SPATIAL AND TEMPORAL VARIATION OF *CENTROCESTUS FORMOSANUS* IN RIVER WATER AND ENDANGERED FOUNTAIN DARTERS (*ETHEOSTOMA FONTICOLA)* IN THE COMAL RIVER, TEXAS.

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ABSTRACT

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Spatial and temporal variations are commonly observed in parasite populations. However, factors that regulate these variations remain unclear. Temperature has been considered an important factor influencing variations in trematode populations, however in a constant thermal ecosystem such as the Comal River, Hays County, Texas, other factors such as variations in hydrology may play a more important role. Populations of *Centrocestus formosanus* were collected quarterly from river water, and in caged and resident fountain darters from 8 different sites of the Comal River for a year to determine the spatio-temporal patterns of *C. formosanus*. The hypothesis that current velocity influences the spatial and temporal variation of *C. formosanus* was also tested. Overall, my results showed *C. formosanus* cercariae and infections occur year-round in the Comal River. While seasonal variation was not pronounced due to relatively constant temperatures, seasonal abundances of the *C. formosanus* population slightly increased during the summer and spring. Heterogeneity of current velocities among the sites

possibly influenced the spatial variations of *C. formosanus* at the Comal River. Although spatial and temporal variations in trematode populations may be influenced by current velocities at the Comal River, other factors such as water volume, photoperiod, and recruitment processes initiated by definitive hosts also may play an important role in structuring the trematode population. In this study, cercariometry was a stronger predictor of infections in caged fish than in resident fish. Considering the accuracy of cercariometry, reduced amount of time, labor, and cost, cercariometry may be the most practical field technique to predict spatial and temporal variations of *C. formosanus* at the Comal River.

INTRODUCTION

A fundamental goal of parasite ecology is to describe and determine causes of spatial and temporal variation of parasite populations in nature (Kennedy 1975, Smith 2001). Many studies show spatial and temporal variations in aquatic ecosystems that undergo seasonal changes in temperature, however few examine whether similar variations occur in constant-thermal ecosystems. Sankurathri and Holmes (1976) showed that temperature differences near a man-made lake are important in influencing the spatio-temporal dynamics of a trematode population. The site that was seasonally covered by ice showed a pronounced temporal change in the trematode population while the other site near a constant-thermal effluent showed a less distinctive seasonal change. The site near the constant-thermal effluent altered the normal seasonal dynamics of the parasite population by allowing the trematode transmission to continue uninterrupted all year.

Springs are also aquatic ecosystems with relatively constant thermal conditions. In general, spring-fed ecosystems have relatively constant physical and chemical conditions (water temperature, pH, and specific conductance) year-round, while others factors (current velocity, flow and maximum water depth) may vary seasonally (Brune 1981, USFWS 1996, Sherwood and Sheath 1999). Pitchford et al. (1969), Pitchford and Visser (1969), Shiff et al. (1975), and Sankurathri and Holmes (1976) suggested that temperature is the most important factor influencing the spatial and temporal dynamics of trematode populations since temperature regulates sporocyst development in snail hosts. In spring-fed ecosystems buffered against seasonal temperature changes, other factors such as hydrodynamics may serve a greater role in regulating the spatio-temporal dynamics of parasite populations. Numerous studies suggest that variations in hydrology (i.e., current velocity, flows, rains) are important in influencing trematode populations in space and time (Theron et al. 1978, Fashuyi 1981, Stables and Chappell 1986, Ouma et al. 1989, Kimura et al. 1994, Sapp and Esch 1994, Sturrock et al. 1994, Yousif et al. 1996, Muhoho et al. 1997, Shinagawa et al. 1999, Sukontason et al. 1999). Other possible abiotic and biotic factors that may contribute to the spatial and temporal variation of trematode populations include variations in habitat structure (Fernandez and Esch 1991a, Williams and Esch 1991, Sapp and Esch 1994, Smith 2001), photoperiod (Glaudel and Etges 1973, Theron 1980, Yousif et al. 1996, Favre et al. 1997), host mobility and behavior (Robson and Williams 1970, Yoshino 1975, Pohley 1976, Irwin 1983, Matthews et al. 1985, Yanohara 1985, Fernandez and Esch 1991a, Fernandez and Esch 1991b, Williams and Esch 1991, Snyder and Esch 1993, Sousa 1993, Kuris and Lafferty 1994, Smith 2001), host density (Pitchford and Visser 1962, Blower and Roughgarden 1989, Yousif et al. 1996, Smith 2001), host susceptibility to infection (Wakelin 1978, Blower and Roughgarden 1989, Williams and Esch 1991, Grosholz 1994), interspecific competition within hosts (Kuris and Lafferty 1994, Lafferty et al. 1994), interspecific competition among hosts (Pointer and Jourdane 2000), host distance from foci (Pitchford and Visser 1965, Rowan 1965, Theron et al. 1977, Terhune et al. 2002), and cercarial predation by fish (Anderson et al. 1978).

At the spring-fed Comal River, the discovery of an exotic ectoparasite (*Centrocestus formosanus*) that severely infects the gills of the federally endangered fountain darter (*Etheostoma fonticola*; Mitchell et al. 2000) has led to the present study to investigate the spatial and temporal patterns of *C. formosanus*. In this study I used 3 different techniques to investigate the spatio-temporal variations in 1) cercarial abundance in river water and 2) prevalence (percent infected hosts) and intensity (cysts/host) in caged fountain darters, and 3) prevalence and intensity in resident fountain darters. I also tested the hypothesis that current velocity influences cercarial abundance in river water and intensity and prevalence in caged and resident fountain darters at the Comal River. I also determined the comparability and practicality of the 3 different techniques used.

MATERIALS AND METHODS

Study Organism

Centrocestus formosanus (Trematoda: Heterophyidae) is an Asian digenetic trematode with a polymorphic life cycle that parasitizes different host species, that include the definitive (birds and mammals), first intermediate (snails), and second intermediate (fishes) hosts (Figure 1). Sexually mature adult trematodes localize in the anterior portion of the small intestines of the vertebrate definitive host (Martin 1958) and lay eggs, which are released into the environment via feces (Yamaguti 1975). Free-swimming miracidium hatch from eggs (Yamaguti 1975) and infect the snail, *Melanoides tuberculata*, the first intermediate host (Scholz and Salgado-Maldonado 2000). The miracidium penetrates the epithelium then migrates to the digestive gland (Martin 1958). The miracidium changes into a mother sporocyst followed by 1 redial generation in the snail (Martin 1958). The rediae produce free-swimming cercariae (Martin 1958) and exit by the snail's exhalent currents (Lo and Lee 1996). Cercariae are carried by water flow and passively enter the gill filaments of a fish via inhalant currents (Balasuriya 1988, Salgado-Maldonado et al. 1995). Upon contact with the gill filament, the cercariae lose their tail (Salgado-Maldonado et al. 1995) then actively migrate to a site on the gill rich in blood and oxygen (Chen 1942, Yamaguti 1975, Madhavi 1986). The larvae then penetrate and encyst in gill filaments (Salgado-Maldonado et al. 1995), mature into an infective metacercaria (Chen 1942, Yamaguti 1975) and the life cycle begins again when they are ingested by a definitive host.

Study Sites

Comal Springs in New Braunfels (29°42’50.6”N;98°08’0.65”W), Texas is the largest spring system in the southwestern United States (George 1952) with an average annual discharge of 284 cfs (8.04 m^3/s; USFWS 1996). Flow tends to fluctuate seasonally with discharge historically high during the winter months and decreasing during the summer months with a uniform hydrochemistry and temperature (23.3 $\pm$ 0.5°C) year-round (USFWS 1996). Water issues from 3 large spring openings flowing into Landa Lake through corresponding spring runs (Figure 2) and numerous small springs bubble up throughout the lake bottom, adding significant inputs to the system (Crowe and Sharp 1997). From Landa Lake, water diverges through the old and new channels. The old channel is the original Comal River streambed while the new channel is a man-made millrace historically used to power cotton gins, grist and flour mills; and later a hydroelectric generating plant (Brune 1981). The old and new channels converge 2.5 km downstream from Landa Lake and the river flows south another 2.5 km before joining with Gaudalupe River.

Preliminary Cage Study

Cages containing fountain darters were placed in the Comal River during the winter of 2001 to determine the rate of infection and how long fountain darters would live in cages. Three months before a caged fish experiment started, 800 fountain darter fry were produced and raised to ~ 25 mm (total length; TL) at the National Fish Hatchery and Technology Center (NFHTC), San Marcos, Texas. Three weeks prior to placing the fish in cages in the river, 25 of 85 fish were sent to U.S. Fish and Wildlife Service Fish Health

Center, Pinetop, Arizona, (PFHC) for examination to ensure that hatchery raised fish were not infected with *C. formosanus* cysts and other parasites that could be introduced into the river. The remaining 60 fish received a 1-h formalin (250 mg/L) prophylaxis treatment and were allowed to recuperate for 1 week. The fish were transported to the Comal River in an 85-L ice chest and then distributed into 5 cages (10 fish per cage) at the Elizabeth Street (ES) site (Figure 2). Groups of 10 fish were transferred with a small net into a 27 cm diameter X 31 cm high cylindrical cage (2.4 mm mesh) that was partly submerged in the river. The cage was closed, submerged completely, and then positioned in a suitable fountain darter habitat within the designated site. Each cage was attached to a nylon rope and weighed down by a 6.8 Kg steel weight. Cages were cleaned of debris and algal build-up every other day. On days 1, 2, 4, 8, and 10, cages were removed and fish were euthanized in a 200-mg/L solution of tricaine methanesulfonate (Finquel ®; Argent Chemical Laboratories, Redmond, Washington). After removing fish from FINQUEL ® solution, the fish were rinsed thoroughly with river water, then preserved in 10% buffered formalin. Originally, fish were to be exposed in the river for 16 d, removing cages on days 1, 2, 4, 8, and 16. However, the preliminary experiment was ended early when all fish in the remaining cages were found dead by day 10. Since 40% of the fish appeared starved and no fish were dead by day 8, fountain darters were removed by day 7 in the full study to reduce starvation and mortalities. In cages, fish accumulated *C. formosanus* infections over time. On days 1, 2, 4, 8, and 10, cysts ranged from 0 – 6, 0 – 6, 10 – 26, 14 – 34, and 16 – 54 cysts per fish respectively. On these same days the mean cyst intensity increased from 2.2 to 33.8 cysts per fish at a rate of 3.5 cysts per fish per day.

Examination of Gills

After fixing each fish in 10% formalin for at least 24 hours, each fish was rinsed with well-water, weighed (g), measured to the nearest mm (TL), sexed, and examined for metacercarial cysts. All gill arches on the right side were removed 1 at a time with iris scissors. The gill arches were examined for parasites using a compound microscope (100 X). To separate the gill filaments and view the metacercariae more clearly under the microscope, a dull probe was pressed against the plastic cover slip. The number of trematode metacercariae per gill and developmental stages of each metacercariae (eyespots, faded eyespots, and X-shaped glands) were recorded (Yamaguti 1975). The total number of metacercariae per fish was estimated by doubling the number of cysts found on the right side gill arches.

Resident Fish Collection

For each seasonal parasite assessment an attempt was made to collect 10 resident fountain darters 23 – 35 mm (TL) from each of 8 sites using a 40 X 40 cm (1.6 mm mesh) dip-net within a 30 min interval. If 10 fish could not be collected at a particular site within the designated time, then the sample size was the number caught. All fish were euthanized with FINQUEL ®, rinsed, preserved, and transported to NFHTC for gill examination as before. During resident fish collections, the presence of *M. tuberculata*, the snail intermediate host of *C. formosanus*, was also noted.

Caged Fish

Sixty fountain darters were examined at PFHC for pathogens and the remaining hatchery reared darters were treated with formalin as before. Two hundred and forty fountain darters were transferred into 24 cages (3 cages per site, 8 sites, and 10 fish per cage). The cages were positioned where resident fountain darters were collected the previous day. At each of the 8 sites, 3 cages were linked together 1 m apart, weighed down, and left in the river for 7 d. The cages were cleaned of debris and algae every other day and removed on day 7. As before, the fish were euthanized with FINQUEL ®, rinsed, preserved, and transported to NFHTC for gill examination.

Preliminary Cercariometry Trials

A diel filtration experiment was conducted during the summer of 2002 at 2 sites (CF and ES; Figure 2) to determine if a diel pattern of cercarial release from snails occurs. Using a modified filtration apparatus (Figure 3) designed by Theron (1979) and Prentice (1984), 3, 10-L samples of river water were collected at each site at 3 h intervals over a 24 h period. After each 3 h collection interval, each filter was removed from the apparatus, placed in a storage dish, stained with 1.5 ml of rose bengal stain and preserved with 3 ml of 10% formalin. The dish was then sealed with parafilm to prevent filters from drying and transferred to NFHTC for examination. In the laboratory, filters were transferred into a petri dish (95 mm diam) that contained a paper counting grid (60 X 60 mm). Drops of water (~ 20 ml) were added to the dish until the screen filter was completely submerged under water. The grid could be viewed through the transparent monofilament filter when light from a fiber optic illuminator was placed above. The stained red cercariae (Figure 5) were then counted under a dissecting microscope (100 X) and the number cercariae/L was calculated for each 10-L sample. The filters were re-used after soaking them overnight in a 10% solution of sodium hydroxide, followed by spraying with a jet of hot water to dislodge cercariae (Prentice 1984). Highest cercarial abundances of *C. formosanus* occurred between 0930 and 1230 hours (Figure 6). These findings agree with earlier findings that more *C. formosanus* cercariae are shed diurnally (Amaya-Huerta and Almeyda-Artigas 1994, Lo and Lee 1996, Zeng and Liao 2000) with a diel pattern similar to other positive phototactic trematode species (Asch 1972, Theron 1980, Kimura et al. 1994). *Haplorchis pumilio* (Digenea: Heterophyidae) cercariae, another exotic trematode (Figure 5B) from Asia, was also collected from the Comal River, but no diel trends were detected (Figure 6).

In laboratory experiments, Kloos et al. (1982) and Prentice (1984) demonstrated that cercarial recovery rates increased (23% to 51% and 60% to 98%, respectively) when formalin was used to kill cercariae before filtration. Cercarial loss was likely due to cercariae wiggling through the filter when no formalin was added to the sample (Prentice 1984). In a preliminary experiment, I placed 50 recently released *C. formosanus* cercariae into 6, 10-L buckets, each containing 5-L of well water. Formalin (0.1%) was added to 3 of 6 buckets, mixed, and then filtered through a filtration apparatus (Figure 6). were stained and examined as before. Cercarial recovery rates from well water were high (80 $\pm$ 7% SE) with formalin treatment and low (12 $\pm$ 2% SE) with no treatment.

A second experiment was conducted using well water to determine: 1) the percent recovery of cercariae on the filters of a newly modified apparatus (Figure 4), and 2) if low and high concentrations of cercariae have an effect on the % recovery. I assumed that the percent recovery from Edwards Aquifer well water in San Marcos, Texas is similar to the Comal River water. To prepare the low and high concentrations of cercarial water, 50 and 500 recently released cercariae were added into separate 10-L buckets containing 5-L samples of well water. Five ml of formalin was added to the 5-L sample to make a 0.1% formalin solution. The sample was stirred (to prevent cercariae from sticking to the walls of the bucket) and poured through the filtration apparatus. The procedure was repeated 5 times. At a cercarial concentration of 10 cercariae/L the mean percent recovery was 76 $\pm$5% SE while at 100 cercariae/L the mean recovery decreased to 62 $\pm$5% SE. However, the percent recovery of cercariae on filters did not significantly ($t = 2.08$; $P = 0.09$) differ between the lower and higher cercarial concentrations.

Cercariometry

Because the highest cercarial densities occurred between 0930 and 1230 hours and because of travel time and logistical constraints, the 8 sites were sampled within 0930 and 1230 hours over a 2-day period (4 sites per day). To avoid stirring sediments from the river bottom and clogging filters, a fiberglass adjustable rod (3.5 m) attached by a clamp to a 10-L bucket was used to collect 5-L samples of river water in front of each darter cage at each site, quarterly. The bucket was lowered to near to the river bottom and raised to the water surface without disturbing bottom sediments. The sample was treated with formalin, stirred, poured through the filtration apparatus and collected on filters, stored and examined as before.

Site Characterization

For each quarterly sample, temperature, dissolved oxygen (D.O. meter model 58, YSI, Yellow Springs, Ohio), current velocity (Marsh-McBirney flow meter, Frederick, Maryland), water depth, and substrate and vegetation composition were recorded at each site. The presence of piscivorous birds was recorded.

Statistical Analysis

All values were log (X+1) transformed to meet the assumptions of parametric data prior to performing linear regressions and correlations. Cercariae from the filtration method were regressed against metacercariae from the caged fountain darter method and metacercariae from the resident fountain darter method to determine how well cercariometry predict infections in resident and caged fountain darters. To determine if spatial and temporal differences in parasite abundance occurs within the Comal River, a 2 factor ANOVA including the factors of site and date was performed.

RESULTS

The trematode community in the Comal River and in fountain darters was composed of *C. formosanus*, *H. pumilio* and an unidentified monogenean *sp.* *Centrocestus formosanus* was the dominant trematode accounting for 97% of the trematode population in the river water, 99% in caged fountain darters, and 99% in resident fountain darters. In the samples collected from the Comal River, the SI site accounted for 54% of cercariae in river water, 72% of cysts in caged fountain darters, and 18% in resident fountain darters. At the SI site, mean cercarial concentration on filters reached as high as 24 cercariae/L and ranged from 0 – 45 cercariae/L (Figure 7A, Appendices 1 – 4). Of the 738 caged fountain darters examined, 55% were infected with *C. formosanus* cysts. At the SI site, mean intensity in caged fountain darters reached as high as 100 cysts/fish (over the 7 d exposure period to river water) with intensities ranging from 0 – 348 cysts per fish (Figure 7B, Appendices 1 – 4). The mean survivorship of all caged darters used in the study was 80%. Of the 235 resident fountain darters examined, 95% were infected with *C. formosanus* cysts. At the SPR1 and CF sites, mean intensities in resident fountain darters reached as high as 581 and 780 cysts/fish, respectively with intensities ranging from 0 – 1394 and 0 – 1662 cysts per fish (Figure 7C, Appendices 1 – 4).

Spatial Variation of Trematodes

Cercarial concentration (mean of the 4 seasons) in the water column significantly (ANOVA, $F = 19.45$, $P < 0.001$, Table 1) differed among the 8 sites in the Comal River. The SI site showed the highest abundance of *C. formosanus* with a mean concentration of 18.2 ± 3.1 SE cercariae/L (Figure 8C). At the SI site, cercarial concentration was significantly (Tukey's studentized range test, $P < 0.001$, Table 2) greater than concentrations at all other sites.

Spatial variation in intensity (cysts/fish) of caged fountain darters was significantly (ANOVA, $F = 11.72$, $P < 0.001$, Table 1) different among the 8 sites of the Comal River. The SI site showed the highest abundance of *C. formosanus* with a mean intensity of 64.4$\pm$16.7 SE cysts/fish in caged fountain darters (Figure 8D). At the SI site, intensity in caged fountain darters was significantly (Tukey's studentized range test, $P < 0.001$, Table 3) greater than all other sites. Spatial variation in prevalence (% infected fish) in caged fountain darters was significantly (ANOVA, $F = 19.96$, $P < 0.001$, Table 1) different among the 8 sites of the Comal River. At the SI site, prevalence in caged fountain darters was significantly (Tukey's studentized range test, $P < 0.001$, Table 4) greater than the SPR1, SPR3 and HS sites.

Spatial variation in intensity of resident fountain darters (cysts/fish) was significantly (ANOVA, $F = 8.26$, $P < 0.001$, Table 1) different among the 8 sites of the Comal River. Of the 8 sites, CF instead of SI, showed the highest abundance of *C. formosanus* with a mean intensity of 788.6$\pm$202.7 cyst/fish (Figure 8E). At the CF site, intensity in resident fountain darters was significantly (Tukey's studentızed range test, $P < 0.05$, Table 5) greater than all sites in the study area. Spatial variation of prevalence of resident fountain darters was not significantly (ANOVA, $F = 1.29$, $P = 0.306$, Table 1) different among the 8 sites of the Comal River. Parasite prevalence in resident fountain darters was high (72% -100%) at the 8 sites (Figure 8E).

Overall *C. formosanus* abundance was highest at the SI site in the water column and in caged fountain darters, but not in resident fountain darters. In resident fountain darters, the highest abundance was at the CF site. Variation in cercarial concentration and intensity among sites was higher than variation associated with prevalence.

Temporal Variation of Trematodes

Centrocestus formosanus occurred at the Comal River year-round (Figure 9B-D). Seasonal variation in cercarial concentration (mean of 8 sites) was significantly (ANOVA, $F = 3.90$, $P = 0.023$, Table 1) different among the 8 sites in the Comal River. Of the 4 seasons, summer and spring showed the highest abundance of *C. formosanus* with a mean concentration (cercariae/L in river water) of 6.6 ± 2.8 and 4.48 ± 2.9 respectively (Figure 9B). Of the 4 seasons cercarial concentration was only significantly (Tukey's studentized range test, $P < 0.013$, Table 2) greater in the summer than the winter.

Seasonal variation in intensity in caged fountain darters was not significantly (ANOVA, $F = 1.52$, $P = 0.238$, Table 1) different in the Comal River. Although, summer and spring showed the highest abundance of *C. formosanus* with mean intensities of 16.0 ± 12.1 and 13.7 ± 10.3 cysts/fish, respectively (Figure 9C). Seasonal variation in prevalence in caged fountain darters was significantly (ANOVA, $F = 5.03$, $P = 0.009$, Table 1) different in the Comal River with summer, fall and spring showing the highest abundance of *C. formosanus* with prevalences of 58, 65, and 63%, respectively (Figure 10B). Prevalence in caged fountain darters in the summer, fall and spring were significantly (Tukey's studentized range test, $P < 0.05$ respectively, Table 4) greater than the prevalence in the winter. The highest prevalence occurred in the fall after the heavy rains of late October and early November.

Seasonal variation in intensity in resident fountain darters was not significantly (ANOVA, F = 1.40, P = 0.273, Table 1) different in the Comal River. Although the highest mean (SE) intensity of 332.6±179.5 occurred in the fall after the heavy rains of late October and early November (Figure 9D). Seasonal variation in prevalence in resident fountain darters was not significantly (ANOVA, F = 0.914, P = 0.452, Table 1) different in the Comal River and prevalence in resident fountain darters was relatively high (92-100%) throughout the year (Figure 10C). The highest prevalence occurred in the fall after the heavy rains of late October and early November.

Effects of Temperature and Current Velocity

The mean temperature of the 8 sites remained relatively constant seasonally at the Comal River, ranging from 22.6±0.3 – 24.2±0.1 C.° The aquifer discharge (cfs) remained above the mean historical discharge of 284 cfs (USFWS 1996) throughout the entire study (Figure 9A). The discharge remained relatively stable throughout the year, except during the fall rains. However, the mean current velocity among the sites significantly (ANOVA, F = 40.24, P < 0.001) differed spatially, ranging from 0.00 – 0.32 m/s (Figure 8A).

Cercarial concentration was inversely related to current velocity although not statistically significant (R^2 = 0.073, P = 0.13, Figure 11A). Intensity and prevalence in caged fountain darters significantly decreased with increased current velocity (R^2 = 0.24, P = 0.004 and R^2 = 0.21, P = 0.008, Figures 11B and D) respectively.

Comparability of Parasite Abundance

Among the 3 techniques used, the caged and filtration technique showed the strongest association. Cercarial concentration and intensity in caged fountain darters showed a strong association that was positive ($R = 0.87$) and significant ($R^2 = 0.75$, $P > 0.001$, Figure 12A). Associations of cercarial concentrations and resident fountain darters were positive ($R = 0.36$) and significant ($R^2 = 0.13$, $P = 0.04$, Figure 12B).

DISCUSSION

The causes of spatial and temporal variation in parasitism is a topic of debate in ecological parasitology (Robson and Williams 1970, Kennedy 1975, Esch 1977, Sousa 1993, Williams and Esch 1991, Snyder and Esch 1993, Fernandez and Esch 1991a, Kuris and Lafferty 1994, Lafferty et al. 1994, Smith 2001). Overall, my results showed *C. formosanus* cercariae and infections to occur year-round in the Comal River. While seasonal variation was not pronounced due to relatively constant temperatures, seasonal abundances of the *C. formosanus* population were slightly higher during the summer and spring. Heterogeneity in current velocities among the sites possibly influenced the spatial variations of *C. formosanus* at the Comal River.

In this study, regressions showed that current velocity negatively influenced the abundance of *C. formosanus* in river water and caged fountain darters. Similarly, Ouma et al. (1989) found significant and negative associations between flow and cercarial concentration. At the SI site where there was little or no current velocity, cercarial concentrations and intensities in caged fountain darters were high. Conversely, at the SPR1 and SPR3 sites where current velocities were high (mean 0.14, range 0.12 – 0.18 m/s and mean 0.15, range 0.11 – 0.20 respectively), concentrations and intensities were low. Webbe (1966) and Stables and Chappell (1986) showed that increased current velocity and flows significantly decreased intensities in host rodents and rainbow trout by decreasing the ability of cercariae from swimming and attaching to hosts. Theron et al. (1977) suggested that the risk of host infection was higher in slow current habitats (~ 0.01 m/s) due to cercariae accumulating in the water column, where as faster current habitats (> 0.10 m/s) diluted cercarial concentration. Upatham (1974) found spatial heterogeneity in different flow habitats using caged rodents, with high infections in pool habitats and low infections in run habitats.

While current velocity may influence trematode abundance, other factors such as snail host densities and distances from transmission sites can also contribute to spatial variations of *C. formosanus*. Theron et al. (1977) showed that cercarial concentration in running water gradually decreased downstream from an infected snail population, unless additional infected snail populations along the river contributed to high cercarial concentration at downstream sites. In my study, cercarial concentrations increased the further downstream samples were collected from a site of high cercarial transmission (SI). At the downstream ES and GS sites, I expected to find low cercarial concentrations due to the relatively high current velocities, dilution effect, and long distance traveled by cercariae. Instead cercarial concentrations were high at the ES and GS sites which may be attributed to the effects of multiple upstream snail populations. Cauble (1998) studied snail assemblages at 18 sites in Landa Lake and found variable densities of *M. tuberculata* with some as high as 3,500 snails/m^2. Thus it is possible that cercariae from different infected snail populations at Landa Lake and as well as in the old and new channels drifted downstream contributing to the high cercarial concentrations at the ES and GS sites.

In caged fountain darters, infections decreased instead of increased the further downstream cages were located from areas of high infection. Radke et al. (1961), Pitchford and Visser (1965), and Rowan (1965) showed similar results in caged rodents. The downstream decrease in host infection may be due to a loss of cercarial infectivity. This loss of downstream infectivity may be associated with the aging of cercariae as they travel downstream. However, this is unlikely since cercariae live up to 110 h at 25 C° (Lo and Lee 1996) and would take ~13 h for cercariae to drift from the SI site to the GS site. Anderson et al. (1978) experimentally showed that small fish greatly reduced cercariae populations by cercarial predation. However, the role of fish predation on cercariae at the Comal River is not known. Alternatively, cercariae may become fatigued over a long distance or mechanically damaged due to turbulence (Radke et al. 1961, Rowan 1965, and Suguira and Ota 1954) thereby reducing infectivity of downstream cercariae.

In this study, the volume of water moving through a site also may have influenced the spatial heterogeneity of infections in caged daters. Warren and Peters (1967) showed marked reductions in trematode intensity of caged rodents by increasing the volume of water 20-fold. Since the volume of water is dependent on the width and depth of a river, these factors may have altered the concentration of cercariae moving through a site. At the Comal River, even though the SI and BI sites were in slow flowing habitats, the width and depth at the BI site was (4 times) wider and (1/4) deeper than the SI site (Figure 2), thereby increasing the overall volume of water and diluting the cercarial concentration at this site. Consequently, cercarial concentrations and intensities in caged fountain darters were significantly less at the BI site than the SI sites. Further studies should be conducted to determine the effects flow and volume on *C. formosanus* in cercarial concentrations and infections in fish.

At spring run sites SPR1 and SPR3, it is not clear why intensities in resident fountain darters were high when cercarial concentrations in river water and intensities in caged fountain darters were low to none. In this study, since resident fountain darters were not confined to cages, it is possible that highly infected resident fountain darters moved from sites of higher infection to sites of lower to no infection. One possible explanation is that high flow velocities from heavy rains moved fountain darters from highly infected sites (SI or other sites in Landa Lake not sampled) to less infected sites downstream (SPR1 and SPR3). However, Meffe (1984) showed that native fish relative to exotic fish were more capable of persisting upstream during floods by properly orienting themselves toward high discharge. Whether fountain darters have a similar adaptation for coping with floods is not known. Alternatively, it is possible that highly infected resident fountain darters migrated away from sites of higher parasite transmission (CF, BI), into refuge sites (SPR1 and SPR3) of lower to no infection.

Although infections in resident darters at the SPR1 site reached intensities greater than 800 cysts per fish, which is considered life threatening to fountain darters (Mitchell et al. 2000), it is not clear why infections in the SPR3 sites did not reach these intensities. However, the highest infected fish from the Comal River were found at the CF site, and CF was closer to the SPR1 site than SPR3, therefore it is possible that fish from the CF site migrated upstream, contributing to the higher infections in the SPR1 site. To better understand the natural movements of infected fountain darters in variable flow regimes more field and laboratory studies should be conducted.

In a non-constant-thermal ecosystem, Shiff et al. (1975) showed that snails completely stop releasing cercariae during the winter due to sporocysts becoming dormant. Sporocyst dormancy thus resulted in a pronounced seasonal variation in trematode abundance. At the Comal River, *M. tuberculata* shed cercariae all year due the relatively constant temperatures (23 – 24 C°) that stayed within the optimal range (15 –

25 C°) for snails to release cercariae (Lo and Lee 1996). Thus, at the Comal River the relatively constant warm temperatures year-round permitted the uninterrupted release of *C. formosanus* cercariae with a less pronounced seasonal variation in the *C. formosanus* population. Sankurathri and Holmes (1976) showed a similar year-round and less pronounced seasonal pattern in a trematode population in a man-made lake near a heated thermal effluent. Further studies should be conducted with *C. formosanus* to determine the effects of temperature on sporocyst development, lifespan and production in *M. tuberculata*.

Since seasonal light regime increased during the summer and spring and was more variable than temperature at the Comal River, light may be an important factor influencing the seasonal abundances of *C. formosanus.* Since this study demonstrated that *C. formosanus* cercariae is phototactic, it is possible that higher and longer sunlight intensities during the summer and spring contribute to the increased release of cercariae by *M. tuberculata* in the Comal River.

Increased evidence suggests that recruitment processes of trematodes initiated by definitive hosts contribute to the spatial and temporal variations of trematodes in aquatic ecosystems (Robson and Williams 1970, Yoshino 1975, Pohley 1976, Irwin 1983, Matthews et al.1985, Yanohara 1985, Fernandez and Esch 1991a, Fernandez and Esch 1991b, Williams and Esch 1991, Snyder and Esch 1993, Sousa 1993, Kuris and Lafferty 1994, Smith 2001). At the Comal River, more Green Herons *Butorides virescens* were observed at the SI site and may explain why the SI site was the highest site of *C. formosanus* transmission. The SI site was a small island, isolated from people and provided a slow flowing, shallow habitat that was ideal for green herons to feed. Smith

(2001) showed that dead mangrove trees provided ideal perching habitats for definitive host birds which increased trematode parasite transmission to snails in a California marsh. In this study I observed more Green Herons in the summer and spring, similar to the abundances of Green Herons observed by NFHTC staff (unpublished data) at the Comal River. In Central Texas, further evidence suggests that the green-back heron is more common in the summer and spring (Travis Audubon Society 1994). The Green Heron is a neotropical bird that breeds north of the tropic of Cancer and winters south of that line (Rappole 1995, Degraaf and Rappole 1995). Since Comal Springs is above the tropic of Cancer, the migratory behavior of Green Heron may link parasite communities from the tropics to the Comal Springs. Given that the migratory Green Heron is a known definitive host for *C. formosanus* in Mexico (Scholz and Salgado-Maldonado 2000), it is likely to serve as a definitive host at the Comal River. Therefore, the seasonal migration of Green Herons to the Comal River may contribute to the increased seasonal recruitment of *C. formosanus* abundance during the summer and spring.

Trematode interspecific competition (within snail hosts) and recruitment processes (initiated by definitive hosts) that structure parasite communities are the center of debate in parasite ecology (Fernandez and Esch 1991a, Fernandez and Esch 1991b, Snyder and Esch 1993, Sousa 1993, Kuris and Lafferty 1994, Smith 2001). At the Comal River, since *C. formosanus* remained the predominant species year-round ($\geq$ 97 % in river water, $\geq$ 99 % in caged and $\geq$ 99 % in resident fountain darters), interspecific competition with other trematodes may not play an important role in structuring the spatial and temporal variation of *C. formosanus* in this ecosystem. Instead, variations in hydrology, temperature, photoperiod, and recruitment processes initiated by definitive hosts appear to play a more important role in structuring the trematode population in this ecosystem.

Comparability of Methods

Three different techniques were used in this study to determine the abundance of *C. formosanus* at different spatial and temporal scales. Here, I evaluate the comparability and practicality of each technique. The advantage of using the dip-net technique to collect resident fountain darters is that the actual infection status of fountain darters in the Comal River can be determined. A disadvantage involves the difficulties of collecting resident fountain darters of a known location and infection history. Fountain darters could become infected at a site of high parasite transmission and move to a site of low transmission. In this study, I found the highest infections in resident darters at and near spring run sites where cercarial concentrations and infections in caged darters were low to none. Another disadvantage of the dip-net technique involves the problem of collecting parasites of known age on the gills of resident fountain darters. Because dead metacercariae accumulate on the gills of fountain darters, the parasite intensity of resident fish captured at a specific point in time is actually an accumulation that occurred over previous months. The problem of collecting parasites of known location and age in resident darters likely led to higher variations in infections compared to the caged fish and filtration technique, thus making it more difficult to statistically determine spatial and temporal patterns of *C. formosanus* with statistical assurance. The advantage to using caged fountain darters is that caged fish are restricted to a specific site and exposed to infection for a known amount of time (7 d). Using caged fountain darters resulted in a 13 fold decrease in standard error relative to the standard error in resident fountain darters. The advantage of using the filter technique is that cercariae were collected from a specific place and time. Since the spatial and temporal scales were much smaller than the scales represented by resident darters, the filtration technique resulted in a 46 fold decrease in standard error relative to the standard error in resident fountain darters.

Among the 3 techniques used, the filtration and caged fish technique showed the strongest association, possibly due to the similar spatial and temporal scales over which parasite populations were assessed. In this study, the increased differences between the spatial and temporal scales and standard errors in cercarial concentration and cysts from resident darters may have resulted in a weaker association between the techniques.

Among the 3 techniques used to detect and quantify *C. formosanus* at the Comal River, the caged fish technique was the most expensive to use due to the extended amount of time, labor, and money required to rear fountain darters. It took ~ 4 months to breed, maintain, and grow fountain darters to ~ 25 mm and 25 h to perform a prophylaxis treatment for external parasites and to allow for recuperation. Not including travel time, it took 45 min to set, remove, and clean 3 cages from 1 site in the river, and 7 min to preserve, dissect, and examine 1 fountain darter for parasites under a microscope. To obtain the parasite intensity in 1 caged fountain darter, it took an estimated 2,906 h. The estimated cost (including labor to rear, set up cages, preserve, dissect caged fountain darter, etc) to quantify intensities in one caged darter was $14.04 ($14,038/year) and $ 940 for cages and supplies (including, formalin, FINQUEL ®, vials, slides, cover slips, etc.). Another disadvantage to the caged fish technique includes cage tampering by park visitors. In this study, I replaced fountain darters in cages twice due to park visitors tampering with cages.

The dip-net technique was less expensive than the caged fish technique since it required less time, labor and money to use. Not including travel time, it took 14 min to capture, preserve, dissect, and examine cysts from 1 resident fountain darter. The estimated cost (including labor to capture resident fountain darters, preserve, dissect fountain darter, etc) to quantify intensities in one caged darter was \$ 2.88 (\$ 922/year) and \$ 430 for the dip-net and supplies (including, formalin, FINQUEL ®, vials, slides, cover slips, etc.). Another disadvantage to using the dip-net technique is that it requires special permits to examine parasites in resident fountain darters.

Cercariometry was also less expensive to use than using the caged fish technique (due to the less amount of time, labor, and money required to rear fountain darters), and not much more expensive than the dip-net technique. Not including travel time, cercariometry took 19 min to filtrate, stain, preserve, and examine one filter for cercariae under a microscope. The estimated cost (including labor to filtrate, stain, preserve, examine filters, etc) to quantify cercariae in one filter was \$ 3.88 (\$ 372/year) and \$ 1,350 for the filter apparatus and supplies (including stain, formalin, storage containers, etc.). Considering the accuracy of filter results, reduced amount of time, labor, and cost to quantify cercariae in filters, cercariometry may be the most practical field technique to predict spatial and temporal variations of *C. formosanus* in the Comal River and in caged fountain darters.

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APPENDIX I

FIGURES AND TABLES

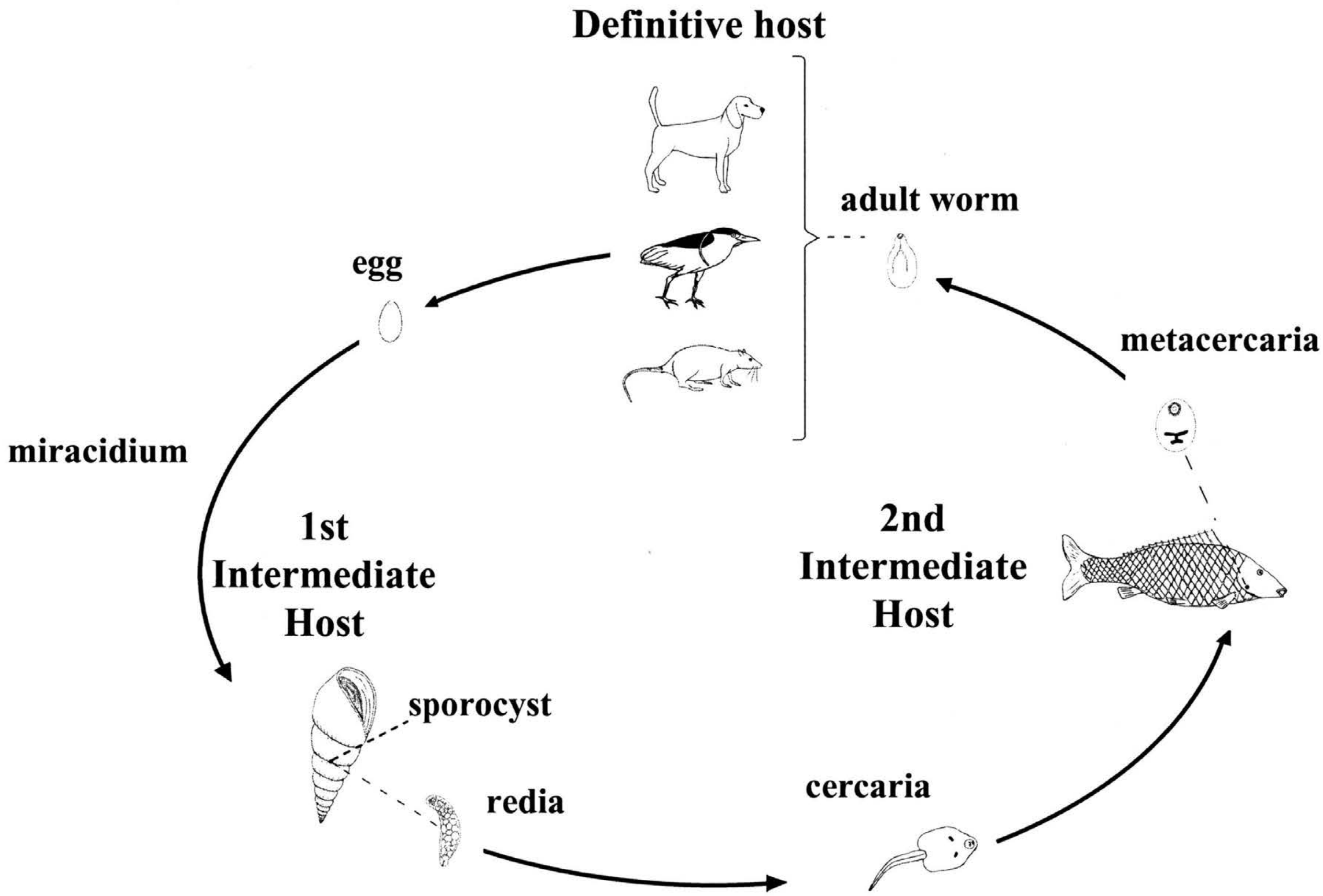

Figure 1. Generalized life cycle of *Centrocestus formosanus.*

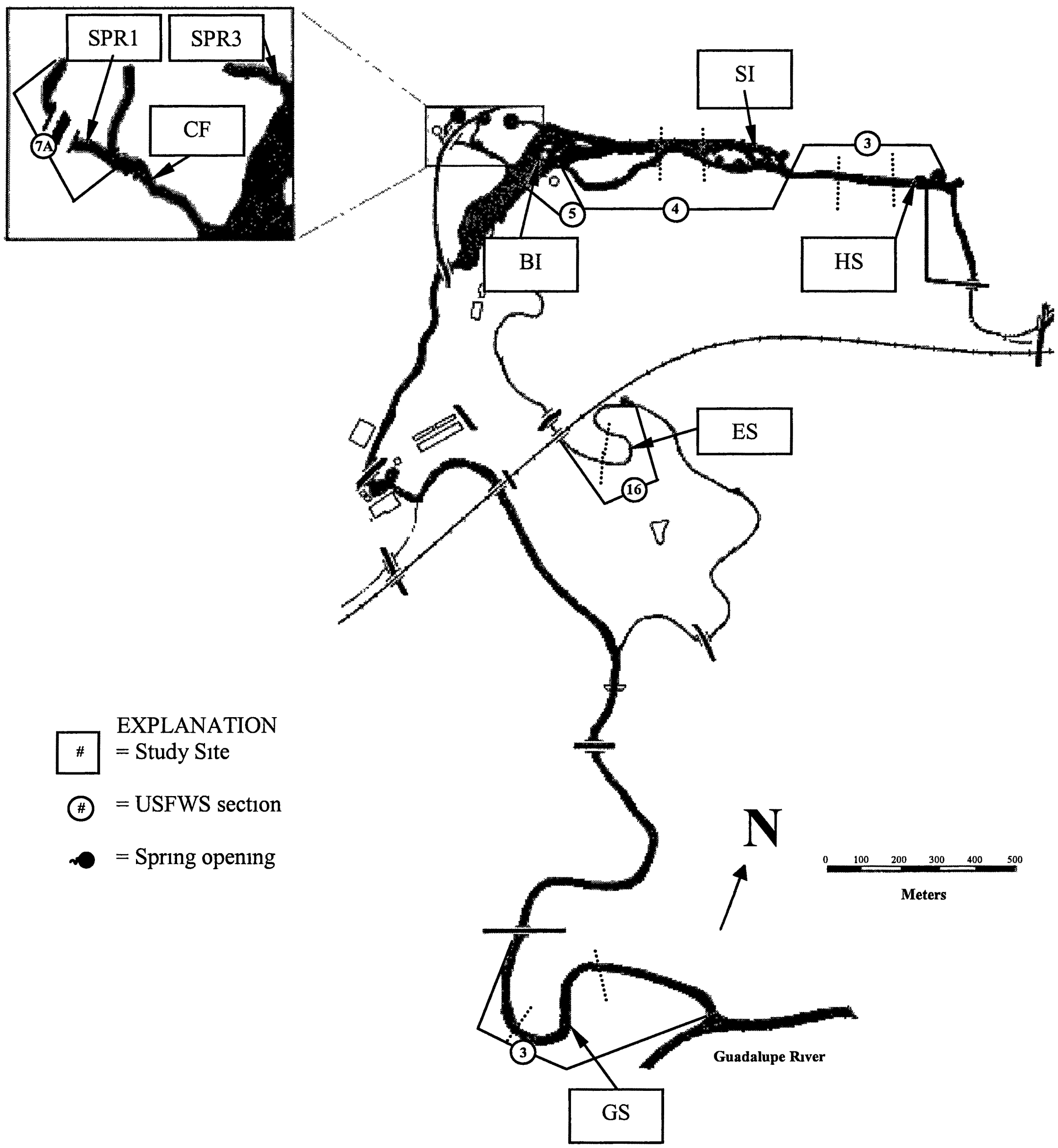

Figure 2. Study sites where *Centrocestus formosanus* were collected in river water, and in caged and resident fountain darters at the Comal River, Comal County, Texas.

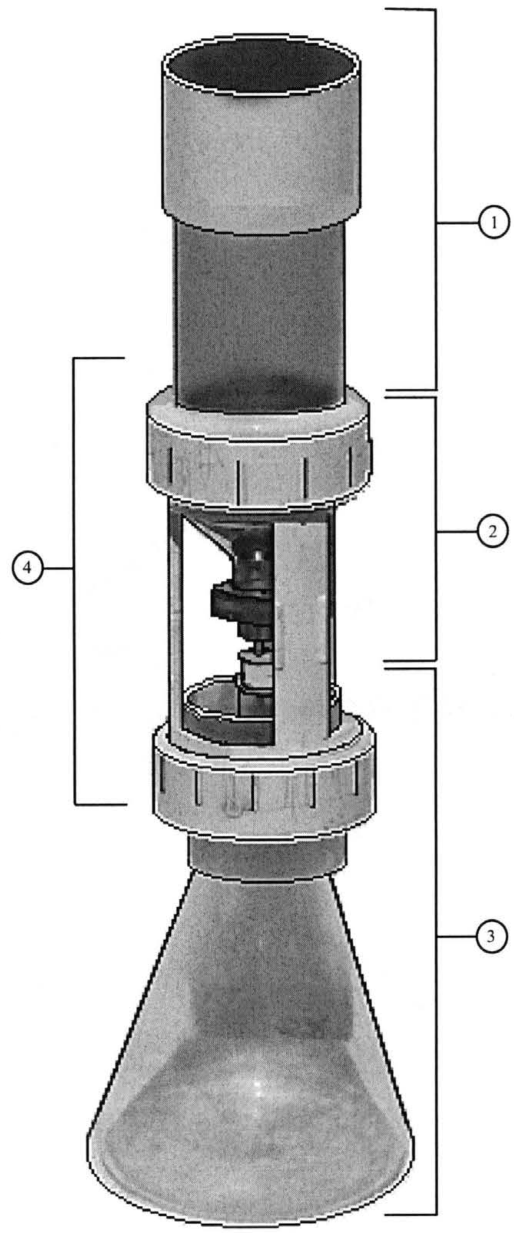

Figure 3. Modified filtration apparatus. 1. prefilters (220μm and 86μm). 2. filter holder with 30 μm monofilament filter. 3. 6-L flask. 4. prefilter support.

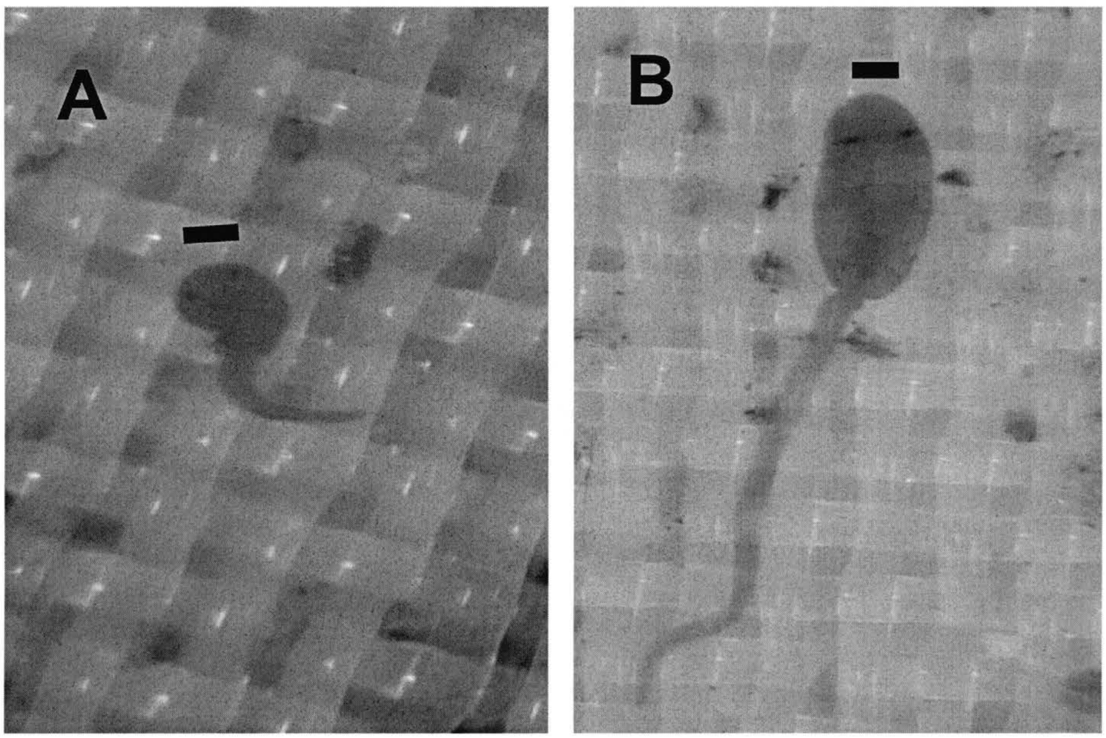

Figure 4. Cercariae recovered on 30 μm nylon monofilament filters from the Comal River. A. *Centrocestus formosanus* cercaria. B. *Haplorchis pumilio* cercaria. The scale bar = 30 μm.

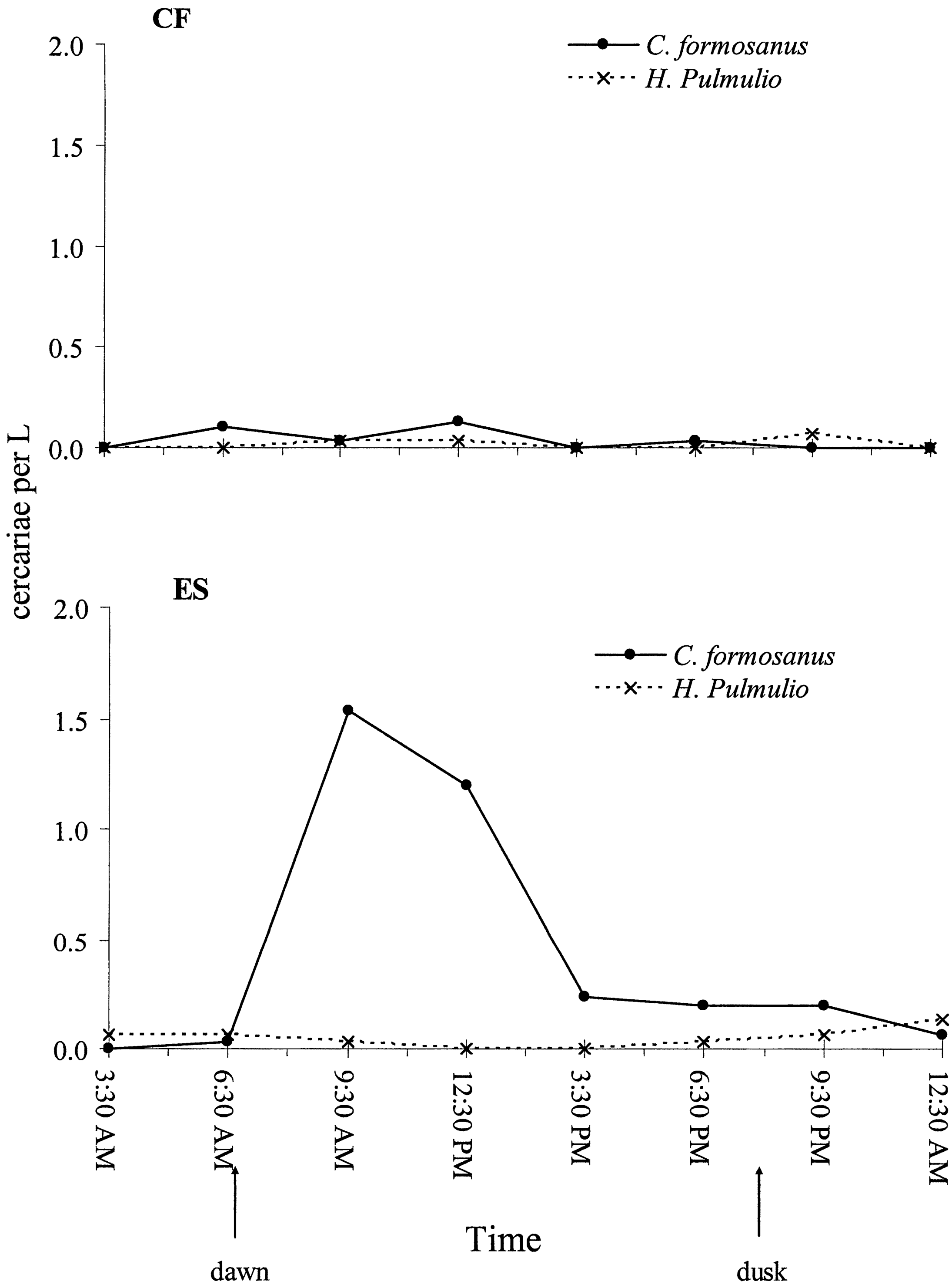

Figure 5. Diel cercarial abundance of *Centrocestus formosanus* and *Haplorchis pulmulio* at sites CF and ES in the Comal River, Comal County, Texas.

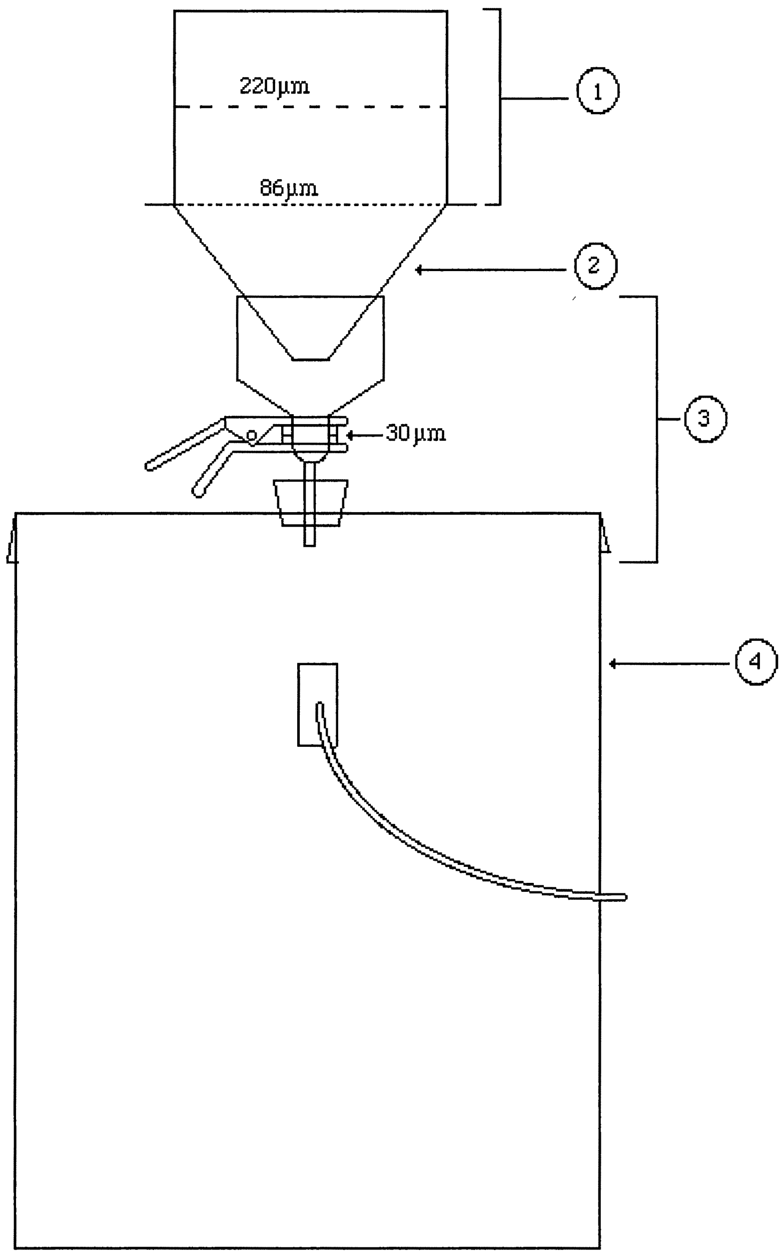

Figure 6. Preliminary filtration apparatus. 1. prefilters. 2. funnel. 3. filter holder with 30 µm monofilament filter. 4. 19-L bucket.

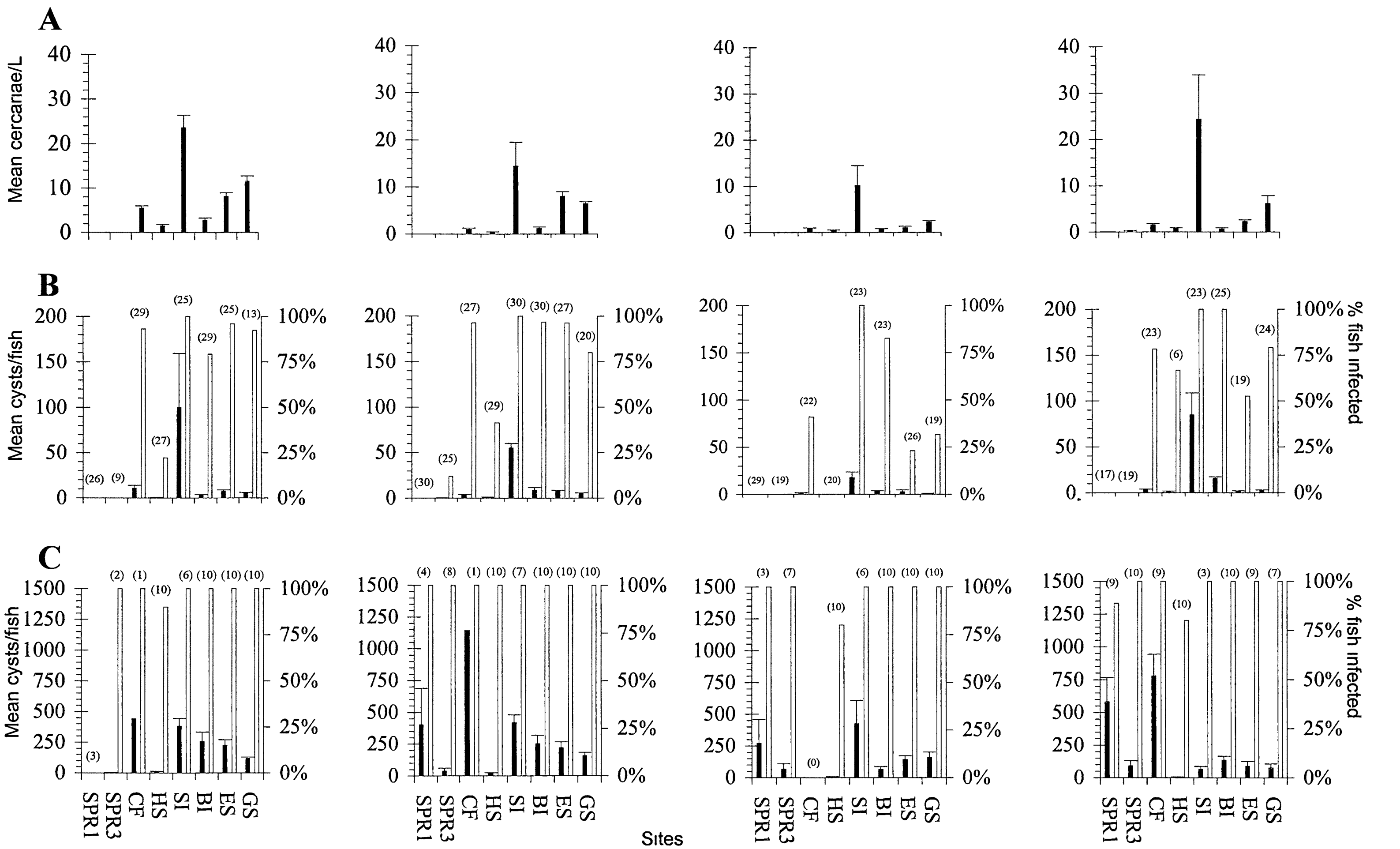

Figure 7 Spatial and temporal abundance of *Centrocestus formosanus* in river water, and in caged and resident fountain darters at eight sites in the Comal River, Comal County, Texas Numbers in parenthesis represent number of fountain darters examined A Mean (±SE) metacercariae (±SE) per caged fountain darter cercarial abundance B Mean (black bars) and mean % infected fountain darters (white bars) C Mean metacercariae (±SE) per resident fountain darter (black bars) and mean % infected fountain darter (white bars) Intensity life threatening when fountain darter infected with > 800 cysts (Mitchell et al 2000)

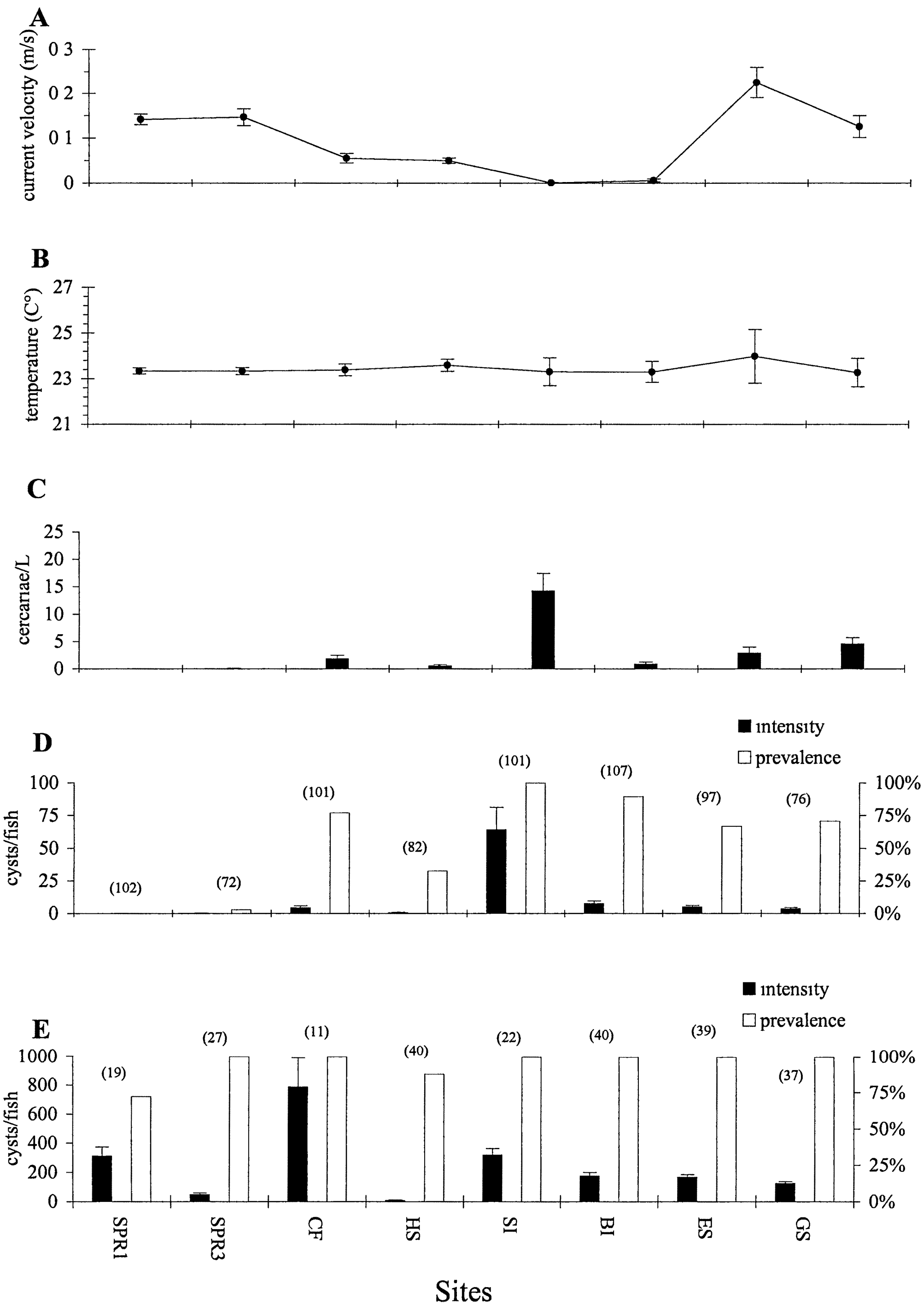

Figure 8 Spatial variation of *Centrocestus formosanus* at eight sites in the Comal River, Comal County, Texas Numbers in parenthesis represent number of fountain darters examined A. Mean (+SE) current velocity in meters per sec B mean (+SE) temperature in degrees celcius C Mean (+SE) cercarial abundance. D Mean (+SE) metacercariae per caged fountain dater and % infected fountain darters E Mean (+SE) metacercariae per resident fountain darter and % infected fountain darters

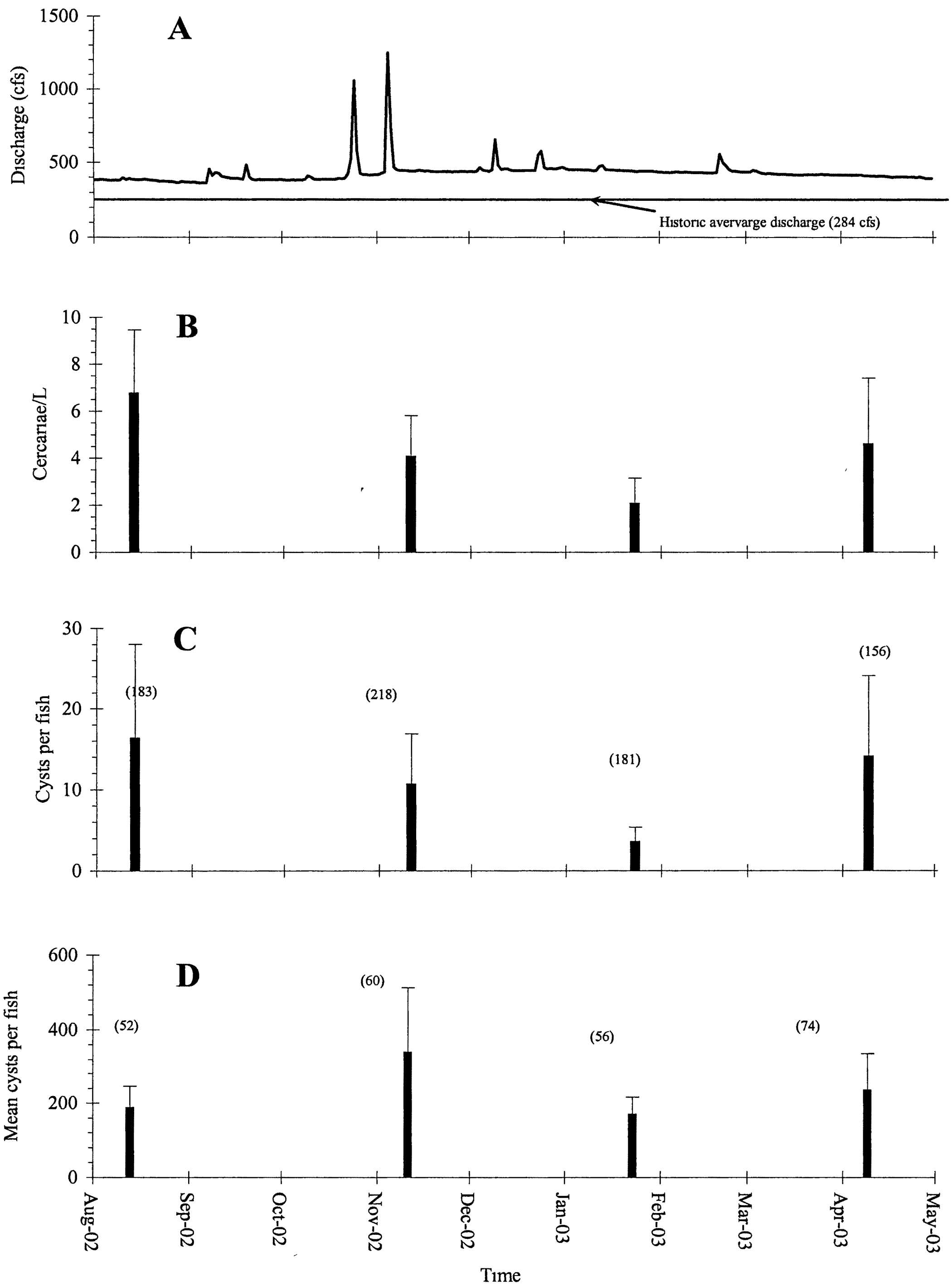

Figure 9 Temporal variation in discharge, cercarial concentration of *Centrocestus formosanus* in river water, and in intensity in caged and resident fountain darters in the Comal River, Comal County, Texas Numbers in parenthesis represent number of fountain darters examined A Mean discharge in cubic feet per second. B Mean (+SE) cercariae in river water per L C Mean (+SE) metacercariae per caged fountain darter D Mean (+SE) metacercariae per resident fountain darter.

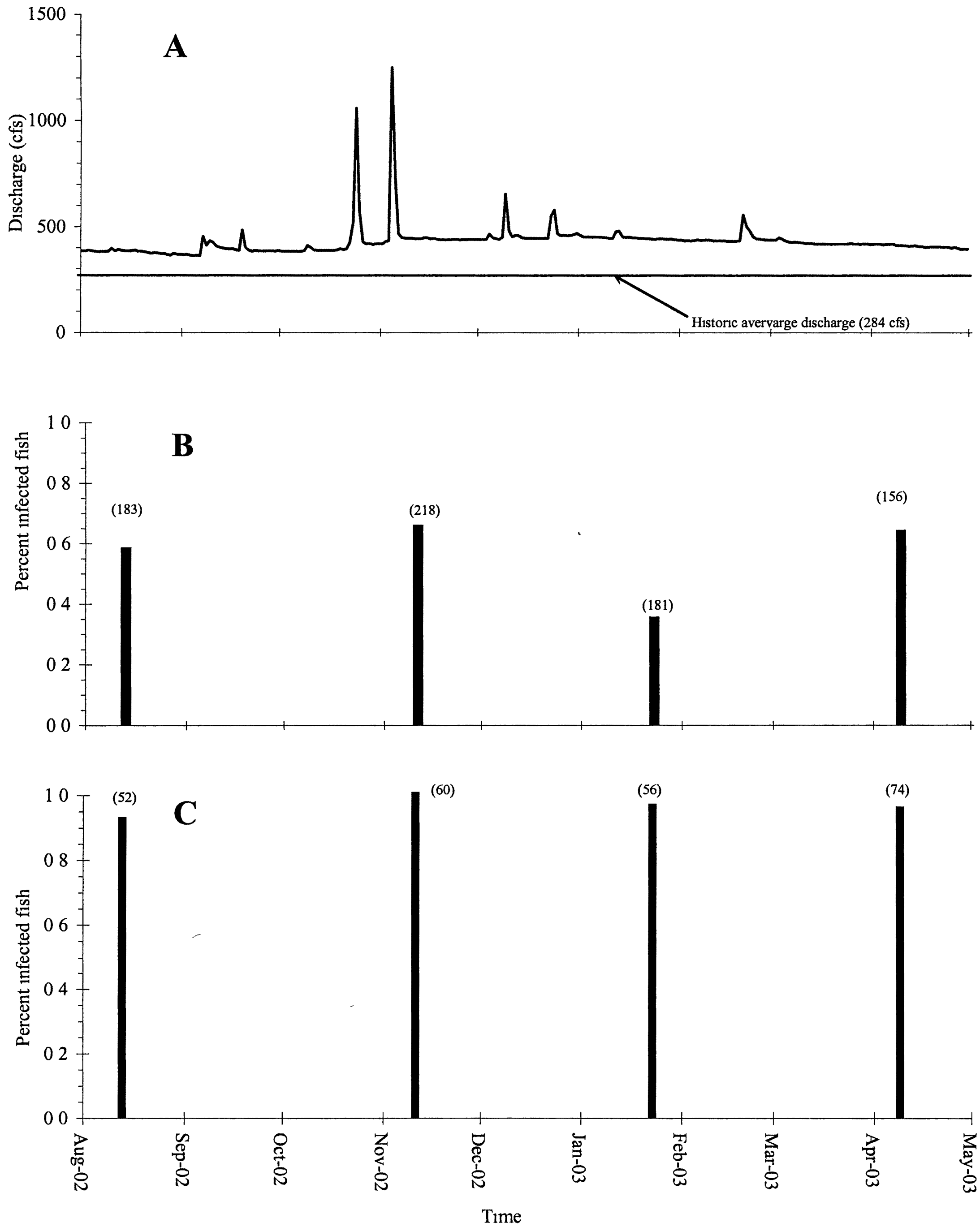

Figure 10. Temporal variation in discharge, cercraial concentration of *Centrocestus formosanus* in river water, and in prevalence in caged and resident fountain darters in the Comal River, Comal County, Texas. Numbers in parenthesis represent number of fountain darters examined A Mean discharge in cubic feet per sec. B Mean % infected caged fountain darters C Mean % infected resident fountain darters

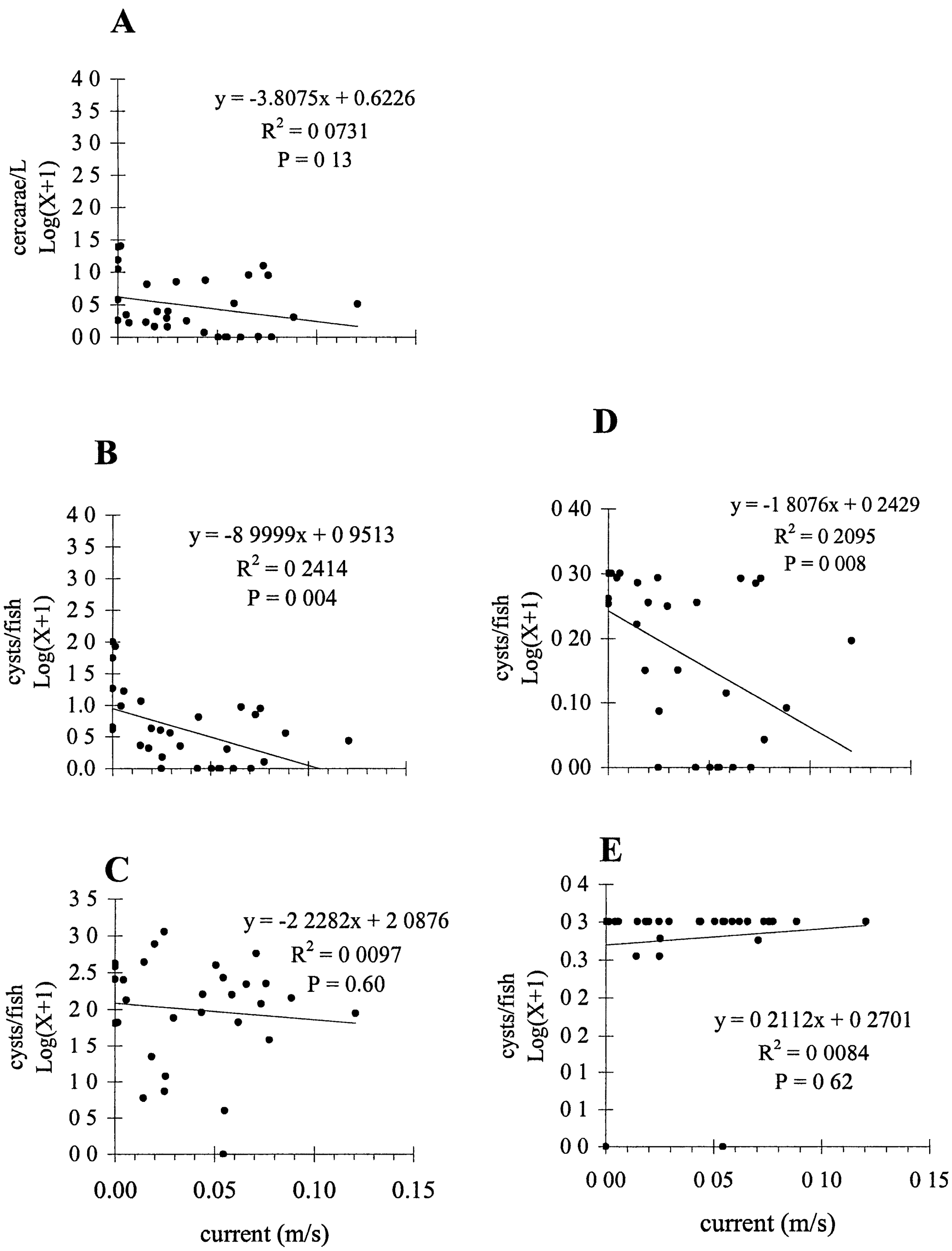

Figure 11. The effects of current velocity (m/s) on *Centrocestus formosanus* abundance. A. Current velocity regressed against cercarial concentration. B. Current velocity regressed against intensity in caged fountain darters C. Current velocity regressed against intensity in resident fountain darters D. Current velocity regressed against prevalence in caged fountain darters. E. Current velocity regressed against prevalence in resident fountain darters.

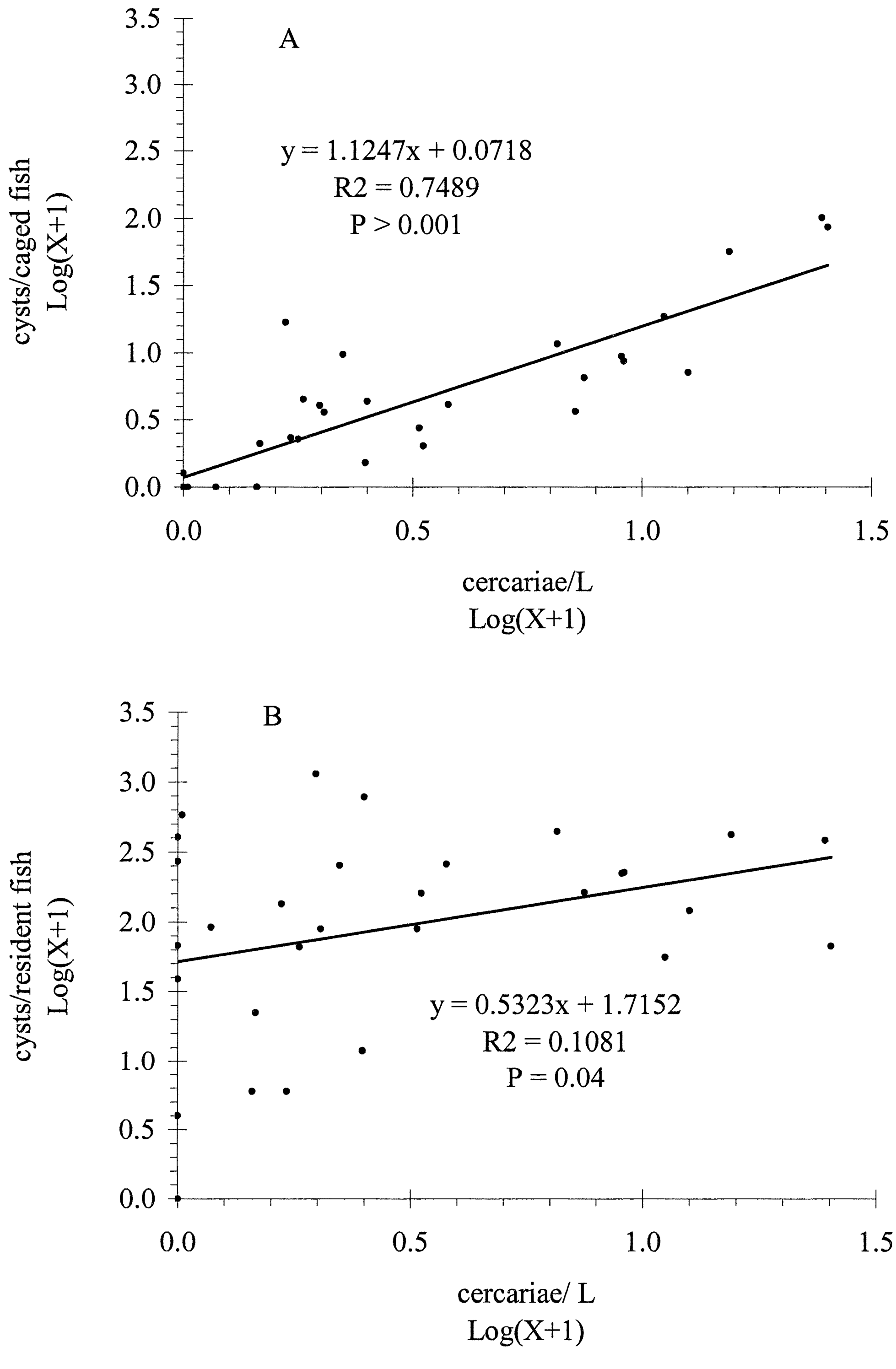

Figure 12 Relationships between cercarial abundance and infection (prevalence and intensity) of resident and caged fountain darters in the Comal River, Comal County, Texas A Cercariae regressed against intensity in caged fountain darters B. Cercariae regressed against intensity in resident fountian darters

Table 1: Spatial and temporal comparisons of *Centrocestus formosanus* abundance at the Comal River, Comal County, Texas, using a two-way ANOVA .

Parasite measurement type	Source of Variation	DF	SS	MS	F	P
Cercarial concentration in river water	Season	3	89.333	29.778	3.904	0.023
	Site	7	1038.5	148.36	19.451	<0.001
	Residual	21	160.18	7.628		
Intensity in caged darters	Season	3	742.27	247.424	1.52	0.238
	Site	7	13348	1906.79	11.717	<0.001
	Residual	21	3417.6	162 742		
Prevalence in caged darters	Season	3	0.447	0.149	5.034	0 009
	Site	7	4.138	0.591	19.962	<0.001
	Residual	21	0.622	0.0296		
Intensity in resident darters	Season	3	97685	32561 6	1.397	0.273
	Site	7	1E+06	192580	8.261	<0.001
	Residual	20	466212	23310.6		
Prevalence in resident darters	Season	3	0.0882	0.0294	0.914	0.452
	Site	7	0.29	0.0414	1.287	0.306
	Residual	20	0.643	0.0322		

Table 2 Spatial and temporal comparisons of cercarial concentration in river water at the Comal River, Comal County, Texas, using an all pairwise multiple comparison procedures (Tukey Test)

Comparison factor		Diff of Means	p	q	P	P<0.050
Season	Summer 2002 vs Winter 2003	4 695	4	4.808	0 013	Yes
	Summer 2002 vs. Fall 2002	2 685	4	2 75	0 241	No
	Summer 2002 vs Spring 2003	2 156	4	2 208	0 421	Do Not Test
	Spring 2003 vs. Winter 2003	2 539	4	2.6	0 284	No
	Spring 2003 vs Fall 2002	0.529	4	0.542	0 98	Do Not Test
	Fall 2002 vs Winter 2003	2 01	4	2.058	0 481	Do Not Test
Sites	SI vs. SPR1	18 148	8	13 142	<0 001	Yes
	SI vs. SPR3	18.107	8	13 113	<0 001	Yes
	SI vs. HS	17.375	8	12.582	<0.001	Yes
	SI vs BI	16.78	8	12 152	<0 001	Yes
	SI vs CF	15 953	8	11 552	<0 001	Yes
	SI vs. ES	13 293	8	9 626	<0 001	Yes
	SI vs GS	11.508	8	8 333	<0.001	Yes
	GS vs SPR1	6 64	8	4.808	0.046	Yes
	GS vs SPR3	6.6	8	4 78	0.048	Yes
	GS vs HS	5 868	8	4.249	0 101	No
	GS vs BI	5.273	8	3.818	0 177	Do Not Test
	GS vs CF	4.445	8	3.219	0 351	Do Not Test
	GS vs ES	1.785	8	1 293	0 981	Do Not Test
	ES vs SPR1	4.855	8	3 516	0.254	No
	ES vs. SPR3	4.815	8	3.487	0.263	Do Not Test
	ES vs. HS	4.083	8	2.956	0 452	Do Not Test
	ES vs BI	3 487	8	2 526	0 636	Do Not Test
	ES vs. CF	2.66	8	1.926	0.864	Do Not Test
	CF vs SPR1	2 195	8	1.59	0 944	Do Not Test
	CF vs. SPR3	2 155	8	1 561	0.949	Do Not Test
	CF vs HS	1.423	8	1.03	0 995	Do Not Test
	CF vs BI	0.828	8	0.599	1	Do Not Test
	BI vs. SPR1	1.368	8	0 99	0.996	Do Not Test
	BI vs SPR3	1.327	8	0.961	0 997	Do Not Test
	BI vs HS	0.595	8	0.431	1	Do Not Test
	HS vs SPR1	0 772	8	0 559	1	Do Not Test
	HS vs SPR3	0.732	8	0.53	1	Do Not Test
	SPR3 vs. SPR1	0 04	8	0 029	1	Do Not Test

Table 3. Spatial and temporal comparisons of intensity in caged fountain darters at the Comal River, Comal County, Texas, using an all pairwise multiple comparison procedures (Tukey Test).

Comparison factor		Diff of Means	p	q	P	P<0.050
Season	Summer 2002 vs Winter 2003	12 74	4	2.83	0.221	No
	Summer 2002 vs Fall 2002	5.68	4	1.26	0 81	Do Not Test
	Summer 2002 vs. Spring 2003	2 256	4	0 5	0.984	Do Not Test
	Spring 2003 vs Winter 2003	10 484	4	2 32	0.377	Do Not Test
	Spring 2003 vs Fall 2002	3 424	4	0.76	0 949	Do Not Test
	Fall 2002 vs. Winter 2003	7 06	4	1 57	0.689	Do Not Test
Sites	SI vs SPR1	64 42	8	10.1	<0 001	Yes
	SI vs SPR3	64.353	8	10 1	<0.001	Yes
	SI vs HS	63.68	8	9 98	<0 001	Yes
	SI vs. GS	60 593	8	9.5	<0.001	Yes
	SI vs CF	59.84	8	9.38	<0.001	Yes
	SI vs ES	59 315	8	9.3	<0.001	Yes
	SI vs BI	56 623	8	8 88	<0 001	Yes
	BI vs SPR1	7.797	8	1 22	0.986	No
	BI vs SPR3	7.73	8	1.21	0 987	Do Not Test
	BI vs HS	7 058	8	1 11	0 992	Do Not Test
	BI vs GS	3.97	8	0 62	1	Do Not Test
	BI vs CF	3.218	8	0.5	1	Do Not Test
	BI vs. ES	2.692	8	0 42	1	Do Not Test
	ES vs SPR1	5.105	8	0.8	0 999	Do Not Test
	ES vs. SPR3	5.038	8	0 79	0 999	Do Not Test
	ES vs HS	4 365	8	0 68	1	Do Not Test
	ES vs. GS	1 277	8	0 2	1	Do Not Test
	ES vs. CF	0 525	8	0 08	1	Do Not Test
	CF vs. SPR1	4 58	8	0 72	1	Do Not Test
	CF vs. SPR3	4 513	8	0 71	1	Do Not Test
	CF vs. HS	3.84	8	0 6	1	Do Not Test
	CF vs GS	0.752	8	0 12	1	Do Not Test
	GS vs SPR1	3 828	8	0.6	1	Do Not Test
	GS vs SPR3	3.76	8	0 59	1	Do Not Test
	GS vs HS	3 088	8	0.48	1	Do Not Test
	HS vs SPR1	0.74	8	0 12	1	Do Not Test
	HS vs SPR3	0 672	8	0.11	1	Do Not Test
	SPR3 vs. SPR1	0 0675	8	0.01	1	Do Not Test

Table 4 Spatıal and temporal comparısons of prevalence in caged fountain darters at the Comal River, Comal County, Texas, usıng an all paırwise multiple comparıson procedures (Tukey Test).

Comparıson factor		Dıff of Means	p	q	P	P<0 050
Season	Fall 2002 vs Wınter 2003	0.304	4	4.993	0.01	Yes
	Fall 2002 vs. Spring 2003	0 0563	4	0.925	0.913	No
	Fall 2002 vs Summer 2002	0 05	4	0.822	0 937	Do Not Test
	Summer 2002 vs Wınter 2003	0.254	4	4 171	0 036	Yes
	Summer 2002 vs. Sprıng 2003	0 00625	4	0.103	1	Do Not Test
	Spring 2003 vs Wınter 2003	0.248	4	4.068	0 042	Yes
Sites	SI vs SPR1	1	8	11.623	<0.001	Yes
	SI vs. SPR3	0.97	8	11.274	<0.001	Yes
	SI vs. HS	0.675	8	7 845	<0 001	Yes
	SI vs. ES	0 33	8	3.835	0.173	No
	SI vs GS	0 292	8	3 4	0 289	Do Not Test
	SI vs. CF	0.23	8	2 673	0 571	Do Not Test
	SI vs. BI	0 103	8	1.191	0 988	Do Not Test
	BI vs SPR1	0 897	8	10 431	<0 001	Yes
	BI vs SPR3	0.868	8	10.083	<0 001	Yes
	BI vs. HS	0 573	8	6.654	0.003	Yes
	BI vs ES	0 227	8	2.644	0.584	Do Not Test
	BI vs. GS	0.19	8	2.208	0.767	Do Not Test
	BI vs CF	0.127	8	1.482	0.961	Do Not Test
	CF vs. SPR1	0.77	8	8 949	<0 001	Yes
	CF vs SPR3	0.74	8	8 601	<0 001	Yes
	CF vs HS	0.445	8	5 172	0.026	Yes
	CF vs. ES	0.1	8	1.162	0.99	Do Not Test
	CF vs GS	0.0625	8	0.726	0 999	Do Not Test
	GS vs SPR1	0 708	8	8.223	<0 001	Yes
	GS vs. SPR3	0 678	8	7 874	<0.001	Yes
	GS vs. HS	0.383	8	4 446	0.077	No
	GS vs ES	0 0375	8	0 436	1	Do Not Test
	ES vs SPR1	0.67	8	7 787	<0.001	Yes
	ES vs. SPR3	0.64	8	7.439	<0.001	Yes
	ES vs. HS	0 345	8	4.01	0.139	Do Not Test
	HS vs SPR1	0.325	8	3 777	0.186	No
	HS vs SPR3	0 295	8	3.429	0.28	Do Not Test
	SPR3 vs. SPR1	0 03	8	0.349	1	Do Not Test

Table 5 Spatial and temporal comparisons of intensity in resident fountain darters at the Comal River, Comal County, Texas, using an all pairwise multiple comparison procedures (Tukey Test).

Comparison factor		Diff of Means	p	q	P	P<0 050
Season	Fall 2002 vs Summer 2002	152.523	4	2.826	0.222	No
	Fall 2002 vs. Spring 2003	104.886	4	1.943	0.529	Do Not Test
	Fall 2002 vs. Winter 2003	92 872	4	1 644	0.657	Do Not Test
	Winter 2003 vs Summer 2002	59.651	4	1.056	0.877	Do Not Test
	Winter 2003 vs. Spring 2003	12.014	4	0.213	0.999	Do Not Test
	Spring 2003 vs Summer 2002	47.636	4	0.882	0.923	Do Not Test
Site	CF vs. HS	775 876	8	9.315	<0.001	Yes
	CF vs. SPR3	737 111	8	8.85	<0 001	Yes
	CF vs. GS	657 859	8	7.898	<0.001	Yes
	CF vs ES	616.966	8	7.407	<0 001	Yes
	CF vs BI	609 426	8	7.317	0.001	Yes
	CF vs. SPR1	473 021	8	5.679	0 013	Yes
	CF vs SI	464.421	8	5.576	0 015	Yes
	SI vs. HS	311 455	8	4.08	0.129	No
	SI vs. SPR3	272.69	8	3.572	0.24	Do Not Test
	SI vs. GS	193.437	8	2.534	0 632	Do Not Test
	SI vs ES	152.545	8	1.998	0 841	Do Not Test
	SI vs. BI	145.005	8	1 899	0 871	Do Not Test
	SI vs SPR1	8.6	8	0 113	1	Do Not Test
	SPR1 vs HS	302 855	8	3.967	0.149	Do Not Test
	SPR1 vs. SPR3	264.09	8	3 459	0.273	Do Not Test
	SPR1 vs. GS	184 837	8	2 421	0.68	Do Not Test
	SPR1 vs ES	143.945	8	1.886	0 875	Do Not Test
	SPR1 vs BI	136.405	8	1.787	0.902	Do Not Test
	BI vs HS	166 45	8	2.18	0.777	Do Not Test
	BI vs SPR3	127.685	8	1 673	0 928	Do Not Test
	BI vs GS	48 432	8	0.634	1	Do Not Test
	BI vs. ES	7.54	8	0.0988	1	Do Not Test
	ES vs HS	158 91	8	2 082	0.813	Do Not Test
	ES vs. SPR3	120.145	8	1.574	0.946	Do Not Test
	ES vs GS	40 892	8	0.536	1	Do Not Test
	GS vs HS	118.018	8	1.546	0.951	Do Not Test
	GS vs SPR3	79 253	8	1.038	0.995	Do Not Test
	SPR3 vs HS	38.765	8	0.508	1	Do Not Test

Table 6. Spatial and temporal comparisons of prevalence in resident fountain darters at the Comal River, Comal County, Texas, using an all pairwise multiple comparison procedures (Tukey Test).

Comparison factor	Diff of Means	p	q	P	P<0.050
N/A	Unable to conduct ANOVA because data was disconnected.				

APPENDIX II

Centrocestus formosanus abundance in river water, and in caged and resident fountain darters from summer 2002 to spring 2003.

Appendix 1. *Centrocestus formosanus* abundance in river water, and in caged and resident fountain darters for summer 2002.

	Filter Study					Caged Fish Study							Resident Fish Study						
Site	No of filters/site	Range of cercariae/5L	Total cercariae/45L	Mean L/filter	SE	No of fish/site	Range of cysts	Total cysts/ caged fish site	Mean cysts/ caged fish	SE	% Infected fish	% Survivorship	No of fish/site	Range of cysts	Total cysts/ resident site	Mean cysts/ resident fish	SE	% infected fish	Mean water velocity (m/s)
SPR1	9	0 - 0	0	0 0	0 0	26	0 - 0	0	0 0	0 0	0%	87%	3	0 - 0	0	0 0	0 0	0%	0 13
SPR3	9	0 - 0	0	0 0	0 0	9	0 - 0	0	0 0	0 0	0%	30%	2	2 - 4	6	3 0	1 0	100%	0 14
CF	9	20 - 41	249	5 5	0 5	29	0 - 30	308	10 6	3 3	93%	97%	1	442 - 442	442	442 0		100%	0 03
HS	9	1 - 13	67	1 5	0 4	27	0 - 4	14	0 5	0 2	22%	90%	10	0 - 24	110	11 0	2 1	90%	0 06
SI	9	50 - 204	1062	23 6	2 8	25	16 - 348	1900	99 8	59 7	100%	83%	6	182 - 596	2288	381 3	63 1	100%	0 00
BI	9	8 - 21	125	2 8	0 5	29	0 - 14	86	3 1	0 7	79%	97%	10	64 - 698	2580	258 0	76 0	100%	0 00
ES	8	29 - 59	317	8 1	0 8	25	0 - 16	192	7 7	1.3	96%	83%	10	86 - 526	2252	225 2	44 7	100%	0 19
GS	9	23 - 82	522	11 6	1 1	13	0 - 14	80	6 1	0 1	92%	43%	10	66 - 150	1078	119 8	9 9	100%	0 18
Total	71		2342			183		2580					52		8756				
Mean				6 6					16 0		60%	76%				180 0		86%	0 09
SD				8 0					34 1							174 5			
SE				2 8					12 1							61 7			

Appendix 2. *Centrocestus formosanus* abundance in river water, and in caged and resident fountain darters for fall 2002.

	Filter Study					Cage Fish Study							Resident Fish Study						
Site	No of filters/site	Range of cercariae/5L	Total cercariae/45L	Mean L/filter	SE	No of fish/site	Range of cysts	Total cysts/ caged fish site	Mean cysts/ caged fish	SE	% Infected fish	% Survivorship	No of fish/site	Range of cysts	Total cysts/ resident site	Mean cysts/ resident fish	SE	% infected fish	Mean water velocity (m/s)
SPR1	9	0 - 0	0	0 0	0 0	30	0 - 0	0	0 0	0 0	0%	100%	4	4 - 1224	1612	403 0	286 0	100%	0 12
SPR3	9	0 - 0	0	0 0	0 0	25	0 - 4	8	0 3	0 2	12%	83%	8	2 - 198	304	38 0	23 1	100%	0 20
CF	9	0 - 11	44	1 0	0 3	27	0 - 8	82	3 1	0 9	96%	90%	1	1144 - 1144	1144	1144 0		100%	0 06
HS	9	1 - 4	21	0 5	0 0	29	0 - 4	32	1 1	0 2	41%	97%	10	5 - 32	214	21 4	4 0	100%	0 05
SI	9	10 - 130	651	14 5	5 0	30	0 - 96	1662	55 4	4 5	100%	100%	7	202 - 638	2928	418 3	64 0	100%	0 00
BI	9	2 - 10	55	1 2	0 3	30	0 - 100	262	8 7	3 2	97%	100%	10	18 - 694	2526	252 6	68 1	100%	0 01
ES	9	29 - 70	361	8 0	1 0	27	0 - 18	226	8 4	0 2	96%	90%	10	56 - 476	2224	222 4	47 2	100%	0 16
GS	9	20 - 38	292	6 5	0 4	20	0 - 14	110	5 5	0 3	80%	67%	10	54 - 326	1608	160 8	26 7	100%	0 11
Total	72		1424			218		2382					60		12560				
				4 0					10 3		65%	91%				332 6		100%	0 09
SD				5 3					18 5							359 0			
SE				1 9					6 6							179 5			

Appendix 3. *C. formosanus* abundancw in river water, and in caged and resident fountain darters for winter 2003.

	Filter Study					Cage Fish Study							Resident Fish Study						
Site	No of filters/site	ange of cercariae/5L	Total cercariae/45L	Mean L/filter	SE	No of fish/site	Range of cysts	Total cysts/ aged fish sit	Mean cysts/ caged fish	SE	% Infected fish	% Survivorship	No of fish/site	Range of cysts	Total cysts/ resident site	Mean cysts/ resident fish	SE	% infected fish	Mean water velocity (m/s)
SPR1	9	0 - 0	0	0 0	0 0	29	0 - 0	0	0 0	0 0	0%	97%	3	14 - 634	814	271 3	186 5	100%	0 13
SPR3	9	0 - 0	0	0 0	0 0	19	0 - 0	0	0 0	0 0	0%	79%	7	4 - 322	468	66 9	43 5	100%	0 15
CF	9	0 - 9	35	0 8	0 2	22	0 - 6	28	1 3	0 4	41%	92%							0 08
HS	9	0 - 5	20	0 4	0 1	20	0 - 0	0	0 0	0 0	0%	83%	10	0 - 26	64	6 4	2 5	80%	0 06
SI	9	10 - 121	458	10 2	4 3	23	4 - 62	406	17 6	6 2	100%	96%	6	40 - 1234	2544	424 0	184 0	100%	0 00
BI	9	2 - 9	37	0 8	0 1	23	0 - 10	80	3 5	0 4	83%	96%	10	12 - 228	650	65 0	21 9	100%	0 00
ES	9	1 - 10	46	1 0	0 3	26	0 - 30	76	2 6	2 1	23%	87%	10	24 - 260	1434	143 4	29 7	100%	0 23
GS	9	6 - 19	105	2 3	0 3	19	0 - 6	18	1 0	0 3	32%	63%	10	22 - 366	1590	159 0	41 5	100%	0 14
Total	72		701			181		608					56		7564				
Mean				1 9					3 25		35%	87%				162 3		97%	0 10
SD				3 4					5 9							143 5			
SE									2 1										
				1 2												54 3			

Appendix 4. *C. formosanus* abundance in river water, and in caged and resident fountain darters for spring 2003.

	Filter Study					Cage Fish Study							Resident Fish Study						
Site	No of filters/site	Range of cercariae/5L	Total cercariae/45L	Mean L/filter	SE	No of fish/site	Range of cysts	Total cysts/ caged fish site	Mean cysts/ caged fish	SE	% Infected fish	% Survivorship	No of fish/site	Range of cysts	Total cysts/ resident site	Mean cysts/ resident fish	SE	% infected fish	Mean water velocity (m/s)
SPR1	9	0 - 1	1	0 02	0 02	17	0 - 0	0	0 0	0 00	0%	57%	9	0 - 1394	5228	580 9	185 9	89%	0 18
SPR3	9	0 - 5	8	0 18	0 18	19	0 - 0	0	0 0	0 00	0%	63%	10	4 - 418	910	91 0	38 6	100%	0 11
CF	9	3 - 12	68	1 51	0 31	23	0 - 12	76	3 3	0 55	78%	77%	9	52 - 1662	7018	779 8	165 2	100%	0 05
HS	9	0 - 9	32	0 71	0 21	6	0 - 2	8	1 3	0 54	67%	20%	10	0 - 14	50	5 0	1 5	80%	0 03
SI	9	24 - 225	1096	24 36	9 64	23	24 - 248	1922	84 9	23 76	100%	77%	3	40 - 108	198	66 0	21 2	100%	0 00
BI	9	1 - 11	30	0 67	0 27	25	2 - 44	398	15 8	1 31	100%	83%	10	38 - 306	1340	134 0	31 4	100%	0 01
ES	9	4 - 16	102	2 27	0 38	19	0 - 6	32	1 8	0 49	53%	63%	9	10 - 284	796	88 4	36 9	100%	0 32
GS	9	14 - 59	277	6 16	1 74	24	0 - 10	60	2 7	0 48	79%	80%	7	6 - 244	534	76 3	31 1	100%	0 07
Total	72		1614			156		2496					67		16074				
Mean				4 48					13 73							227 7			0.10
SD				8 27					29 22							286 62			
SE				2 92					10 33							108 33			

VITA

Valentin Cantu, son of Maria Theodora Cantu and Enrique Cantu, was born in San Antonio, Texas on July 18, 1970. He grew up in Uvalde, Texas and graduated from Uvalde High School. As an undergraduate of Southwest Texas State University, he worked for the U.S. Fish and Wildlife Service (USFWS) as a research technician. He received a Bachelor of Science in Aquatic Biology with a Minor in chemistry from Southwest Texas State University in December 2000. While pursuing his master degree in Aquatic Biology at Texas State University, he continued to work for the USFWS as a Fisheries Biologist and was also employed by BIOWEST.

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