THE EFFECTS OF SPRING FLOW ON THE ABUNDANCE OF HETEROPHYID CERCARIAE IN THE COMAL RIVER, NEW BRAUNFELS, TX

THESIS

Presented to the Graduate Council of
Texas State University-San Marcos
in Partial Fulfillment
of the Requirements

for the Degree

Master of SCIENCE

by

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San Marcos, Texas

December 2007

ACKNOWLEDGEMENTS

I would like to express the deepest appreciation to my committee members, Dr. David Huffman, Dr. Tom Brandt, and Dr. Alan Groeger for their patience and guidance throughout my graduate career. I would also like to thank the entire staff at the San Marcos National Fish Hatchery and Technology Center for their assistance and use of their facilities. Additionally, I would like to thank Ed Oborny and Bio-West Inc. who have been abundantly helpful.

I am deeply indebted to Valentin Cantu, Kelly McDermott, and Pamela Holmes for their encouragement and time spent in the field. Also it is difficult to overstate my gratitude to Tiffany Kuhlman and Erin O'Docharty. Thank you so much for your support and your friendship. I could not have done it without you.

Lastly, and most importantly, I wish to thank my parents, John and Mary Bolick. Thank you for encouraging me to go to graduate school in the first place, and all your love and support.

This manuscript was submitted on October 24, 2007.

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CHAPTER I

INTRODUCTION

Setting and History

Comal Springs in New Braunfels, Comal County, Texas is the largest spring system in Texas (Heitmuller and Williams 2006) with a mean annual discharge of 8.04 cms (USFWS 1996). It consists of four major springs, all of which are fed by water from the Edwards Aquifer. These springs are impounded to form Landa Lake, which constitutes the headwaters of the Comal River.

The Comal River is one of two rivers supporting wild populations of the endangered fountain darter, *Etheostoma fonticola* (Schenck and Whiteside 1976). *Etheostoma fonticola* requires clean clear water, adequate stream flows and prefers vegetated floor habitats and constant water temperatures (USFWS 1996). The fountain darter was listed as an endangered species on October 13th, 1970, and its local extinction in the Comal River was most likely caused by the drought of the mid 1950s (USFWS 1996). This severe drought resulted in a cessation of discharge from the Comal Springs that lasted from June until November of 1956 (USFWS 1996). The Comal River was later repopulated with 457 adult fountain darters collected from the nearby San Marcos River (USFWS 1996).

In 1996, while collecting fountain darters from the Comal River for a USFWS refugium program, workers observed that the gills of many fish were heavily infected with trematode metacercariae that had not been seen before in the river. The metacercariae have since been identified as *Centrocestus formosanus* (Mitchell et al. 2000) and the source of the infection has been traced to cercariae emerging from the exotic red-rimmed melania snail, *Melanoides tuberculata* (Salgado-Maldonado et al. 1995; Scholz and Salgado-Maldonado 2000). The trematode is now a concern because of its potential to negatively affect U.S. hatchery fishes and fish in the wild (McDermott 2000; Mitchell et al. 2000, 2002).

Study Organism

The parasite *Centrocestus formosanus* (Trematoda: Heterophyidae) is an invasive digenetic trematode that was originally described in Taiwan and is widely distributed in Asia (Scholz and Salgado-Maldonado 2000). It is now cosmopolitan in many warm waters of the world, including the spring-fed San Antonio, Comal, and San Marcos rivers of central Texas.

The life cycle of digenetic trematodes involves at least two hosts, one is a vertebrate and the other is usually a mollusk (Roberts and Janovy Jr. 2000). The *C. formosanus* life cycle has three stages (Figure 1). Vertebrates that have been shown experimentally to be suitable definitive hosts for *C. formosanus* include birds, dogs, cats, mice, and occasionally amphibians (Yamaguti 1975; Salgado-Maldonado et al. 1995). In central Texas the natural definitive host seems to be the Green Heron, *Butorides virescens* (Kuhlman 2007). In the Green Heron, the adult trematodes localize in the colon where they lay eggs that are later passed through host feces (Yamaguti 1975; Kuhlman 2007).

One complicating factor that may ultimately change the assignment of blame in this issue was discovered by Kuhlman (2007) while attempting to recover *C. formosanus* adults from piscivorous birds taken in the area. Not all heterophyid specimens recovered could be confidently assigned to *C. formosanus*, and indeed, many adult specimens are now suspected of being in the genus *Phagicola* (Kuhlman 2007). This being said, the fountain darters in the Comal River are heavily impacted by metacercariae that are definitely heterophyid. Two species of heterophyid cercariae were seen during the study, *Centrocestus formosanus* (until resolved) and *Haplorchis pumilio*, which both utilize the same snail host (Lo and Lee 1996). Reference to cercariae from this point forward will refer to *C. formosanus* cercariae, unless noted otherwise.

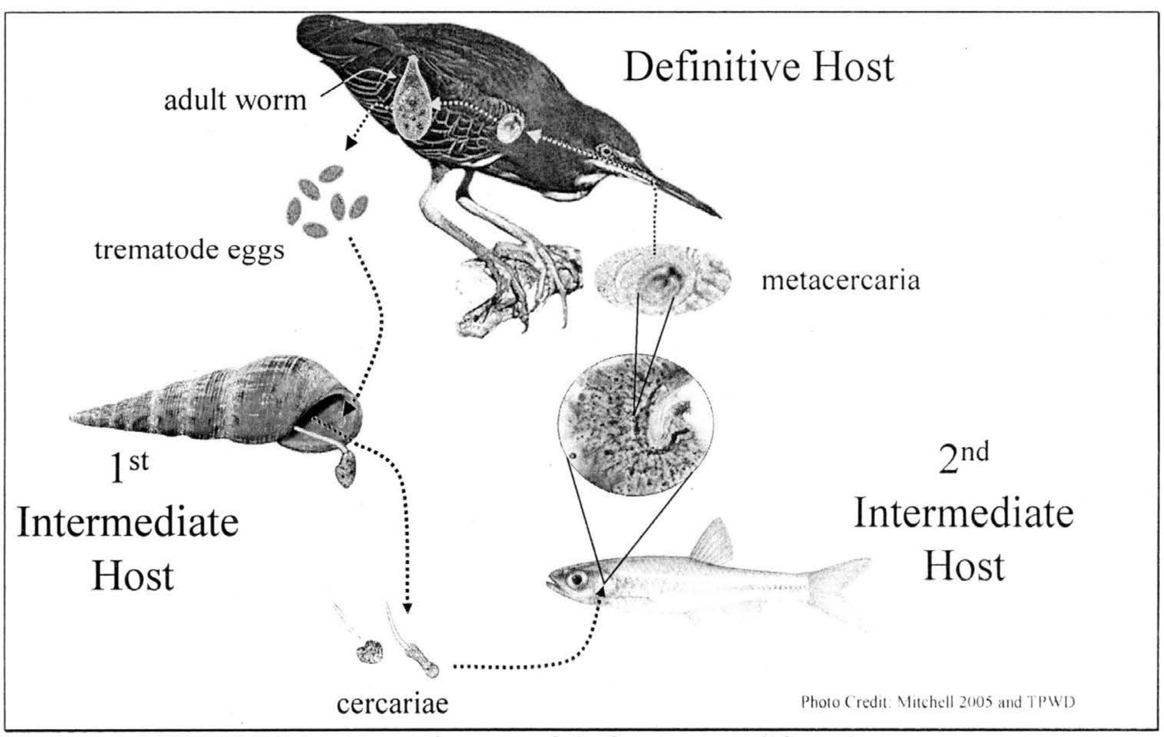


Figure 1. Diagram of *C. formosanus* lifecycle.

The first intermediate host for *C. formosanus* in the Comal River is *Melanoides tuberculata* (Mitchell et al. 2005) an exotic snail found in tropical, subtropical, and temperate regions (Amaya-Huerta and Almeyda-Artigas 1994). The snail has an elongate conical shell with the length being more than twice the width (Duggan 2002; Mitchell and Brandt 2005). The shell color is dark brown with reddish brown basal whorls, giving it the common name the red-rimmed melania (Duggan 2002). *Melanoides tuberculata*, which has the capacity to colonize rapidly and densely in many types of habitats (Amaya-Huerta and Almeyda-Artigas 1994), was first reported from Texas waters in 1964 (Murray 1964).

Centrocestus formosanus can infect the snail via two routes: The snail can ingest the eggs and the miracidium later hatches inside the host, or the eggs can hatch in the water and the free-swimming miracidium can penetrate the snail (Schell 1970; Roberts and Janovy Jr. 2000). Inside the snail, the miracidium metamorphoses into a sporocyst, followed by asexual reproduction within the sporocyst that results in many rediae larvae (Roberts and Janovy Jr. 2000). Both the redia and the sporocyst are able to produce cercariae (Schell 1970), which emerge through the exhalent respiratory current of the snail as free-swimming larvae (Lo and Lee 1996) that are infective to fishes, the second intermediate host (Martin 1958; Yamaguti 1975). This process results in embryonic amplification in which thousands of cercariae are produced (Kiesecker 2002).

While the cercariae are in the water column, they can infect several species of fish. The route of infection is by passively entering the mouth of the fish with the respiratory current and then making contact with the gills (Salgado-Maldonado et al.

1995). The cercariae immediately beginning attaching to the gill filaments, cast off their tails, and begin cyst formation (Salgado-Maldonado et al. 1995).

The metacercarial cysts occur on gill filaments in areas closely associated with bloods vessels (Madhavi 1986). Severe gill lesions and cartilage hyperplasia due to metacercariae have reportedly caused respiratory problems leading to death of infected fish (Balasuriya 1988; Velez-Hernandez et al. 1988; Alcaraz et al. 1999; Mitchell et al. 2000). The life cycle is completed when an infected second intermediate host is consumed by a suitable definitive host.

Relevant Previous Work

Following the first report of this parasite in central Texas, various strategies have been employed to monitor the abundance and impact of the parasite in the Comal River. Mitchell et al. (2000) monitored the mean intensity (number of cysts per fish) and prevalence (percent of fish infected) in the red-rimmed melania and fish in both the San Marcos and Comal rivers from 1997 to 1998. Although these studies revealed the severity of *C. formosanus* infections, no consistent seasonal trends in parasite abundance were reported.

In 2003, Cantu examined the spatial and temporal variation of *C formosanus* in the Comal River by studying cercarial abundance in river water, and intensity and prevalence of the parasite in caged fountain darters. Cantu (2003) observed a significant positive association between the abundance of cercariae in the water near a cage and the number of cysts actually acquired by the caged darters.

Cantu's estimates of cercarial abundance in river water were based on only three samples taken right along side the cages, because he was concerned only with estimating how much infection pressure the caged darters had experienced. Consequently, Cantu's cercarial abundance estimates cannot be used as estimates of the overall total abundance of cercariae drifting through the entire cross section of the stream at his study sites.

Cantu (2003) also recommended that for studies designed to monitor changes in parasite abundance in the ecosystem over time, sampling the cercarial drift using the filtration technique would be more practical than counting metacercarial cysts on fish. He found that the filtration technique requires fewer resources, provides results with better precision, and does not require the sacrifice of an endangered fish.

Later, Lozano (2005) attempted to determine the minimum sampling effort required to obtain a stable estimate of the number of cercariae drifting through a site. He collected 45 cercarial samples along a single cross-section of the Comal River, but determined that five samples per cross section would be sufficient to accurately represent total cercarial drift for the rest of his study. Lozano (2005) also examined the relationship between his estimates of cercariae drift through the site relative to stream discharge at the site using a modification of Buchanan and Somers (1969) standard procedure for estimating wading discharge.

In the mid 1950's, Comal Springs ceased flowing and this is thought to be the most likely cause of the local extinction of darters in the Comal River (USFWS 1996). The species was successfully reintroduced using 457 darters, but in 1996, a drought brought stream discharge down to 2.35 cms (USGS), a level considered low enough to jeopardize survival of the darters again (Table 2 in USFWS 1996). During 1996 biologists began to find fish with swollen gills. This was caused by reaction to heavy infections with metacercariae of a trematode later identified as *C. formosanus*. In a follow-up study prompted by this development, Mitchell et al. (2000) determined that infection with these metacercariae could severely and permanently damage the respiratory system of individual darters, and estimated that infection intensity exceeding 800 cysts per fish was life threatening to the darters. Some of the darters collected from the Comal River at this time had many more than 800 metacercarial cysts per fish (as many as 1500+, Mitchell et al. 2002) indicating that the darter population was not only jeopardized by low stream discharge, but this threat was substantially compounded by the presence of this parasite.

Statement of the Problem

Infection pressure on fountain darters is a function of cercarial density (Cantu 2003). If the stream discharge rate passing by shedding snails is reduced by say, 50%, there will be a consequent proportionate doubling of cercarial density (count/L) downstream from the snails, essentially doubling the infection pressure on the darters downstream from the snails. Thus, during a drought that reduces discharge rates in the Comal River, darters downstream from shedding snails will experience increasing infection pressures as flow decreases. Thus, the presence of the parasite in the Comal ecosystem is expected to cause the darters to become threatened with local extinction at higher discharge rates (less severe drought) than if the parasite were absent.

Additionally, there is anecdotal evidence that the emergence rate of *C. formosanus* cercariae into the drift community of the Comal River does not remain constant when discharge rates change, but may increase when discharge from the springs decreases (T. Brandt, U.S. Fish and Wildlife, personal communication). If cercarial emergence rate is independent of current velocity, then as discharge rates change cercarial density would double if the discharge rate was reduced by 50%. But, if cercarial emergence rate increases as stream discharge decreases then reducing stream discharge by 50% would more than double the cercarial density and infection pressure on the darters. That would mean that, during a drought, the existing wild population of darters would not just experience an increase in cercarial infection pressure proportionate to volumetric reduction of water, but a compounded increase in infection pressure caused by the hypothetical increase in cercarial emergence rate caused by reduced discharge.

Since discharge rate goes down during an extended drought, and since the respiratory efficiency of the fish is now impacted by the trematode, the wild population could possibly go extinct earlier in a drought and at much higher discharge rates than those thought to have caused the previous extinction. So, if cercarial emergence rate does indeed increase as spring discharge decreases, then the presence of this worm in the Comal River may exacerbate the negative impact of drought on the survival of wild populations of the fountain darter beyond what would be expected from simple proportionate increase in cercarial density.

Project Objectives

The objectives of this project are:

1. To study cercarial drift in the Comal River over a period of at least 1 year to determine if there is evidence that cercarial shedding rate is increased when discharge decreases, and
2. To examine other physiochemical variables to determine if any of them may help explain any trends discovered in objective 1.

CHAPTER II

MATERIALS AND METHODS

Preliminary Study

A preliminary study was conducted from January to April 2006 in which six sites along the Comal River were sampled. A transect was established at each site and 10 equidistant points along the transect were selected for sampling. Three 5-L water samples were taken at each of the 10 points, one approximately 10 cm from the bottom, one at 60% depth, and one approximately 10 cm from the surface. This resulted in 30 water samples taken at each site. The difference between the sample taken at 60% depth and the sample taken at 10 cm from the surface was virtually indistinguishable, and therefore the final design did not include a sample 10 cm from the surface. The design of the preliminary study could not be extended into a long-term study because it would have required resources that exceeded the resource budget. Therefore, budget constraints required that the number of sampling sites be reduced to three.

Sampling Sites

Three sites along the Comal River were selected for this study (Figure 2). The Houston Street site (HS, 29.720777° Lat, -98.128097° Long) was located directly below Spring Five, which is one of the larger springs in this portion of the river. The next downstream site, Liberty Avenue (LA, 29.718766° Lat, -98.130305° Long) was located 300 m downstream from the Houston Street site at the west end of Liberty Avenue. These two sites were chosen because the springs that provide discharge through this stretch of the river were expected to exhibit the earliest reduction in flow as a drought progresses. Approximately 750 m downstream from LA, the flow is impounded by a dam which forms Landa Lake. Water flows from Landa Lake into two channels: the New Channel and Old Channel.

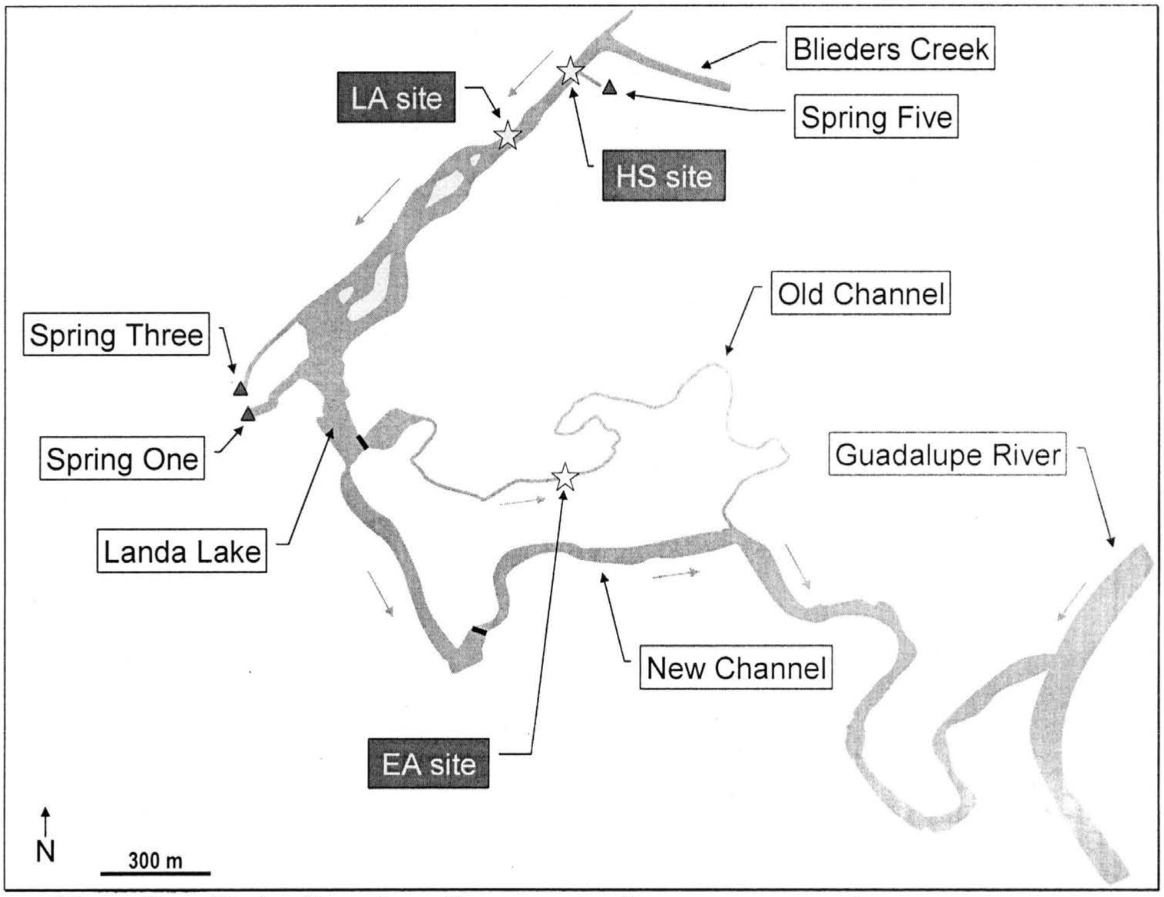


Figure 2. Study sites where *Centrocestus formosanus* cercariae were collected from the Comal River.

The third site, Elizabeth Avenue (EA, 29.710133° Lat, -98.128703° Long) is located in the Old Channel. The EA site is historically one of the most stable areas in the river because a culvert regulates flow from Landa Lake into the Old Channel, in order to reduce scouring from floods events. The Old Channel and New Channel converge 2.5 km downstream from Landa Lake and the river flows south another 2.5 km before the confluence of the Comal River with the Guadalupe River (USFWS 1996).

Site characterization data collected from each of the three sites included wading discharge, current velocity (Marsh-McBirney portable flowmeter, Model 2000, Frederick, Maryland), temperature, and dissolved oxygen (DO meter Model 58, YSI, Yellow Springs, Ohio), while total stream discharge was obtained from USGS. At times of sampling, degree of insolation ("sunny," "partly cloudy," "mostly cloudy," or "overcast") and the presence of piscivorous birds was also recorded.

Sampling Protocol: Final Design

A transect was established across the river at each site, from left bank to right bank (facing downstream), and six equidistant points along the transect were selected for sampling (Figure 3). Two 5-L samples of water were taken at each of the six points, one at approximately 10 cm from the bottom and one at sixty percent depth from the surface (V6). If the depth at a transect point was too shallow for two samples to be taken, then only one sample was taken at sixty percent depth.

Each of the three sites was sampled every two weeks for 12 months (June 2006-2007) between 0930 and 1230 hours (when possible), unless weather or flooding prohibited. The morning time period was chosen because snails infected with *C. formosanus* have been reported to release more cercariae diurnally than nocturnally (Lo and Lee 1996), which is similar to other positively phototactic trematode species (McClelland 1965). The six equidistant points along the transect were sampled in an alternating pattern in order to randomize diel effects over the sampling interval (Figure 3). Sampling was also conducted on sunny days when possible because cercarial abundance differed significantly between sunny and cloudy days (Lozano 2005).

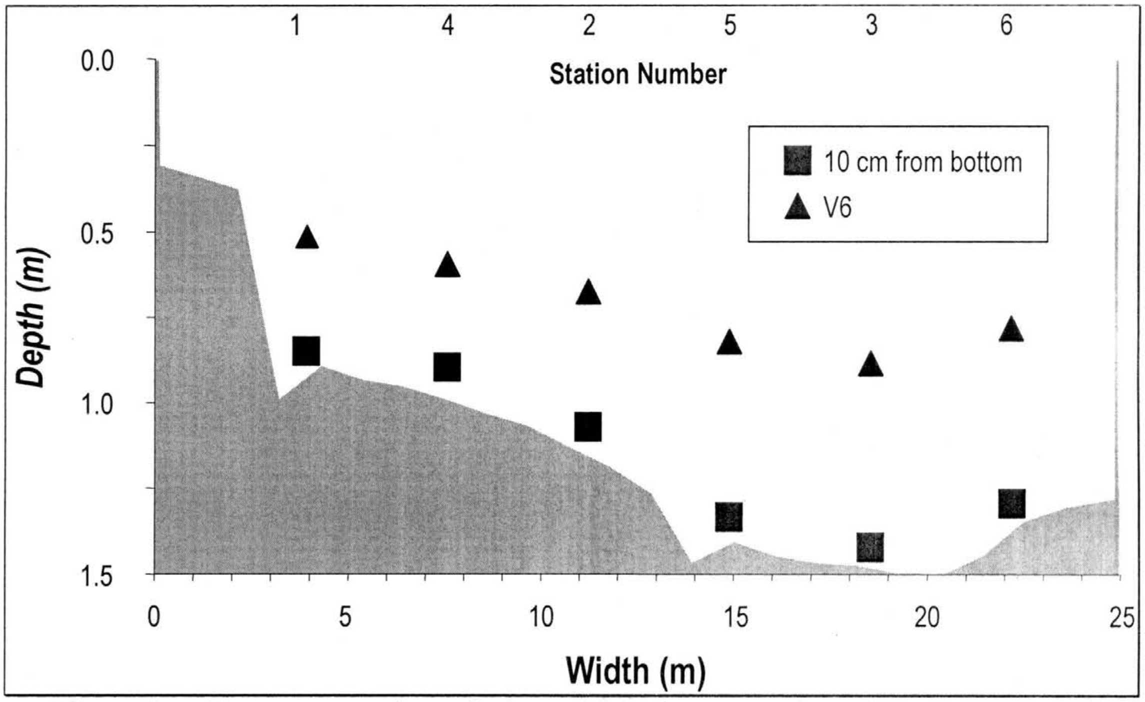


Figure 3. Downstream view of a hypothetical cross section of the Comal River illustrating the locations where samples were collected. The station number represents the sequence in which samples where collected.

A battery operated pump (Attwood aerator pump, Model A500, Lowell, Michigan) was used to transfer 5-L of river water through a 1/4" flexible tube into a 10-L bucket. To collect cercarial samples at various depths, the pump was attached to a flow rod. Current velocity at each cercarial sample point was taken first, while allowing any sediment disturbed during wading to settle. After current velocity was recorded, the pump opening was then pointed into the current to collect the water samples.

The LA and HS sites were sampled on the same date, since they are on the same stretch of the river, with LA sampled first followed by HS. To allow for cercarial sampling to be completed in the recommended diel timeframe, recording of wading discharge was delayed until after all water samples for the day had been collected. The EA site was sampled within one day of the other two sites.

Cercarial Counts

Immediately after collection, the cercariae in each water sample were fixed by adding 5 ml of formalin to the 5 L sample of river water to make 0.1% formalin solution. This was done to prevent cercariae from squeezing themselves through the filter during the filtration process. The sample was then stirred and poured through a filtration apparatus (Figure 4) modified from Theron (1979) and Prentice (1984). Cercariae in the water sample were small enough to pass through two prefilters with 220 μm and 86 μm mesh, respectively, but then collected on a 30 μm final filter. The waste water was collected in a 22.7 L waste container below the final filter and taken back to the laboratory to be treated with formalin neutralizer for proper disposal (Detox Formalin Neutralizer, Scientific Device Laboratory, Des Plaines, Illinois).

After all the water from a sample had passed through the apparatus, the final filter was removed, placed in a petri dish, stained with 1.5 ml of Rose Bengal, and preserved with 3 ml of 10% formalin on site. The petri dish was then sealed with Parafilm (Pechiney Plastic Packaging, Chicago, Illinois) to prevent desiccation until analysis. Sampling at each site typically resulted in 12 final filters.

At the laboratory, a paper counting grid (60 X 60 mm) was placed into a petri dish (95 mm diameter) and a final filter from a water sample was placed on the grid.

Water (~ 20 ml) was then added to the dish to help reduce glare. All cercariae from each final filter were counted under a dissecting microscope (100 X) and the total number was recorded.

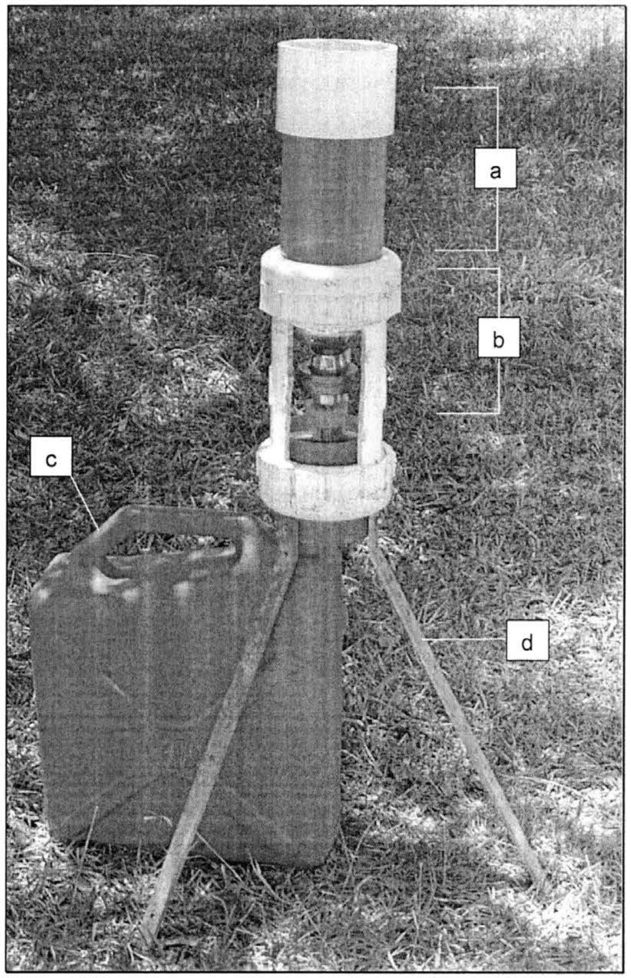


Figure 4. Modified filtration apparatus. a) prefilters (220μm and 86μm). b) sample filter mount with 30 μm monofilament filter. c) 22.7 L formalin collection container. d) filter apparatus support.

To prepare for the next sampling session, the filters were soaked in a 10% solution of sodium hydroxide and then sprayed with hot water to dislodge cercariae (Prentice 1984) so that the filters could be reused.

Effort Required

During the one year study period (apart from the preliminary study), a total of 48 days were spent in the field and 336 hours were expended collecting samples. HS and EA each resulted in 12 samples per visit and 11 samples were collected at LA per visit. This resulted in a total of 840 water samples (4200L) collected during the study.

During the one year period, (apart from the preliminary study), a total of 42 days were spent in the laboratory. Approximately 15 minutes was required to count the number of cercariae per filter, therefore 180 hours total was spent counting cercariae. The number of *C formosanus* cercariae counted was 17,563 and the number of *Haplorchis pumilio* cercariae counted was 132. Therefore, the total number of cercariae counted during the study was 17,695.

Numerical Methods

The number of *C. formosanus* cercariae per liter was calculated by dividing the total number of cercariae counted from a final filter by 5L. The number of cercariae/L was transformed to the meet parametric assumption of normality. The transformation used was the square root of the number of cercariae plus three eighths $\sqrt{N / \mathrm{L}+3/8}$ (Zar 1999). Means are reported in the form: mean ± SE (min-max).

Cercarial abundance (number of cercariae/L) will be regressed independently against total stream discharge, wading discharge, current velocity, temperature, and dissolved oxygen to determine if cercarial abundance varies in response to changes in any of these variables. Regression analyses were tailored to the data post hoc to find a trendline that best fits the model. Regressions used in this study were linear and third order polynomial.

Total stream discharge and wading discharge were recorded in cubic meters per second (cms). Total stream discharge values were obtained from USGS, based on a gage located below the confluence of the Old Channel and New Channel (Lat 29°42'21", Long 98°07'20"), while wading discharge was calculated at each site at the time of sampling. Wading discharge calculations were adapted from the method of Buchanan and Somers (1969). Wetted stream width was divided into approximately 25 equal segments (n=25). Stream depth was then recorded at the junctions of the segments resulting in n-1 depths (Figure 5). If stream depth at the segment junction was 0.76 m or less, one velocity measurement was taken at 60% depth. If depth exceeded 0.76 m, two velocity measurements were taken, one at 20% depth and the other at 80% depth from the surface. Later, partial discharge at each segment was calculated by multiplying depth at junction × segment width × mean velocity. The partial discharges from a stream cross section were summed to determine the wading discharge for the cross section on that sampling date.

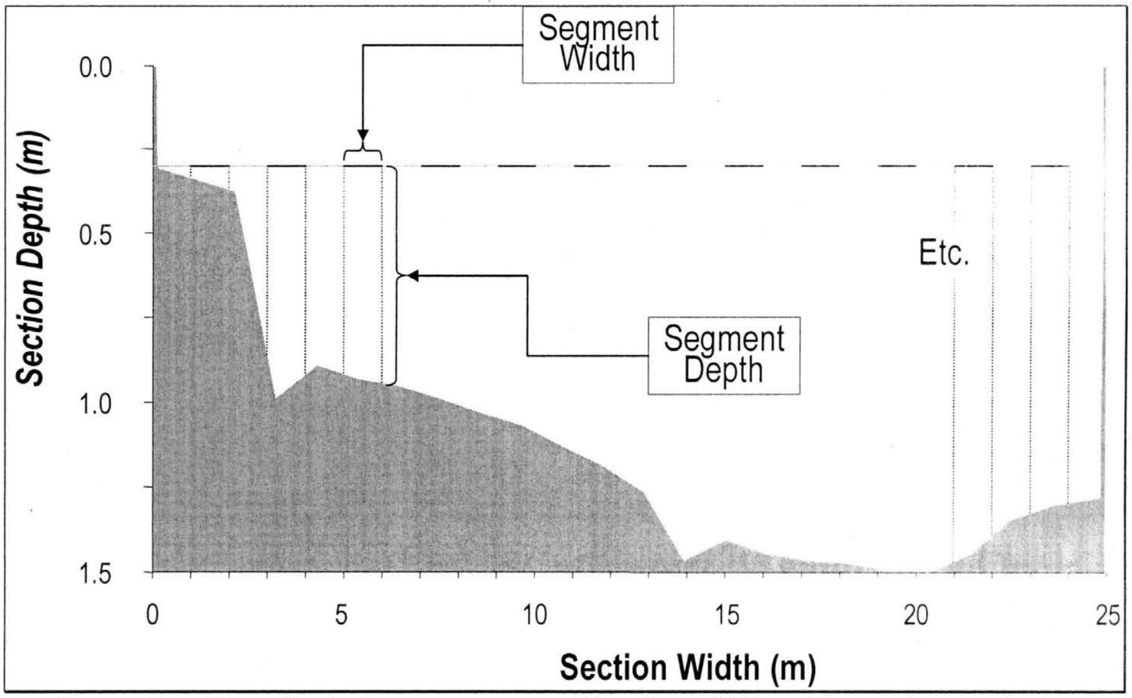


Figure 5. Hypothetical cross section of the Comal River illustrating method of computing wading discharge.

Current velocity was recorded in meters per second (m/s), temperature in degrees Celsius (°C), and dissolved oxygen in milligrams per liter (mg/L). Percent oxygen saturation in water was calculated using the temperature and dissolved oxygen data (WOW 2004a).

Data in this study were recorded in metric units, the equivalents to imperial units are as follows: 1 m^3=35.315 ft^3, 1 m=3.281 ft, 1 L=0.264 gal, and 1 °C=33.8 °F (WOW 2004b).

CHAPTER III

RESULTS

Descriptive Statistics

The mean total stream discharge during the study was 7.61 cms ± 0.04 (5.78-12.49). Total stream discharge during the study was slightly lower than the historical mean (total annual mean from 1933 to 2005 is 8.58 cms).

The mean wading discharge for HS was 0.38 cms ± 0.01 (0.02-0.82). The mean wading discharge for LA was 0.45 cms ± 0.02 (0.12-0.93). The mean wading discharge for EA was 1.42 cms ± 0.01 (1.17-1.62).

The mean current velocity recorded along with the water samples taken at HS was 0.02 m/s ± 0.001 (0.00-0.08). The mean current velocity for samples at LA was 0.02 m/s ± 0.001 (0.00-0.08). The mean current velocity for samples at EA was 0.19 m/s ± 0.01 (0.003-0.44).

The mean temperature during the study was 23.48 °C ± 0.04 (21.0-26.1). The mean amount of dissolved oxygen during the study was 7.30 mg/L ± 0.04 (5.5-10.4).

During the study the mean number of cercariae was 4.18/L ± 0.16 [0.00(in nine samples)-60.6] across all sites. The mean number of cercariae per cross section ranged from 0.48-12.32/L, and by using wading discharge to calculate the number of liters per cross section this indicates that there could be between 1.21 and 1,813 cercariae passing through a cross section at one time.

During the 48 days spent in the field, Green Herons were observed nine times across all sites, with two being the greatest number observed on one sampling date. The LA site had the greatest number of observations during the one year period with a total of seven.

Overall Counts

Effects of discharge

Linear regression was used to search for effects of discharge on cercarial abundance. The number of *C. formosanus* cercariae decreased as total stream discharge increased [$p(F_{1,838}\geq 46.38)<0.0001$]. However, because only about 5% of the variance in cercarial counts could be explained by total stream discharge (Figure 6, R^2=0.052), the variable was dismissed as a useful predictor of cercarial abundance. The number of *C. formosanus* cercariae increased as wading discharge increased [$p(F_{1,780}\geq 206.24)<0.0001$]. However, because only about 21% of the variance in cercarial counts could be explained by wading discharge (Figure 6, R^2=0.209), the variable was dismissed as a useful predictor of cercarial abundance.

Seasonal Effects

Nonlinear regression was used to search for effects of season on cercarial abundance, and a seasonal relationship was observed [$p(F_{3,836}\geq 17.60)<0.0001$]. The minimum cercarial count predicted by the regression was in March, and the maximum predicted cercarial count was in November. Even though this model had a very small *p* value, season was dismissed as a useful predictor of cercarial abundance because only about 6% of the variance in cercarial counts could be explained by season (Figure 7, R^2=0.059).

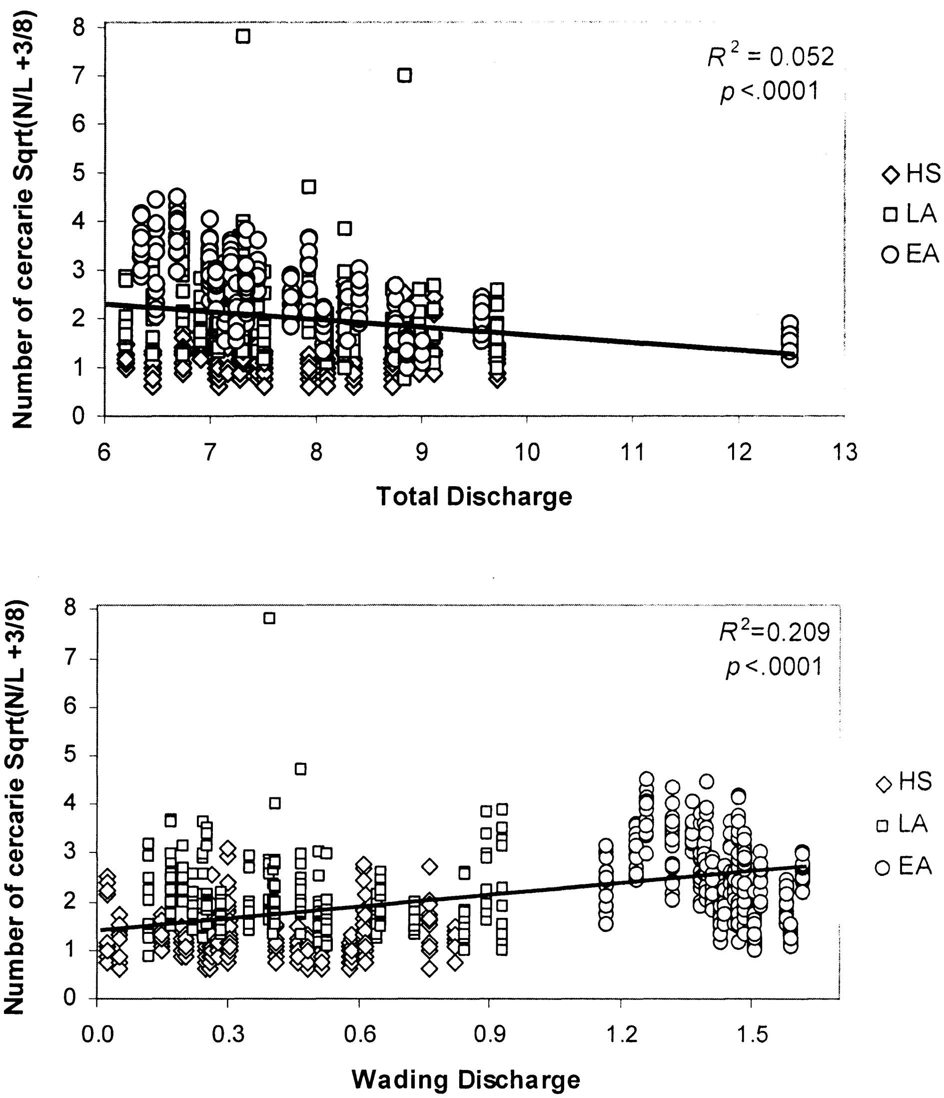


Figure 6. Effects of total and wading discharge (cms) on the abundance of *C. formosanus* cercariae.

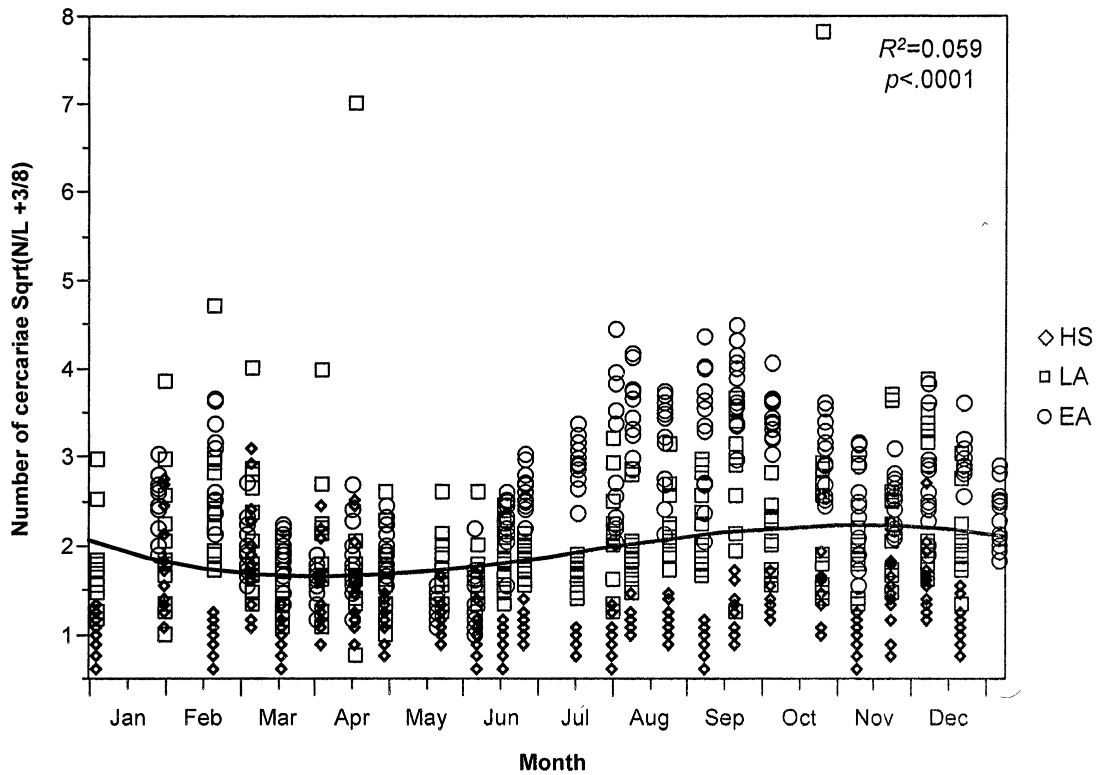


Figure 7. Effects of season on the abundance of *C. formosanus* cercariae

Effects of Site

One-factor ANOVA was used to search for effects of site on cercarial abundance. The mean number of *C formosanus* cercariae counted at EA [6.75/L ± 0.024 (.60-19.80)] was found to be higher than LA [4.53/L ± 0.33 (0.20-60.60)], followed by HS [1.31/L ± 0.08 (0.00-9.20); $p(F_{2,837} \geq 280.64) < 0.0001$]. Therefore, it appears that the further downstream a site was, the higher the cercarial abundance. Table 1 provides the ANOVA table and means table for the transformed counts.

Effects of Insolation

One-factor ANOVA was used to search for effects of insolation on cercarial abundance. The degree of insolation on a sampling date was subjectively classified as "sunny," "partly cloudy," "mostly cloudy," and "overcast." However, because insolation is a random factor, unequal allocation of these four categories among the three sites complicated the analysis, and some insolation categories were never recorded at some of the sites. Consequently, all sampling dates with insolation recorded as "overcast," "mostly cloudy," or "partly cloudy" were subsequently pooled into a new category referred to as "cloudy" from this point forward. This resulted in two categories for insolation, "sunny," and "cloudy."

After these modifications, the mean number of *C. formosanus* cercariae counted on sunny days [4.49/L ± 0.23 (0.00-60.6)] was found to be higher than on cloudy days [3.72/L ± 0.19 (0.00-18.60); $p(F_{1,838} \geq 4.38) = 0.037$]. Table 2 provides the ANOVA table and means table for the transformed counts.

Table 1. a) One factor ANOVA table for the effects of site on the abundance of *C. formosanus* cercariae. b) Transformed means table for effects of site using one factor ANOVA.

a

Source of Variation	DF	SS	MS	F	*p*
Site	2	260.078	130.039	280.640	<0.0001
Error	837	387.838	0.463		
Total	839	647.916			

b

Site	Number	Mean	Std Error
HS	288	1.224	0.040
LA	264	2.073	0.042
EA	288	2.552	0.040

Table 2. a) One factor ANOVA table for the effects of insolation on the abundance of *C. formosanus* cercariae. b) Transformed means table for the effects of insolation using one factor ANOVA.

a

Source of Variation	DF	SS	MS	F	*p*
Insolation	1	3.367	3.367	4.378	0.037
Error	838	644.550	0.769		
Total	839	647.916			

b

Insolation	Number	Mean	Std Error
Cloudy	337	1.869	0.048
Sunny	503	1.998	0.039

Interaction between Insolation and Site

The analyses of insolation and site effects both resulted in very small *p* values; however, the reported *p* values may be erroneous because of possible interactions between insolation and site due to the unequal allocation of insolation categories. In order to determine if there was a significant interaction between these two variables, a two factor ANOVA with replication was executed. A two factor ANOVA requires a balanced data set (equal sample sizes) and so a random subset of sunny days was taken to equal the total number of cloudy days. Though this reduced the degrees of freedom for the analysis, the resulting cell size in the model was 96 sunny/cloudy pairs. The analysis did not reveal a significant interaction between insolation and site (Table 3 p=0.722).

Table 3. Insolation and site effects on the abundance of *C. formosanus* cercariae using a two factor ANOVA.

Source of Variation	DF	SS	MS	F	p
Weather	1	6.109	6.109	15.879	<0.0001
Site	2	186.203	93.101	242.008	<0.0001
Interaction Wx:Site	2	0.251	0.125	0.326	0.722
Error	570	219.281	0.385		
Total	575	411.843			

Effects of Variables by Site

The abundance of *C. formosanus* cercariae was regressed against several variables within each site. Total stream discharge was found to be a poor predictor of cercarial abundance at all three sites because 40% or less of the variance in cercarial counts could be explained by total stream discharge (Figure 8). The R^2 value for total stream discharge at EA (R^2=.407) was the highest value of any regression in the study, and while it was high compared to all other R^2 values calculated, it was not consistent across all sites. Also, the trend at EA is being driven by a single event of high flow (12.49 cms), which if considered an outlier would bring the R^2 value down to 7.4%. Wading discharge was found to be a poor predictor of cercarial abundance at all three sites because 20% or less of the variance in cercarial counts could be explained by wading discharge (Figure 9).

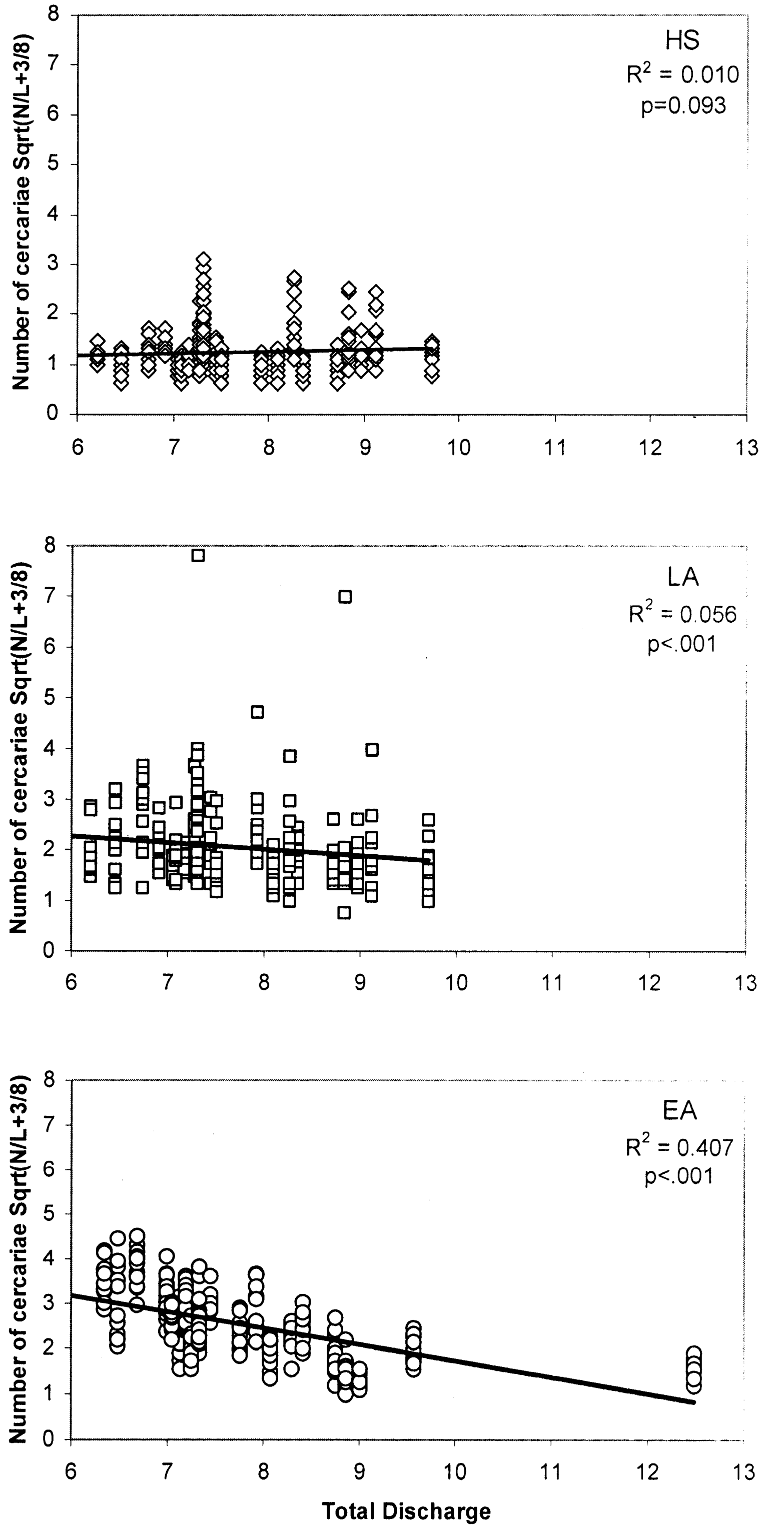


Figure 8. Number of *C. formosanus* cercariae regressed against total stream discharge (cms) at each site.

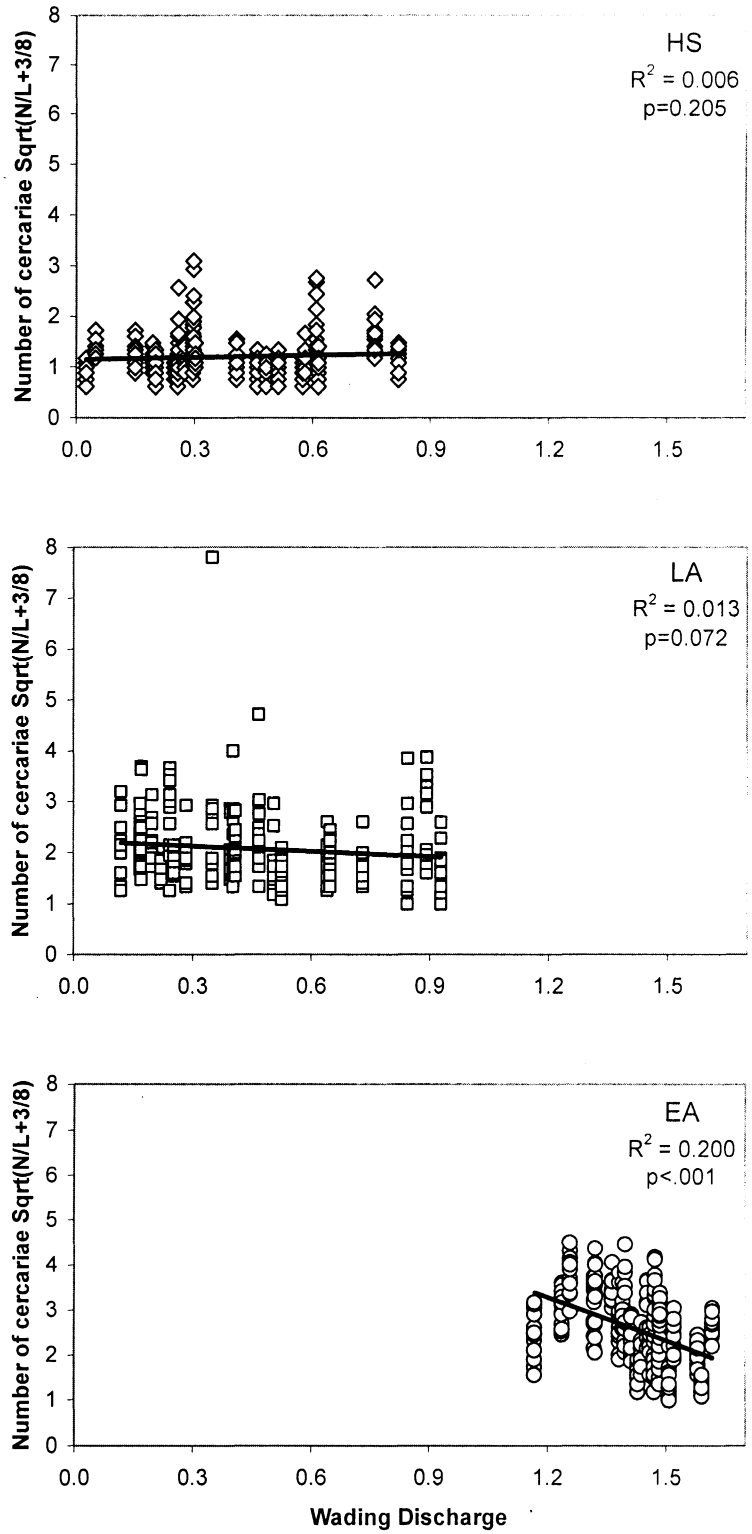


Figure 9. Number of *C. formosanus* cercariae regressed wading discharge (cms) at each site.

Current velocity was found to be a poor predictor of cercarial abundance at all three sites because 0.4% or less of the variance in cercarial counts could be explained by current velocity (Figure 10).

Temperature was found to be a poor predictor of cercarial abundance at all three sites because 12.2% or less of the variance in cercarial counts could be explained by temperature (Figure 11).

Dissolved oxygen was found to be a poor predictor of cercarial abundance at all three sites because 9.5% or less of the variance in cercarial counts could be explained by dissolved oxygen (Figure 12).

Percent oxygen saturation was also found to be a poor predictor of cercarial abundance at all three sites because 7% or less of the variance in cercarial counts could be explained by percent oxygen (Figure 13).

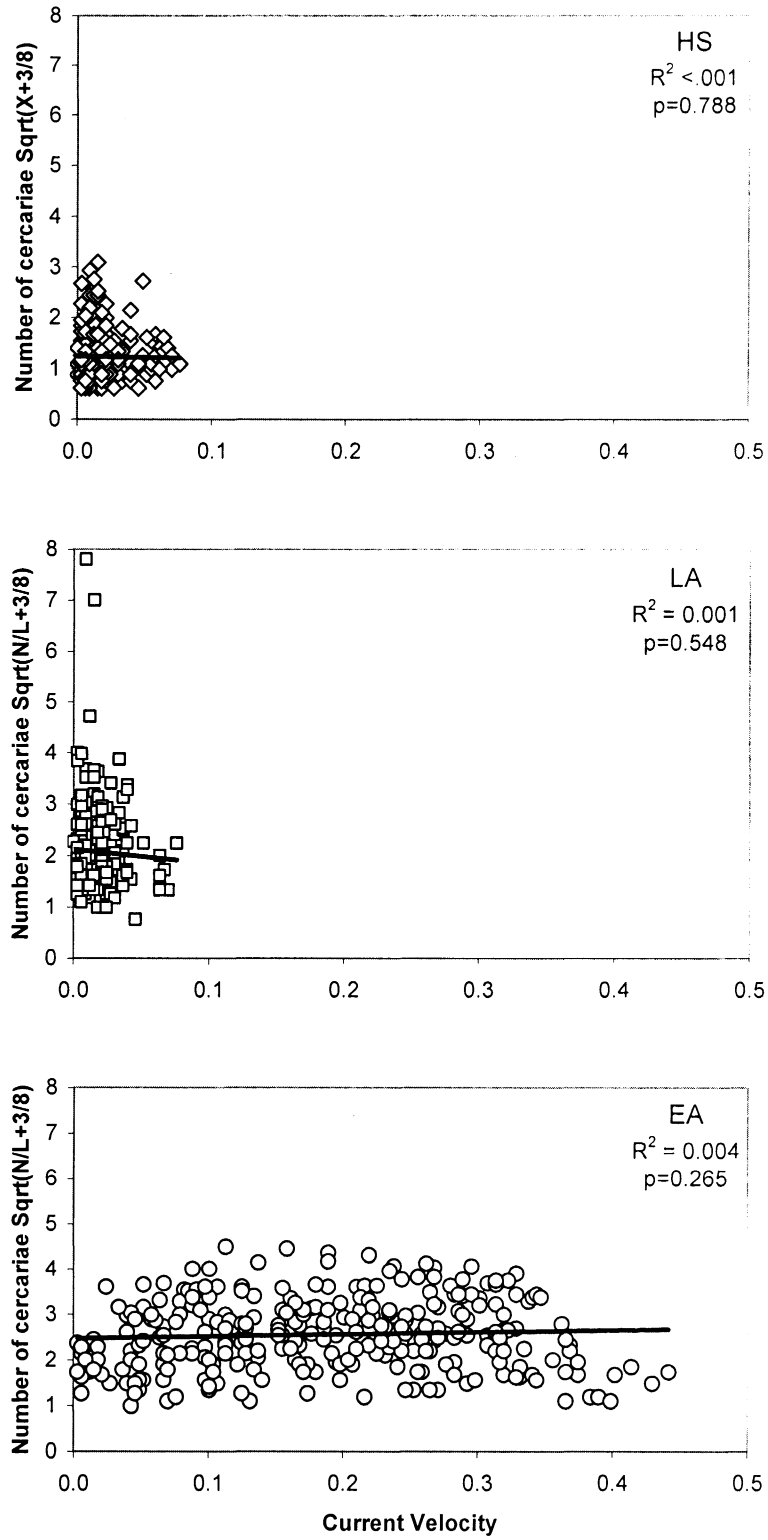


Figure 10. Number of *C. formosanus* cercariae regressed against current velocity (m/s) at each site.

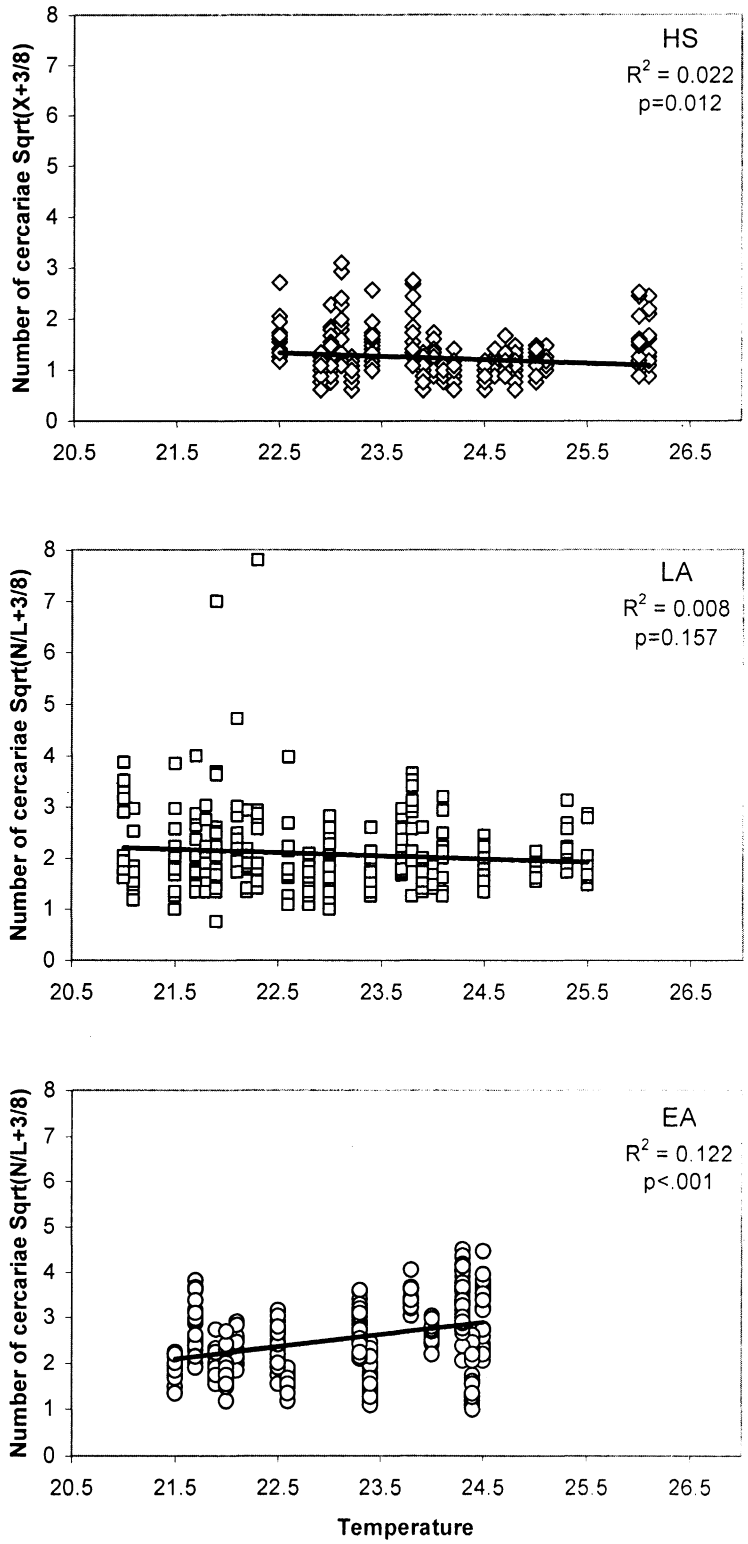


Figure 11. Number of *C. formosanus* cercariae regressed against temperature (°C) at each site.

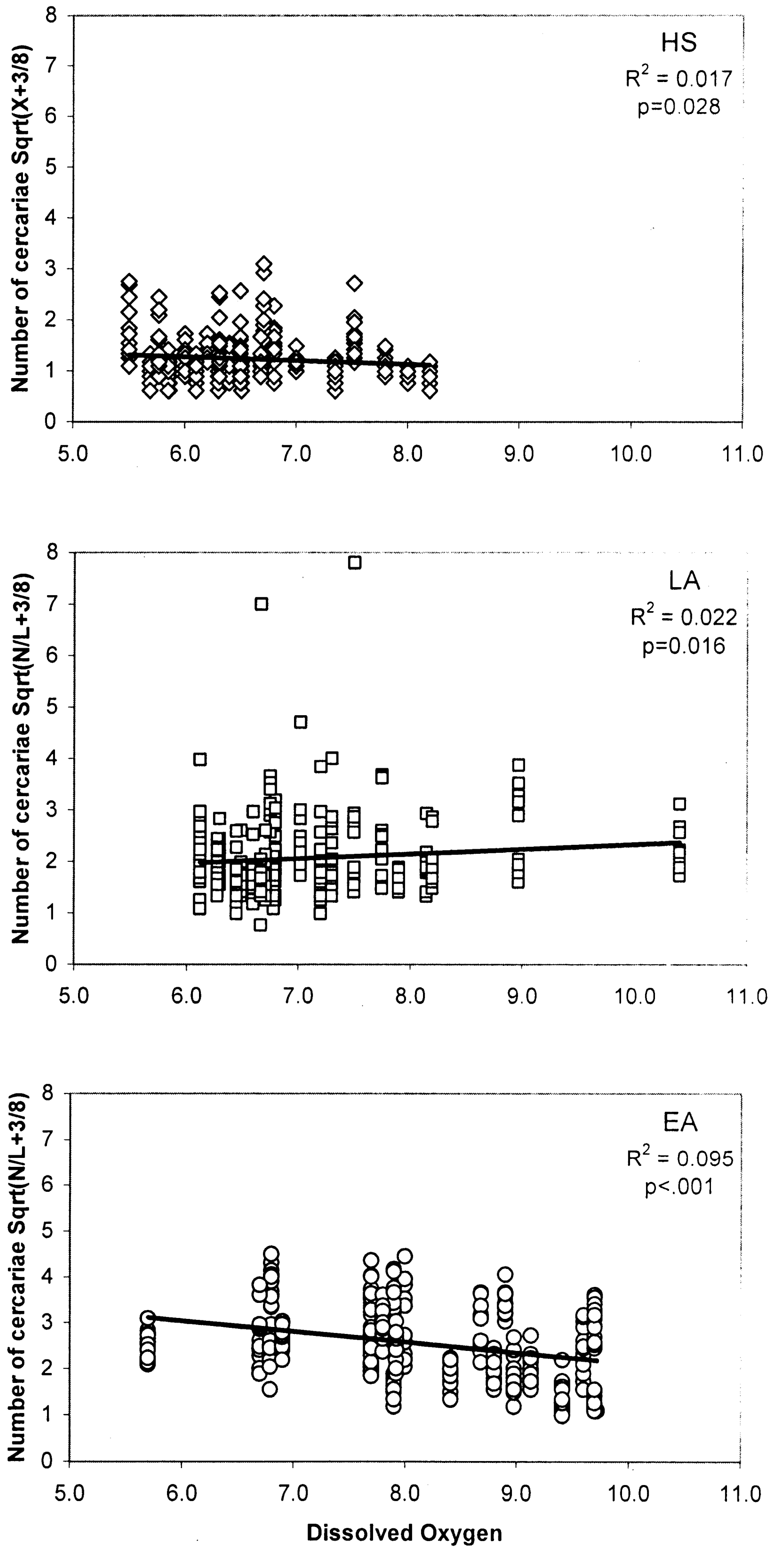


Figure 12. Number of *C. formosanus* cercariae regressed against dissolved oxygen (mg/L) at each site.

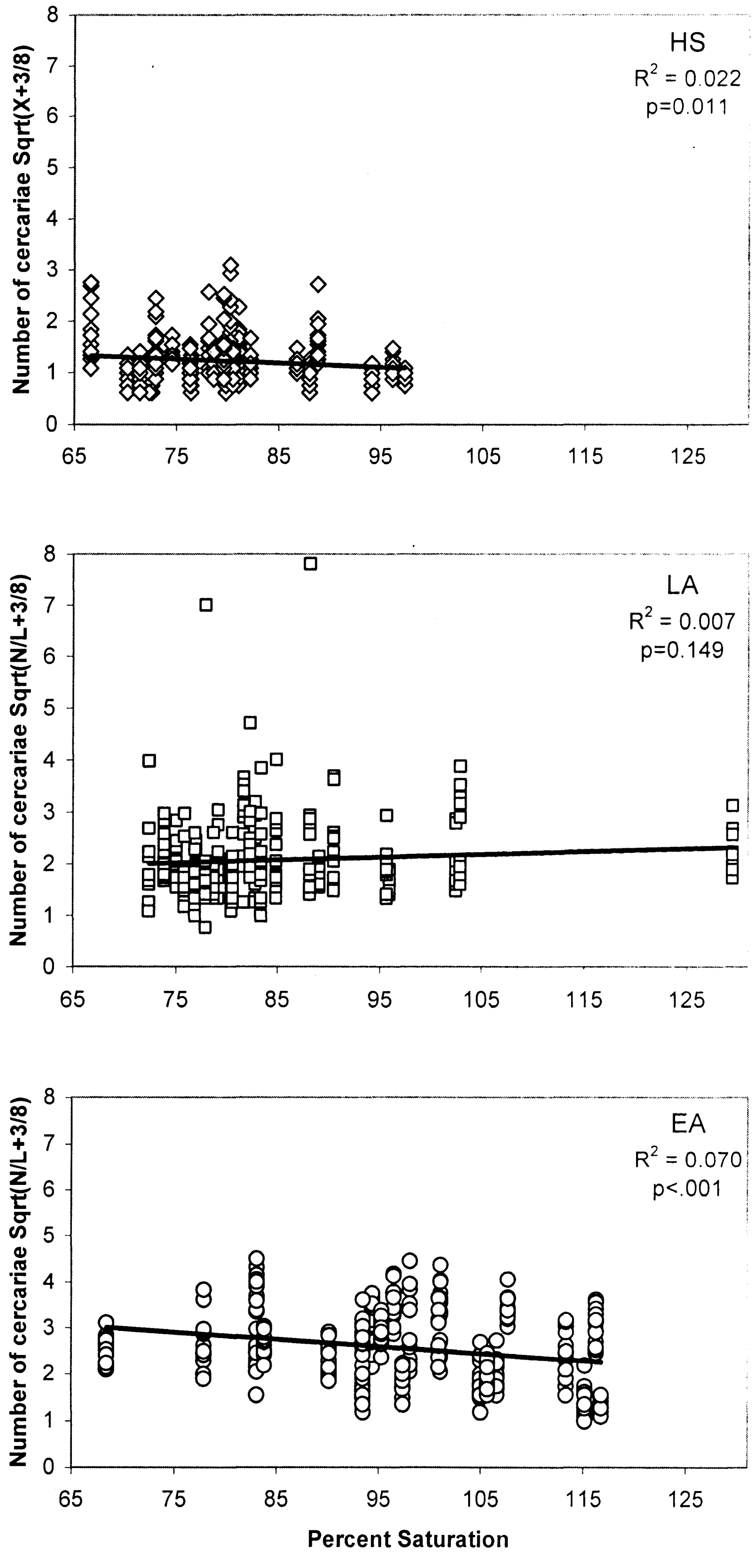


Figure 13. Number of *C. formosanus* cercariae regressed against percent saturation at each site.

Removing the Confounding Effects of Site and Insolation

Cercarial abundance differed by site with increasing numbers of cercariae as distance downstream increased. Cercariae drifting through a site could come from any point upstream, and this makes it difficult to explore relationships between cercarial abundance and discharge-related effects, especially at EA, because of the uncertainty about the location where a given cercaria was originally shed.

Since the LA and EA sites receive water from upstream, the data from these sites are likely to be contaminated by cercarial-laden drift from upstream. Therefore, the cercarial count data from the uppermost site, HS, was considered to be more responsive to local conditions, and so data from this site became the focus of subsequent analyses.

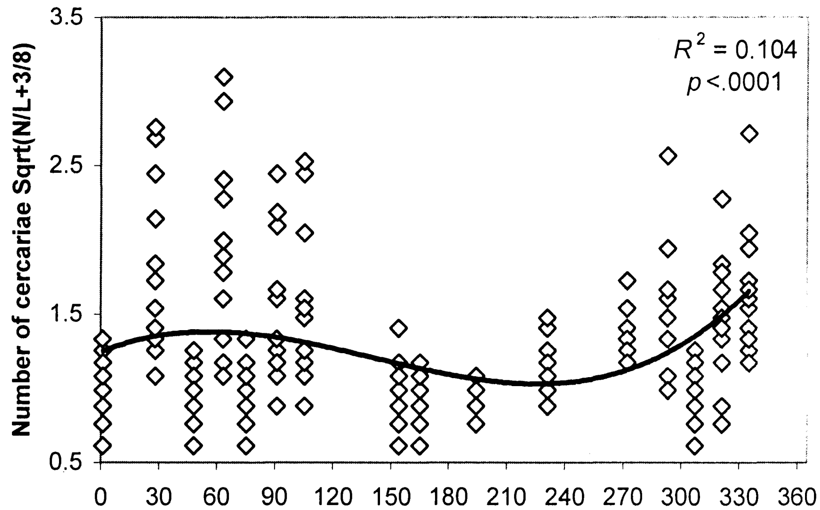
Because insolation had an affect on cercarial count and the focus is now HS, cercarial abundance collected on sunny days only was regressed against stream variables at the HS site. Even after removing cumulative and insolation effects, all variables were found to be poor predictors of cercarial abundance because 16.68% or less of the variance in cercarial counts could be explained by any variable (Figures 14-20).

Removing the Confounding Effects of Season

Even after the dataset was restricted as described above, there were still seasonal patterns in the distribution of cercarial abundance. Thus, any apparent trends in cercarial abundance related to other variables could still be confounded by covariance with season. The seasons generally having the highest cercarial abundance were late fall through late spring (Julian days 250-120), and so only these sample dates were included in the final analyses in an attempt to reduce confounding effects of uncontrolled interaction between season and the other variables.

Though these efforts to reduce the potentially confounding effects of site, insolation, and season reduced the degrees of freedom for the analysis by half, the resulting model still had 144 degrees of freedom. However, even after removing site effects, insolation effects, and seasonal effects, all variables were found to be poor predictors of cercarial abundance because 13.36% or less of the variance in cercarial counts could be explained by all variables (Figures 14-20).

a



b

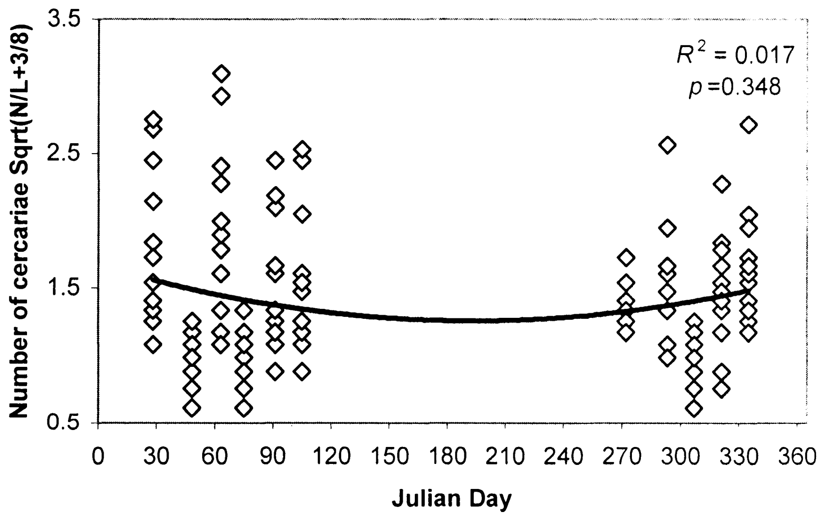
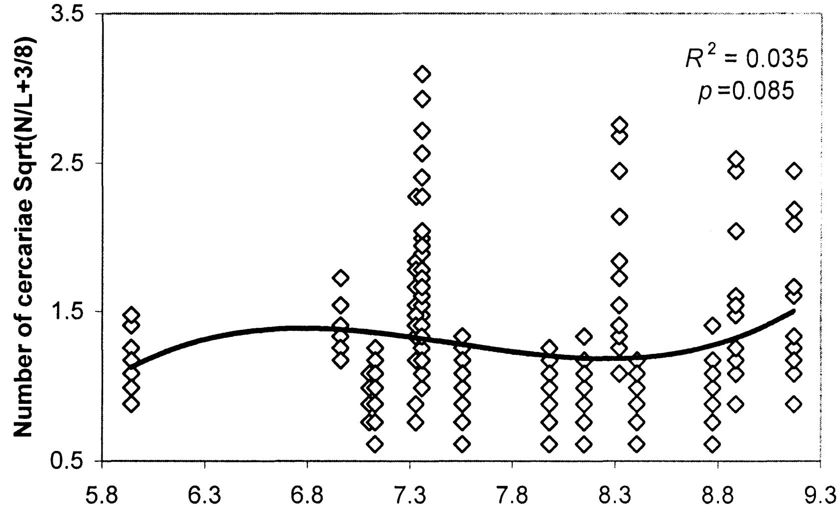


Figure 14. Number of *C. formosanus* cercariae at HS regressed against a) Julian day on sunny days only and b) Julian day on sunny days only and during times of peak emergence.

a



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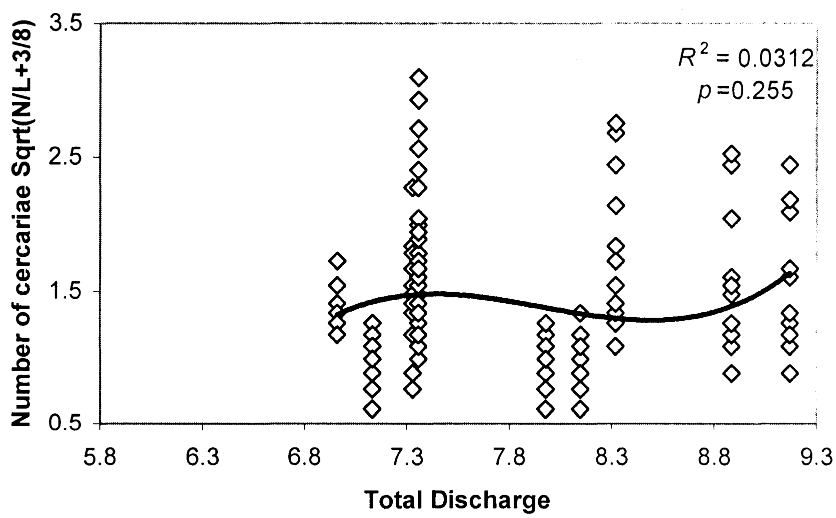
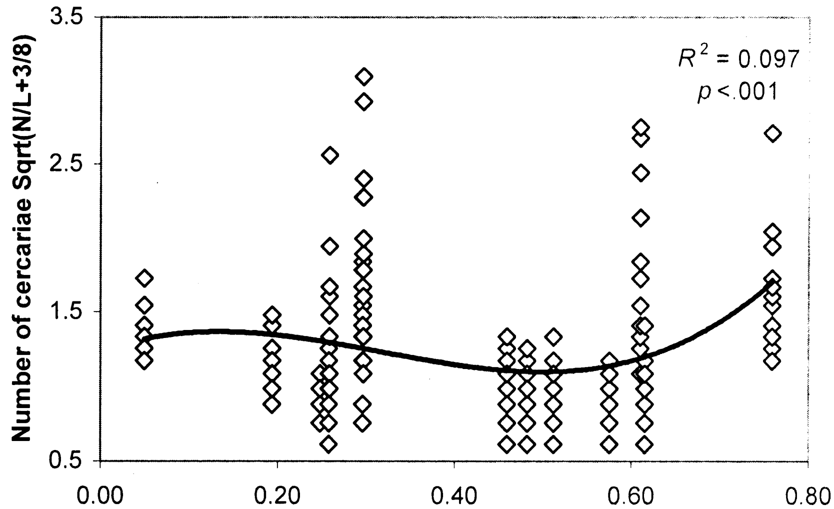


Figure 15. Number of *C. formosanus* cercariae at HS regressed against a) total stream discharge (cms) on sunny days only and b) total stream discharge on sunny days only and during times of peak emergence.

a



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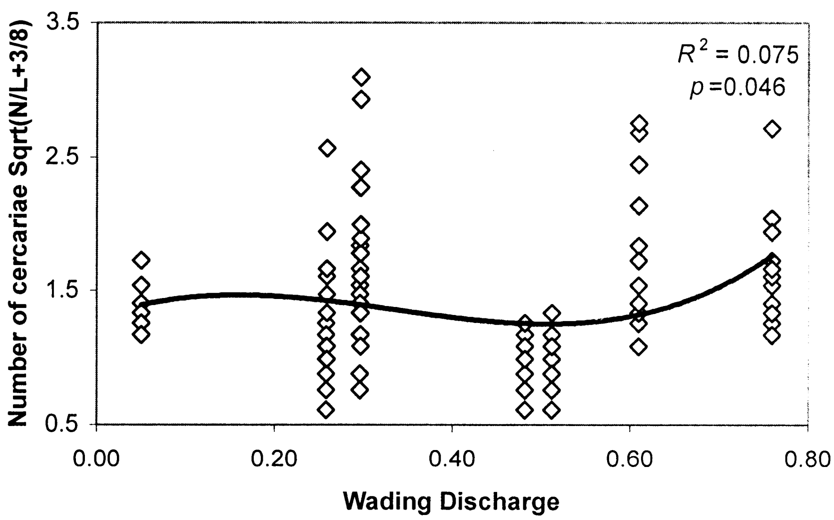
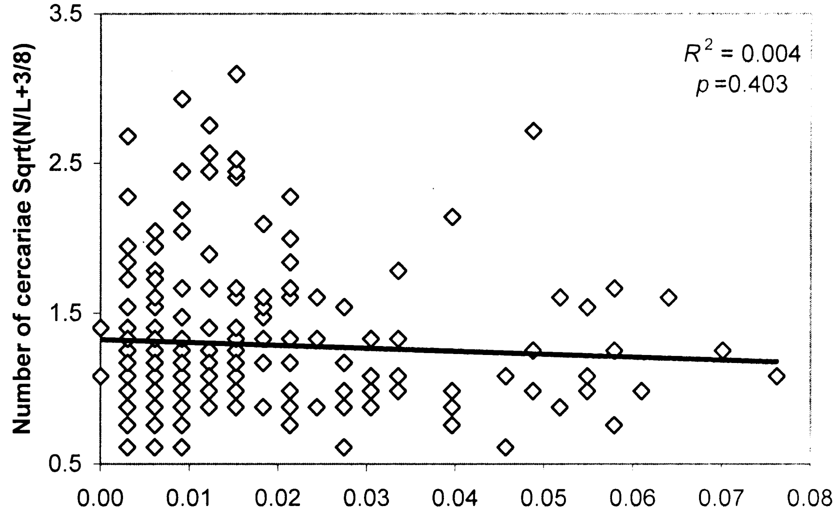


Figure 16. Number of *C. formosanus* cercariae at HS regressed against a) wading discharge (cms) on sunny days only and b) wading discharge on sunny days only and during times of peak emergence.

a



b

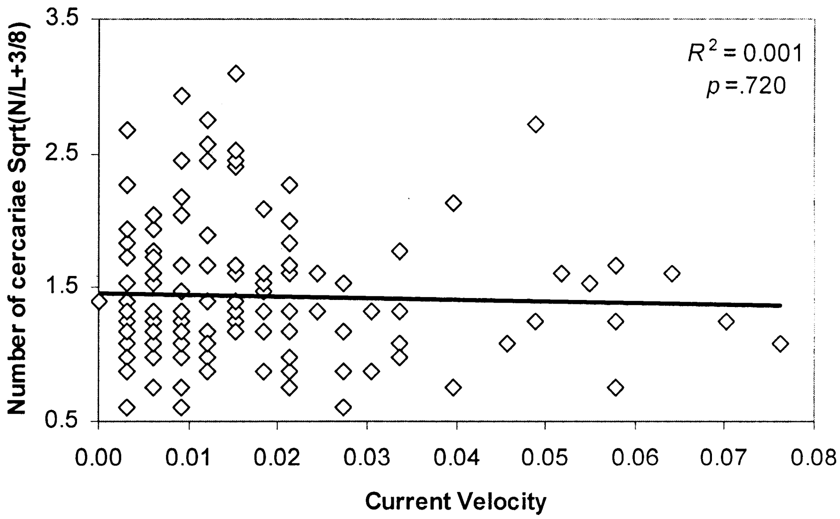
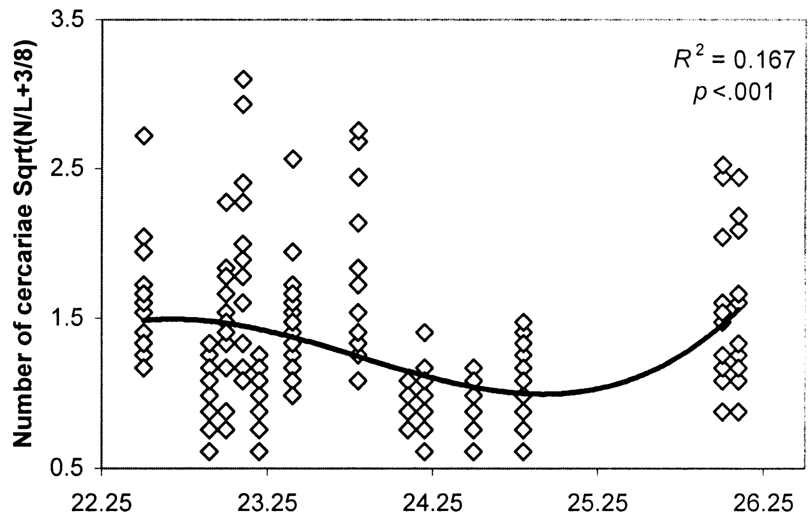


Figure 17. Number of *C. formosanus* cercariae at HS regressed against a) current velocity (m/s) on sunny days only and b) current velocity on sunny days only and during times of peak emergence.

a



b

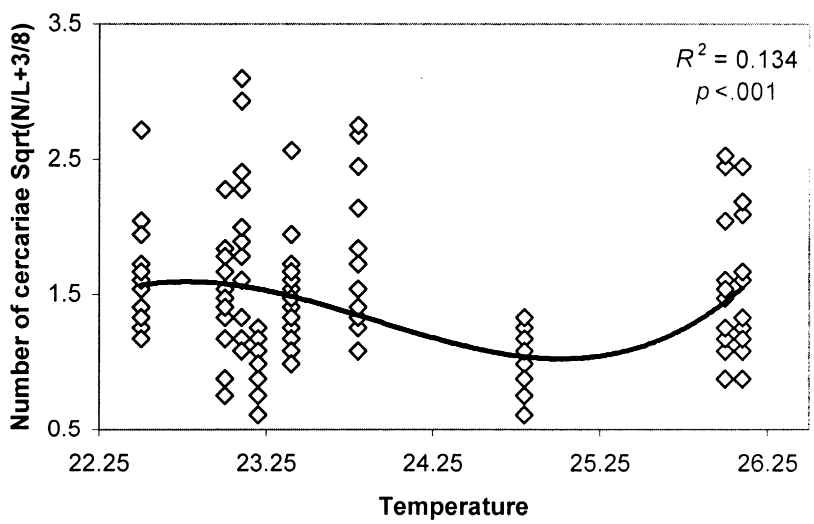
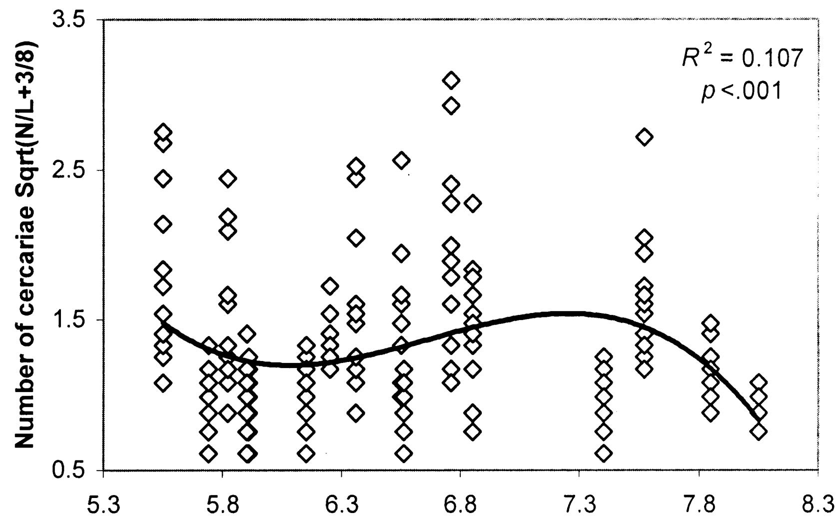


Figure 18. Number of *C. formosanus* cercariae at HS regressed against a) temperature (°C) on sunny days only and b) temperature on sunny days only and during times of peak emergence.

a



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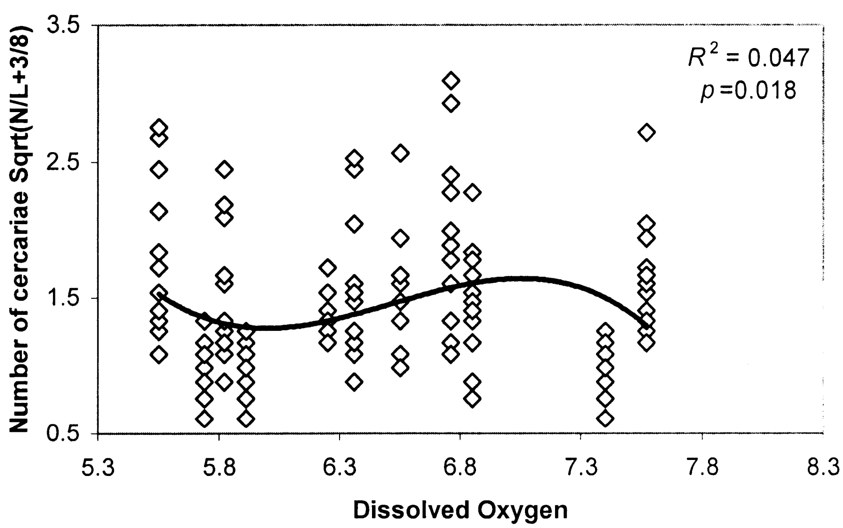
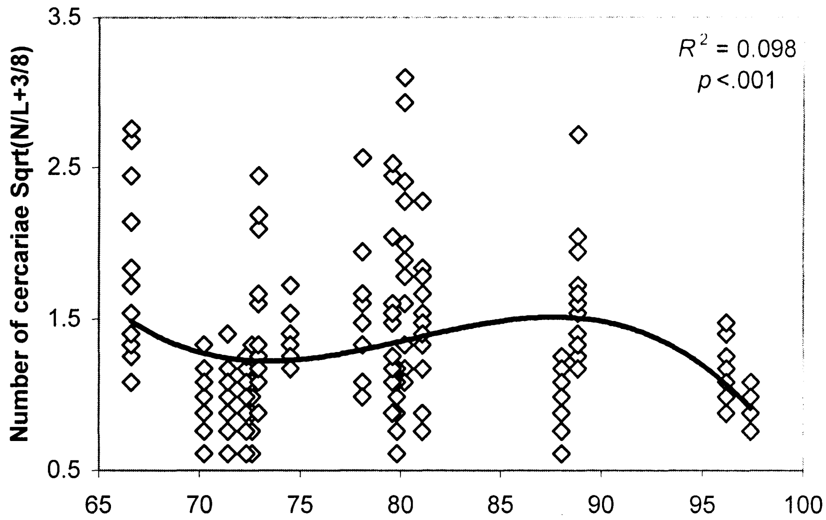


Figure 19. Number of *C. formosanus* cercariae at HS regressed against a) dissolved oxygen (mg/L) on sunny days only and b) dissolved oxygen on sunny days only and during times of peak emergence.

a



b

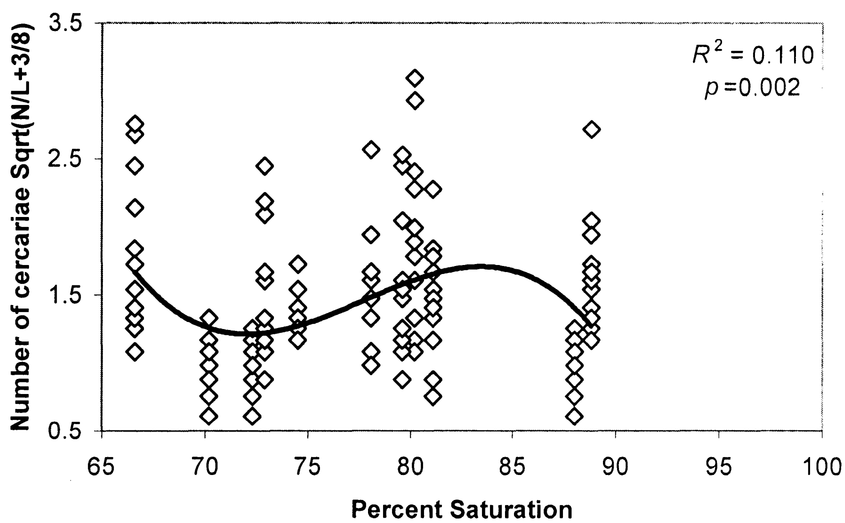


Figure 20. Number of *C. formosanus* cercariae at HS regressed against a) percent oxygen saturation on sunny days only and b) percent oxygen saturation on sunny days only and during times of peak emergence

CHAPTER IV

DISCUSSION

Objectives and Expected Results

The objectives of this study were to determine (1) if cercarial emergence rate in the Comal River increased with decreasing discharge, and (2) if discharge or any other site-characterization variable could be used to predict cercarial abundance. This information was expected to contribute to the management of fountain darters in the Comal River.

Conclusions

Sites in this study with the stillest waters (HS and LA) had lower cercarial abundance than the faster flowing site (EA). Given a snail shedding cercariae at a constant rate, the cloud of cercariae around that snail would be denser if that snail were in the slow flowing sites than if it were in the fastest flowing site. However, since the life expectancy of *C. formosanus* cercariae in the drift (100% survival for 50 hours at 25°C, Lo and Lee 1996) exceeds the transit time from the most upstream site (HS) to the most downstream site (EA), cercariae collected at EA were not only from snails shedding at the EA site, but also from many snails shedding cercariae upstream from EA. This cumulative effect swamped any tendencies for cercarial abundance to be higher in slow-flow conditions at HS and LA. For this reason, the HS site, which is near the very beginning of the river, was thought to have the least contaminated data regarding any effects of local conditions on cercarial abundance. Furthermore, total stream discharge measurements were always reported from the USGS gage downstream from the furthest downstream study site, therefore total stream discharge reports may not be representative of discharge effects on snails at the HS site. Wading discharge, on the other hand, was recorded locally, and was expected to be more representative of the current velocity experienced by snails shedding cercariae at the HS site at the times cercarial samples were collected. On these grounds, total discharge was eliminated as a useful variable for predicting cercarial abundance. and any observed trends between total stream discharge and cercarial abundance were dismissed.

Another potentially confounding factor was uncontrollable variation in insolation. Sunny days had higher cercarial counts than cloudy days, which agreed with the results of a previous study (Lozano 2005). Light is the key stimulus in emergence of *Schistosoma mansoni* and *S. haematobium* cercariae (McClelland 1965) and *C. formosanus* cercariae is similar in that emergence can take place in the dark, but the numbers are greatly reduced (Lo and Lee 1996). In order to eliminate potentially confounding effects of insolation on cercarial abundance, all analyses were performed only on data collected on days recorded as sunny.

Even when data were restricted as described above, there were seasonal patterns in the distribution of cercarial abundance, and so only data collected from late fall through late spring (Julian days 250-120) were included in the final analyses.

With the above restrictions in place, there was still a sufficient range of wading discharge at the HS site (0.05-0.76 cms) to provide evidence of any discharge-related effects on cercarial abundance, but no useful association between wading discharge and cercarial abundance was observed.

One troubling caveat remains in the filtered HS data. The cercarial abundance data for Julian days 1 – 90 alternated dramatically between successive sampling days (Figure 14), and no cause for such alternation could be found in the other variables recorded.

Predictors of Cercarial Abundance

In all of the regressions none of the variables explained a large proportion of the variance in cercarial abundance and therefore all variables were dismissed as useful predictors of cercarial abundance.

One reason for this outcome could be due to the fact that discharge did not decrease into drought conditions during the study. Spring Five flowed during the entire year of sampling. If Spring Five, which is the main water source for the upper sites, had stopped flowing, then perhaps cercarial abundance would have changed more dramatically with wading discharge.

Implications

The results of this study imply that the wild fountain darter population in the Comal River will not experience increased cercarial infection pressure due to reduced discharge within the observed range of this study.

Suggestions for Future Research

The results of this study now provide baseline data to which future cercarial samples can be compared, but this study also raises a number of questions that would benefit from more research.

A study utilizing a living stream could be useful in discovering if there is a relationship between cercarial abundance and discharge while having control over variables. For example, a living stream study could be designed to explore a wider range of discharge values and simulate severe drought conditions that were not observed in the field. Interactions between variables could also be managed and therefore the outcome could be less confounded.

The issue of how many species may be represented in the metacercariae and cercariae remains an interesting challenge, and must be resolved before the results of this study can be used to develop management strategies. Studies such as this can still provide information that may be useful in mitigating the exacerbating effects of theses parasites on the fountain darter. The resolution of the taxonomic questions will not threaten the validity of this study, but may provide a more enlightened perspective on any results uncovered in this study.

LITERATURE CITED

Alcaraz, G., G. P. De Leon, P. L. Garcia, V. Leon-Regagnon, and C. Vanegas. 1999. Respiratory responses of grass carp *Ctenopharyngodon idella* (Cyprinidae) to parasitic infection by *Centrocestus formosanus* (Digenea). Southwestern Naturalist 44(2):222-226.

Amaya-Huerta, D., and R. J. Almeyda-Artigas. 1994. Confirmation of C*entrocestus formosanus* (Nishigori, 1924) Price, 1932 (Trematoda: Heterophyidae) in Mexico. Research and Reviews in Parasitology 54(2):99-103.

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.

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.

Cantu, V. 2003. Spatial and temporal variation in C*entrocestus formosanus* cercarial abundance and metacercarial infection in endangered fountain darters (E*theostoma fonticola*) in the Comal River, Texas. Master's Thesis. Texas State University-San Marcos.

Duggan, I. C. 2002. First record of a wild population of the tropical snail M*elanoides tuberculata* in New Zealand natural waters. New Zealand Journal of Marine and Freshwater Research 36:825-829.

Heitmuller, F. T., and I. P. Williams. 2006. Compilation of historical water-quality data for selected springs in Texas, by ecoregion. U.S. Geological Survey Data Series 230.

Kiesecker, J. M. 2002. Synergism between trematode infection and pesticide exposure: A link to amphibian deformities in nature? Proceedings of the National Academy of Sciences 99(15):9900-9904.

Kuhlman, T. A. 2007. The predominant definitive avian hosts of the invasive Asian trematode, C*entrocestus formosanus*, in the headwaters of the Comal, San Antonio, and the San Marcos rivers of central Texas. Master's Thesis. Texas State University, San Marcos.

Lo, C. T., and K. M. Lee. 1996. Pattern of emergence and the effects of temperature and light on the emergence and survival of Heterophyid cercariae (*Centrocestus formosanus* and *Haplorchis pumilio*). The Journal of Parasitology 82(2):347-350.

Lozano, C. 2005. The effects of turbulence and snail populations on the abundance of C*entrocestus formosanus* cercariae in the Comal River, Comal County, TX. Undergraduate thesis. University of Texas, San Antonio.

Madhavi, R. 1986. Distribution of metacercariae of *Centrocestus formosanus* (Trematoda: Heterophyidae) on the gills of *Aplocheilus panchax*. Journal of Fish Biology 29(6):685-690.

Martin, W. E. 1958. The life histories of some Hawaiian heterophyid trematodes. Journal of Parasitology 44(3):305-318.

McClelland, W. J. F. 1965. The production of cercariae by S*chistosoma mansoni* and *S. haematobium* and methods for estimating the numbers of cercariae in suspension. Bulletin of the World Health Organization 33:270-276.

McDermott, K. 2000. Distribution and infection relationships of an undescribed digenetic trematode, its exotic intermediate host, and endangered fishes in springs of west Texas. Master's Thesis. Southwest Texas State University, San Marcos.

Mitchell, A. J., and T. M. Brandt. 2005. Temperature tolerance of red-rim melania melanoides tuberculatus, an exotic aquatic snail established in the United States. Transactions of the American Fisheries Society 134(1):126-131.

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(1):55-59.

Mitchell, A. J., 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(8):11-16.

Mitchell, A. J., 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(4):283-289.

Murray, H. D. 1964. *Tarebia granifera* and M*elanoides tuberculata* in Texas. In Annual Report for the American Malacological Union :15-16.

Prentice, M. A. 1984. A field-evolved differential filtration method for recovery of schistosome cercariae. Annals of Tropical Medicine and Parasitology 78(2):117-127.

Roberts, L. S., and J. Janovy Jr. 2000. Gerald D. Schmidt and Larry S. Roberts' Foundations of Parasitology. 6th edition. McGraw-Hill Science, Boston.

Salgado-Maldonado, G., M. I. Rodriguez-Vargas, and J. J. Campos-Perez. 1995. Metacercariae of *Centrocestus formosanus* (Nishigori, 1924) (Trematoda) in freshwater fishes in Mexico and their transmission by the thiarid snail *Melanoides tuberculata*. Studies on Neotropical Fauna & Environment 30(4):245-250.

Schell. S.C. 1970. How to know the trematodes. WM. C. Brown Company Publishers. Dubuque, Iowa.

Schenck, J. R., and B. G. Whiteside. 1976. Distribution, habitat preference and population size estimate of *Etheostoma fonticola*. Copeia 1976(4):697-703.

Scholz, T., and G. Salgado-Maldonado. 2000. The introduction and dispersal of *Centrocestus formosanus* (Nishigori, 1924) (Digenea: Heterophyidae) in Mexico: A review. American Midland Naturalist 143(1):185-200.

TPWD (Texas Parks and Widlife Department). Texas Shiner (*Notropis amabilis)*.Available: http://www.tpwd.state.tx.us/huntwild/wild/species/texasshiner/ (November 2007).

Theron, A. 1979. A differential filtration technique for the measurement of schistosome cercarial densities in standing waters. Bulletin of the World Health Organization, 57(6):971-975.

USFWS (U.S. Fish and Wildlife Service). 1996. San Marcos and Comal Springs and Associated Aquatic Ecosystems (revised) Recovery Plan. Albuquerque, New Mexico. Available: http://www.fws.gov/southwest/es/Documents/R2ES/San_Marcos_&_Comal_Springs_Aquatic_Ecosystems_Revised_Recovery_Plan_Feb_14_1996.pdf (June 2006).

USGS (U.S. Geological Survey). Texas Water Data. Available: http://waterdata.usgs.gov/tx/nwis/nwisman/?site_no=08169000&agency_cd=USGS (January 2006).

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 C*entrocestus formosanus*. Journal of Fish Diseases 21:229-232.

WOW (Water on the Web). 2004a. Monitoring Minnesota Lakes on the Internet and Training Water Science Technicians for the Future - A National Online Curriculum using Advanced Technologies and Real-Time Data. University of Minnesota-Duluth. Available: http://waterontheweb.org/under/waterquality/oxygen.html (August 2007).

WOW (Water on the Web). 2004b. Monitoring Minnesota Lakes on the Internet and Training Water Science Technicians for the Future - A National Online Curriculum using Advanced Technologies and Real-Time Data. University of Minnesota-Duluth. Available: http://waterontheweb.org/resources/unitConverter.html (August 2007).

Yamaguti, S. 1975. A synoptical review of life histories of digenetic trematodes of vertebrates with special reference to the morphology of their larval forms. Keigaku Publishing Company, Tokyo, Japan.

Zar, J. H. 1999. Biostatistical analysis. 4th edition. Prentice Hall Inc, Upper Saddle River, NJ.

VITA

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