snail removal when sampling ceased. It is not known if these effects would persist. The persistence of low cercarial numbers after snail removal is dependent on snail re-colonization and reproduction rates.

As a result of the potential impact of gill parasites under low flows, *C. formosanus* cercarial concentrations should be monitored in multiple areas along the Comal River on a semi-annual basis, and more frequently when spring flow drops below 150 cfs. The frequency of monitoring should correlate with the rate of decreasing spring flow. Results from this study, as well as Cantu (2003), suggest that monitoring should take place during mid-morning (9:00 AM and 10:00 AM) to capture the daily peak in cercariae release

within the Comal River. At the least, collection times should be similar at a given site, or between any samples to be compared.

Monitoring should focus on areas with high density of *M. tuberculatus*. To identify such areas, a system-wide survey of snail population density needs to be conducted. Then, to assess the influence of these areas on density of drifting cercariae, cercarial density should be examined just upstream and just downstream of each snail hotspot. Periodically, the river should be examined for new areas of high *M. tuberculatus* densities and those areas should be included in each monitoring event.

Given that the density of drifting cercariae has declined in recent surveys (USFWS in preparation), a system-wide intensive snail removal effort may not be necessary at this time. However, this type of effort may be necessary if snail abundance and/or gill parasite concentrations increase over time or are exacerbated by low flows. The exact density of drifting cercariae which becomes problematic to fountain darters is also unclear. Continued monitoring of the fountain darter population is necessary to examine correlations between cercariae densities and fountain darter infections (documented by inflamed gills). Additionally, water temperature may also play a role in cercariae release (McClelland 1965, Lo and Lee 1996) as Lo and Lee (1996) showed that as temperature increases above 15 °C, cercariae release begins and increases exponentially. As such, continued water quality monitoring is also important.

SUMMARY AND RECOMMENDATIONS

The results of this study confirm that removing *M. tuberculatus* from the Comal River will result in a decrease in *C. formosanus* cercariae in the water column. However, several unknowns remain regarding magnitude and duration of benefits from snail removal in the Comal system, and thus, specific study recommendations are presented to the EARIP as follows:

- evaluate alternative methods for snail removal;
- evaluate magnitude of snail removal necessary to affect downstream conditions; and
- evaluate the long-term benefit of snail removal;

Additionally, although cercarial densities may be abating, *C. formosanus* still poses a threat to fountain darters in the Comal River, especially during low-flows. As such, continued monitoring is essential along with targeted conservation efforts focused on reducing levels of infection pressure from the parasite where possible. The following activities and monitoring recommendations are presented to the EARIP as follows:

- continue existing water temperature monitoring;
- conduct a system-wide survey of snail population density and cercarial concentrations;
- based on system-wide survey, determine whether a system-wide removal effort is necessary, and if so, conduct said effort;

- based on the system-wide survey, design and implement a monitoring program for cercarial concentrations to be monitored in multiple areas along the Comal River on a semi-annual basis, and more frequently when spring flow drops below 150 cfs; and
- continue existing monitoring of the fountain darter population to examine correlations between cercariae densities and fountain darter impacts in the wild.

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