
Final Report to the Utah Reclamation Mitigation and Conservation Commission

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*Centrocestus formosanus cercariae.*

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Table of Contents

Executive Summary ................................................................. 3

Introduction .............................................................................. 4

Methods .................................................................................. 6

Prevalece, Magnitude and Duration of Cercariae Release .............. 6
June Sucker Exposures ............................................................... 7
Praziquantel Treatment of Metacercariae .................................... 8
Phototaxis Test ......................................................................... 9
Sand Filtration Test ................................................................. 10
Ultraviolet Light Test ............................................................. 11
Life cycle ................................................................................. 11

Results .................................................................................... 12

Prevalece, Magnitude and Duration of Cercariae Release .............. 12
June Sucker Exposures ............................................................... 14
Praziquantel Treatment of Metacercariae .................................... 15
Phototaxis Test ......................................................................... 16
Sand Filtration Test ................................................................. 16
Ultraviolet Light Test ............................................................. 17
Life cycle ................................................................................. 17

Discussion ............................................................................... 18

Acknowledgments ..................................................................... 21

Literature Cited ....................................................................... 22
Executive Summary

The proposed construction of a June sucker hatchery at Goshen or Gandy Warm Springs raised concerns about the potential impact of the trematode parasite, Centrocestus formosanus, on fish produced there. The parasite is released from the exotic snail Melanoides tuberculata and has been blamed for severe losses of fish among aquaculture facilities in the tropics. This report summarizes research on the effects of C. formosanus on June sucker and on potential control methods including drug treatment, sand filtration, and phototaxis. The objectives of the research were to learn more about the biology of the parasite, to determine if June sucker were susceptible, and to explore control methods for the snail and C. formosanus.

Several experiments were conducted at the Fisheries Experiment Station, Logan, Utah. The prevalence of C. formosanus in snails and the duration of cercariae release were examined. At Goshen Warm Springs and Fish Springs National Wildlife Refuge, 2% and 2.6% of snails were infected, respectively. Cercariae release varied among infected snails and from week to week. Extrapolating production, cercariae produced by a single snail could reach 1,344 - 41,592 in 24 hrs. June sucker fry exposed to 25 to 200 cercariae per fish in a series of tests suffered significant mortality at doses as low as 25 cercariae/fish, but we were unable to get any metacercarial cysts produced. In another test, we attempted to complete the complex life cycle of this trematode by infecting mice and domestic ducklings with naturally infected Gambusia. These attempts were unsuccessful. The drug praziquantel was given in a bath solution to kill metacercariae encysted in the gills of naturally infected Gambusia. Concentrations of 5 mg/L for 6 hr or 2.5 mg/L for 12 hr were not effective. Ultraviolet light was evaluated by varying the amount of time (0 to 10,000 sec) cercariae were exposed to UV irradiation (28.34 mW/cm² at 257 nm); 99% of the cercaria were alive in all the treatments except for the 10,000 second trial, where 0% of the cercaria were found alive. Sand filtration was evaluated as a water treatment method. The June suckers in the sand filter treatments were not infected, but neither were the positive controls, compromising interpretation of the test. Positive phototaxis behavior of the cercariae was documented in a test in which cercariae were given a choice of light or dark. This behavior could be used to concentrate cercariae and apply a lethal treatment or to capture them in light traps. Water treatment, preferably filtration, is recommended before any water is used for June sucker culture. June suckers were susceptible to infection, however, no effective treatment was found.
Introduction

Fish health professionals across the U.S. and Mexico have become increasingly concerned about the invasion of an exotic digenetic trematode, *Centrocestus formosanus* (Mitchell et al. 2005). The trematode has been blamed for heavy losses (estimated at US$3.5 million) by tropical fish producers in Florida (Francis-Floyd et al. 1997; Mitchell et al. 2005). *Etheostoma fonticola*, a darter federally listed as endangered, has struggled with infections intensities as high as 50 to 191 metacercariae per gill arch or over 800 per fish (Mitchell et al. 2000). Balasuriya (1988) reported *C. formosanus* to be a major cause of a low (8%) survival rate of cultured cyprinids from infected ponds. Native to Asia, the parasite was first reported in Mexico in 1985 (Salgado-Maldonado et al. 1995; Scholz and Salgado-Maldonado 1998). The parasite was discovered in Utah in 2003 (Wilson 2003).

Like other digenetic trematodes, the parasite life cycle involves three different hosts: snail, fish, and bird or mammal (Ginetsinskaya 1988; Fig. 1). The exotic red-rimmed snail (*Melanoides tuberculata*) and a Hawaiian snail *Stenomelania newcombi* are the snail hosts reported to date for *C. formosanus* (Martin 1958; Salgado-Maldonado et al. 1995). *M. tuberculata* inhabits at least 17 warm springs throughout Utah, including the Goshen and Gandy Warm Springs, proposed recovery hatchery sites for June sucker (*Chasmistes liorus*) rearing (Rader et al. 2003). *M. tuberculata* prefer temperatures of 18 to 32°C (Mitchell and Brandt 2005). This tropical prosobranch out-competes many native snail species, and is an obligate intermediate host to several digenetic trematode parasites, including *C. formosanus* (Mitchell et al. 2005). Cercaria, the infective stage in the trematode life cycle that is capable of infecting fish, are shed from the snail. Unlike many species of cercariae that encyst in a fish’s muscle tissue, *C. formosanus* encysts in the lamellae of the gills, impacting respiration (Velez-Hernandez et al. 1998). Alcaraz et al. (1999) showed a 10% decrease in oxygen consumption in infected grass carp (*Ctenopharygodon idella*).

For documented fish hosts, *C. formosanus* has parasitized over 40 species of fish including channel catfish (*Ictalurus punctatus*), fathead minnow (*Pimephales promelas*), golden shiner (*Notemigonus crysoleucas*), and white bass (*Morone chrysops*) (Mitchell et al. 2002; Salgado-Maldonado et al. 1995). Chen (1942) found *Rana limnocharis* and *Bufo melanostictus*, to be susceptible to this parasite as well.
The continuing spread of *M. tuberculata* throughout Utah is a growing problem. Not only are they hosts to the parasitizing trematode *C. formosanus*, they also out compete native snail populations. Rader et al. (2003) found *M. tuberculata* to be the most abundant snail in 19 out of 20 of the springs surveyed in the Bonneville Basin. Over a five-year period, this invasive mollusk has become the most dominant animal in terms of numbers and biomass in the entire spring complex at the Fish Springs National Wildlife Refuge. In St. Johns River in Florida *M. tuberculata* had reached 10,000 snails m$^{-2}$ seven years following its introduction (Thompson 1984). It is important to note that co-evolution occurred between *C. formosanus* and its hosts within *M. tuberculata*’s native range, and not with hosts outside of the indigenous area. Therefore, *C. formosanus* could have a more severe impact on species outside of its native range.

Utah is home to several endemic warm water fish that may be affected by *C. formosanus* including the least chub (*Iotichthys phlegethontis*), the leatherside chub (*Snyderichthys copei*), and the June sucker. Currently the least chub and leatherside chub abundance and range are declining and are considered species of ‘special concern’, and the June sucker is classified as federally endangered. The Utah native Columbia spotted frog (*Rana luteiventris*) may be susceptible to *C. formosanus* as well.
The proposed construction of a June sucker hatchery at Goshen or Gandy Warm Springs raised concerns about the impact of the trematode on fish produced there. This report summarizes research on the effects of *C. formosanus* on June sucker and on potential control methods including drug treatment, sand filtration, and phototaxis. The objectives of the research were to learn more about the biology of the parasite, to determine if June sucker were susceptible, and to explore control methods for the snail and *C. formosanus*.

**Methods**

Several experiments were conducted at the Fisheries Experiment Station, Logan, Utah, which are the subject of this report. The prevalence of *C. formosanus* in snails and the duration of cercariae release were examined. Another experiment was conducted exposing June suckers to a given number of cercariae and examining their gills for infection. In another test, we attempted to complete the complex life cycle of this trematode by infecting mice and ducklings with naturally infected *Gambusia*. Yet another test evaluated high doses of the drug praziquantel to kill metacercariae encysted in the gills of naturally infected *Gambusia*. Praziquantel is widely used for treating fish infected with monogenetic trematodes and other types of infections (Noga 1996; Eissa 2002). Another experiment looked at the phototaxis behavior of the cercariae, in hopes to concentrate cercariae in the water column and apply a lethal treatment. Ultraviolet light and sand filtration were also evaluated as potential control methods.

*Prevalence, Magnitude, and Duration of Cercariae Release*

We collected 600 *Melanoides tuberculata* from Goshen Warm Springs (southern Utah County), as well as several hundred snails from Gandy Warm Springs (northwestern Millard County) and the Fish Springs National Wildlife Refuge (FSNWR), Juab County. Each population of snails was housed in separate 20-gallon aquaria with oxygenated well water at 20°C, their optimum temperature (Mitchell 2005; Fig.2).

Snails were scanned for infection by placing ten snails in a beaker filled with just enough water to cover them. The beaker was placed in a dark box for 1 hr, and then transferred to a source of artificial light at 1000 lux for 2 hrs (Lo and Lee 1996; Umadevi and Madhavi 1997). Three 1 ml aliquots...
were taken from the sample and scanned using a light microscope. If cercariae were observed, the snails were segregated into individual test tubes and scanned again to isolate the infected snail.

The number of cercariae shed from each infected snail was monitored once per week for 4 months to determine cercarial production. Each individual snail was placed in 3.4 ml well water and exposed to 1000 lux light for 2 hrs. Three 25 µl aliquots were observed under a light microscope and number of cercariae counted. The average number of cercariae per aliquot was extrapolated to get the total number in 3.4 ml. A Kruskal-Wallis test was used for comparing total cercariae production among individual snails. Ordinary linear least-squares regression was used to test for trends in each snail’s weekly production of cercariae.

**June Sucker Exposures**

*Trial 1*— In our first trial experiment we attempted to infect June suckers with three different doses of cercariae freshly harvest from *Melanoïdes*. Ten fish were put into 100 ml beaker of aerated water and left for 30 minutes (Fig. 3). Fish were exposed to either 100 cercariae/fish, 200 cercariae/fish, or 0 cercariae/fish. Two replicates of each treatment were conducted. Temperature was 21°C. Mean fish weight was 41 mg and mean length was 17.5 mm. After exposure the fish were transferred to aquaria in a recycle system.

The leftover water following exposures was decanted into 50 ml centrifuge tubes and 1.85 ml of 10% formalin was added to each tube. Tubes were centrifuged at 1000 rpm/10 min. Afterwards 100µl was pipetted out from the bottom of the tube and examined for any remaining cercariae.

*Trial 2*— The second experiment on 4 August 2004 used a smaller dose per fish, assuming the 100 and 200 cercariae doses were lethal to the small fry. We exposed 10 fish per replicate to 0, 25, 50 and 100 cercariae/fish. The volume of water was reduced to 40 ml, exposing fish for 30 min with aeration. Temperature was 21°C. Fish were transferred to 10 gallon aquaria set up on a recycle system. Due to fish being impinged in the drain, the mortality assessment was compromised and the survivors were harvested 5 days after exposure. Fish were stored in formalin and three heads from each treatment were analyzed for any sign of migrating cercariae and metacercariae.

Figure 3. Beakers used for exposure of June suckers
Trial 3—One last experiment was conducted 11 August 2004 in an attempt to successfully infect June suckers with Centrocestus formosanus. Again, 10 fish in each replicate (3 replicates per treatment) were exposed to 0, 25, 50 or 100 cercariae/fish for 30 min. Only 35 ml of water was used, with intermittent air pulses to give the cercariae a better chance of encountering a fish. Temperature was 19ºC. The aquaria set up to house each treatment were modified to a static system with supplemental aeration in hopes of reducing deaths due to impinging on the outflow tube screen. Each tank was cleaned twice a week and 5 fish from each treatment were harvested every 7 days for 4 weeks. One set of gills from each fish was checked for cercariae and metacercariae.

Praziquantel Treatment of Metacercariae

The objective of this experiment was to determine if praziquantel could kill encysted metacercaria of C. formosanus. Two tests were conducted on naturally infected mosquitofish (Gambusia affinis) harvested from FSNWR, Utah. The fish were held in 20 gallon aquaria for several weeks at 17°C until the drug treatment was given. Before the first experiment, a wet mount of the gills from four fish was microscopically examined for metacercariae to ensure natural infection was present. In addition, a histological section of gill, stained with Masson’s trichrome, was created (Fig. 4).

In the first test on 13 December 2004, ten fish were placed in each of three replicate buckets (30 fish per treatment) for the drug bath treatments. Praziquantel treatment doses were 0.66 mg/L for 12 hrs, 1.32 mg/L for 6 hrs, and 0.0 mg/L for 6 hrs (control). Since the drug was diluted in ethanol, the control received 1 ml of ethanol as well. Buckets contained 7.6 L of wellwater that was aerated during the exposure via airstones. The water temperature during exposure was 14°C. Fish were transferred to 20 gallon aquaria within a recycle system after drug treatment. Fish were fed a flake feed (Tetramin®) twice a day until harvested. The recycle system water quality was measured prior to introducing fish (temperature, ammonia, nitrite, and pH). Fish were harvested after 3 days and one gill from each fish examined under a light microscope. Each metacercarial cyst was observed for at least 2 min, watching for movement of metacercariae. If no movement...
was observed, the metacercaria was pronounced dead. Necrosis of the parasite was also looked at and recorded. The remaining gills were preserved in 10% neutral-buffered formalin for possible histology.

In the second test, ten fish were placed into 4 L aerated water bath for each replicate of each treatment. Two different doses of praziquantel were used: 5 mg/L x 6 hr, 2.5 mg/L x 12 hr. A control group was treated with 1 ml 70% ethanol for 12 hr. The water temperature during exposure was 14°C. After the allotted time per treatment, fish were placed into 20-gallon aquaria on a recycle system and fed a Tetramin diet twice a day until harvested. Five fish from each treatment were sampled on day 3 and day 7 after exposure. A wet mount consisting of one gill from each fish was analyzed under the microscope. Each cyst was observed for at least 2 minutes watching for movement of metacercariae.

Phototaxis Test

The objective of this study was to determine if the cercariae of *C. formosanus* exhibits phototaxis behavior. If a positive phototaxis behavior is found, it could be used to concentrate the parasite into a specific area of the water column, then apply a treatment such as ultraviolet light, filtration, or sonication to eradicate the parasite.

Freshly harvested cercariae were placed in half-dark/half-clear (HDHC) boxes to observe which side they preferred. All-dark and all-clear boxes were used as control treatments. Three replicates of each treatment were conducted in clear plastic boxes 11 X 7.5 cm by 1.2 cm deep. The dark side of the box was blackened out using several coats of black spray paint. Clear plastic wrap and a bisecting string were placed in the bottom of each container. Boxes were filled with 90 ml of water, and 5,000 freshly harvested cercariae, and left under 1100 lux of artificial light for 3 hrs. The string underneath the plastic wrap, when pulled taut, bisected the box, allowing the water to fall to each side. The water from each side was pipetted out into appropriately labeled vials. Each vial was treated with 10 µl fluorescein diacetate (FDA) solution (100 µl FDA stock solution (5 mg/ml acetone) diluted in 8 ml deionized water), incubated for 45 minutes, and filtered through an 8 µm Nuclepore membrane. Live cercariae were counted using a fluorescence microscope.

For statistical analysis, a significance level of $\alpha = 0.05$ was used for each test. Percentages of cercariae on the clear side (or A side) were arc-sine transformed prior to analysis. A one-way ANOVA was used to compare percentages among treatments. Tukey’s Least Significant Difference test was subsequently used to determine which treatment means were significantly different from each other. Statistical analyses were conducted using SPSS software (SPSS 1993).
Sand Filtration Test

The sand filtration system was designed using three 20-gallon tanks on a recycle system. With the exception of sand size, the design of the sand filter system is as described in Arndt and Wagner (2004). Briefly, 10 cm of gravel was placed in the bottom of a capped section of 15.2 cm diameter PVC pipe, which was placed above the tanks. The gravel was topped with 18 cm of sand 250-300 µm in size. A head box received water from a sump collecting water from the aquaria after passage through high-surface area media for aeration and ammonia and nitrite stripping. A manifold delivered water from the headbox to the sand filters. A float valve was connected above the sand to stop the flow of water if the sand filter backed up.

Ninety June suckers were placed into 9 tanks (10 fish/tank) one week before starting the experiment to acclimate. The front of each tank was covered with black plastic to prevent spooking the fish. Fish were fed a razorback diet twice a day and the water was kept at an average of 17.2°C. Three treatments were used in triplicate for this experiment: a positive control, negative control, and the sand filters. The positive control and sand filter treatments were exposed to cercariae, while the negative control was not.

Cercariae for each treatment were freshly harvested the day of exposure from a tank of 16 infected snails by siphoning out 15 L of water after the water had been agitated thoroughly. From this sample, three 300 ml samples were stained with 20 µl fluorescein diacetate (FDA) stock solution (100µl FDA diluted in 8 ml deionized water), filtered through an 8 µm Nuclepore filter, and counted under a fluorescence microscope. The three-sample average was extrapolated out to estimate the total number of cercariae. The cercariae total was divided equally between the two exposure treatments. Using a chicken-waterer, infected water (7.6 L) was dripped into the head box of the positive control and the sand filter treatment over a period of approximately 45 minutes. This procedure was repeated three times per week for two months.

Biweekly water exchanges were implemented for each system. The sand filter treatment also required a weekly back flush to clean the extra debris that had accumulated in the filter. The back flush pumped fresh water up and out of the pipe into a waste bucket. The protocol was followed as described in Arndt and Wagner (2004).

Four fish from each tank were harvested and placed in formalin at the end of two months. One week later, the rest of the fish were harvested and pickled in formalin. Each individual gill from both sides of the head was cut out and placed on a slide for wet mount analysis. The gills were then placed back into 70% alcohol for possible histology.
**Ultraviolet Light Test**

An experiment using UV light was conducted as a means of eradicating cercaria living in the water. To test this, we exposed approximately 21,000 freshly harvested cercaria directly to UV light using four different treatments 10, 100, 1,000, and 10,000 seconds. The mean UV irradiation per second was 28.34 mW/cm² at 257 nm. The change in time increased the irradiation exposure on the cercaria to 283.4, 2,834, 28,340, and 283,400 mW/cm², respectively. After the allotted amount of time, three 50 ml samples were taken from each treatment and stained with fluorescein diacetate (FDA) and propidium iodide (PI). The samples were then filtered using an 8 μm Nuclepore® membrane and observed with a fluorescence microscope. Cercariae staining green were considered alive, while those staining red were considered dead.

Due to the increase in temperature from UV treatment, a separate test was conducted with cercaria to evaluate the effect on survival of temperature per se at the levels observed in the UV tests. For example, in the 10,000 sec treatment, temperature increased from 14 to 28°C. The vital stains noted above were used to assess viability of cercariae in a water bath that matched the increase observed in the UV test (Fig. 5).

**Life Cycle**

Attempts were made to complete the life cycle of *Centrocestus formosanus* by infecting mice or ducks with metacercaria encysted in *Gambusia* gills. Two mice were obtained from a local pet store. A fecal sample from the mice was analyzed prior to feeding metacercariae. Six eggs were found in an agitated water/feces floatation, which were identified as pinworm eggs (genus *Aspiculuris*). Both mice were treated with Ivermec® (Ivermectin and propylene glycol mixture) at 2 mg/kg (50 μl of solution given orally). A second treatment was given 3 days later. A week later, one mouse was fed three June suckers left over from the exposure study. Two days after feeding, a fecal float was examined for trematodes, but none were found. Similar results were observed after 3, 4, or 5 weeks. After 5, 8, 9 and 10 weeks, the mouse was fed three small
Gambusia from Fish Springs, which were known to be infected. These were mixed together with peanut butter for feeding. Commercial feed was also provided in excess during their tenure in the laboratory. Fecal samples were examined periodically from both mice. Finally the mice were euthanized and scrapes of the intestine were examined microscopically.

Ducks were also evaluated as potential hosts for *C. formosanus*. Three domestic ducklings were fed *Gambusia* infected with the parasite. They were given 20 per day for 8 days. The feces were examined after sedimentation treatment using standard veterinary techniques (Fowler and Miller 1999). After several months, the ducks were euthanized and scrapes of the intestine were examined microscopically.

**Results**

*Prevalence, Magnitude, and Duration of Cercariae Release*

At Goshen Warm Springs only 14 out of 600 snails, or 2%, were infected. Only 8 snails or (2.6%) were found with *C. formosanus* from FSNWR and 0% were infected from Gandy. We also found another trematode released from *Melanoides* snails that we identified as *Haplorchis* sp. The percent of infected snails may be misleading due to intermittent shedding of cercariae from individual snails. The number of cercariae shed over a 2-hr period on a given day varied between snails as well as within individual snails (Table 1). One snail released 56 cercariae in 2 hrs one day and 1733 cercariae in 2 hrs the next day. Variation in the number of cercariae shed between snails was also significant, with some snails that continually shed less *C. formosanus* than others (Fig. 6).

Although only a small percentage of snails were infected with *C. formosanus*, the rate of infectivity may be higher due to *M. tuberculata*’s unpredictable cercarial shedding patterns. Nonetheless, the potential to have high cercariae densities in water supplies is likely due to the sheer number of cercariae released daily from one infected snail. Extrapolating this information, one snail could release from 1,344 - 41,592 cercariae in 24 hrs. The common carp (*Cyprinus carpio*) was easily infected with *C. formosanus* cysts when exposed to 100 cercariae for 1 hr (Lo and Lee 1996). Indeed, our sampling of *Gambusia affinis* from Fish Springs National Wildlife Refuge has indicated that 100% (*n = 200*) of the fish are infected.
Table 1. The number of *Centrocestus formosanus* cercariae shed from each individual snail A-O for each of nine weeks, after exposure to 1000-lux artificial light for 2 hrs. Weekly production averages and 9-week totals are also presented. The x indicates that the snail was not observed.

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Figure 6. Mean number of *Centrocestus formosanus* cercariae produced per week per individual snail (± SE, n = 9).
June Sucker Exposures

Trial 1— All the fish from each treatment were dead the following morning (100%), but only one control fish was dead (5%). This may have been due to number of cercariae infecting each fish. However, interpretation of the results was complicated by problems with water quality in the recycle system. Nitrite levels were high, reaching 0.33 mg/L and unionized ammonia was higher than recommended (0.102 mg/L). For counts of remaining cercariae in containers after exposure, there were 233 and 111 (23% and 11%) cercariae for the 100 cercariae/fish treatment, compared to 192 and 273 (9% and 14%) cercariae remaining in the 200/fish treatment. So, actual infection doses were less (77-89/fish or 172-182/fish, respectively), but still potentially lethal to June sucker fry.

Trial 2— Within a half hour of the last exposure, 3 fish were dead in the 100 cercariae/fish treatment. The average mortality rate after 18 hr was significantly higher in the 100 cercariae/fish treatment (43%) than among controls (0 to 10%, Table 2). After 96 h, cumulative mortality rates were not significantly different among treatments (Table 2). Water quality tests indicated that the recycle system pH (8.0 to 8.5), temperature (20.5°C), and unionized ammonia (0.00 to 0.012) was likely not responsible for deaths. There were migrating cercariae found in each fish examined that had not yet transformed into metacercariae. The number of cercariae found per fish head ranged from 12 to 33, 5 to 10, and 0 to 3 in the 100, 50, and 25 cercaria/fish treatments, respectively. Controls had no cercaria in the gills. Means for each treatment are presented in Table 2.

<table>
<thead>
<tr>
<th>Treatment</th>
<th># Cercariae found/fish head</th>
<th>Mortality after 18 hr % ± SD</th>
<th>Mortality after 96 hr % ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>15.0 ± 4.2 b</td>
<td>43.3 ± 15.3 a</td>
<td>56.7 ± 15.2</td>
</tr>
<tr>
<td>50</td>
<td>16.7 ± 14.2 b</td>
<td>10.0 ± 17.3 b</td>
<td>20.0 ± 34.6</td>
</tr>
<tr>
<td>25</td>
<td>2.2 ± 2.2 ab</td>
<td>20.0 ± 17.3 ab</td>
<td>40.0 ± 36.0</td>
</tr>
<tr>
<td>0</td>
<td>0.0 ± 0.0 a</td>
<td>3.3 ± 5.8 b</td>
<td>3.3 ± 5.8</td>
</tr>
</tbody>
</table>

Trial 3— There was no sign of cercariae or metacercariae found in any of the gills from the fish harvested. The gills of many fish secrete copious amounts of mucus that may hinder the
penetration of *C. formosanus* cercariae. Although we found that *C. formosanus* cercariae are lethal to June sucker fry, in order to firmly establish cercariae of *C. formosanus* will encyst in the gills of June suckers, another experiment should be conducted. Lo and Lee (1996) found on average only 45% of cercariae that successfully invaded the gills of *Cyprinus carpio* developed into cysts.

*Praziquantel Treatment of Metacercariae*

The *Gambusia* sampled before the experiment were heavily infected. In the first test, praziquantel treatments 0.66 mg/L for 12 hrs or 1.32 mg/L for 6 hrs concentrations of at the observed doses had little effect on metacercariae encysted in the gills of *Gambusia*. On each sample day, 90% metacercariae were still active from the 5 mg/L x 6 hr treatment. On day 3, 100% of the 2.5 mg/L x 12 hr treatment metacercariae were active, while 90% were active on sample day 7 (Fig. 7). Only one cyst from sample day 3, treatment 5 mg/L x 6 hr, showed visible signs of necrosis (Fig. 8). Because most of the metacercaria were active, histology was not conducted.

![Figure 7. The percent of metacercariae cysts active after praziquantel treatments.](image-url)
Phototaxis

A positive phototaxis behavior was found for *C. formosanus* cercariae. One hundred percent of the total cercariae in the HDHC were found on the clear side of the box while cercariae were randomly dispersed in the other two treatments (Fig. 9). An ANOVA showed a significant difference among treatments. The post hoc test indicated a significant difference between the HDHC and the other two treatments, but no difference between the all-dark and all-clear treatments.

Sand Filtration

Previous studies by Arndt and Wagner (2004) showed that sand filtration could eliminate triactinomyxons of *Myxobolus cerebralis* from infected water (the parasite that causes whirling disease). The objective of this study was to test sand filtration as a means of eradicating cercariae from infected water. The number of cercariae harvested for each exposure varied significantly (Fig. 10). The most cercariae harvested were 2250/fish on January 20th, with the lowest harvest at 40/fish on January 31st. The two-month average was 305 cercariae/fish.

Although one fish from the positive control may have had one encysting cercariae on the dorsal gill, the remaining samples showed no sign of metacercariae. Some gills were difficult to cut due to the small size of the fish, thus a small portion of the gill was lost. Due to the negative results, histology was not conducted.
**Ultraviolet Light Test**

On average, 99% of the cercaria were alive in all the treatments except for the 10,000 second trial, where 0% of the cercaria were found alive (Fig. 11). The parasite may have died due to a 14 °C rise in temperature during the 10,000 second duration. However, our temperature test showed that 91% of cercariae were alive at the end of the treatment (Fig. 11).

**Life Cycle**

The fecal samples from the mice were all negative for any trematodes. Fecal samples from the ducklings were similarly devoid of trematodes. Examination of the intestines did not reveal any trematodes either, indicating that at least the fecal sampling techniques were not at fault. The time frame for sampling should not have been a problem since Chen (1942) reported recovery of adult trematodes within a week of feeding white rats. Chen (1942) also reported recovery of the parasite from cats and humans, indicating that the primary host specificity is broad. Research with another trematode (*Echinostoma caproni*) in mice indicated that excystation of ingested metacercaria takes place within 1-2 hr (Fried et al. 2001). The reason for our failure to recovery any trematodes remains unclear. The metacercariae in the gills were checked before feeding at least on one occasion and they were moving. With the mice, the Ivermectin treatment may have been a complicating factor, but the
ducks were not treated with any drugs in our care. It is conceivable that they were treated before
we purchased them, preventing infection.

**Discussion**

The control measures evaluated provided mixed results. Praziquantel is highly effective for
several species of monogenean trematodes and tapeworms (Bylund and Sumari 1981; Székely
and Molnár 1991). However, the doses used in this experiment were relatively high compared to
the wide range of doses reported throughout the literature (Noga 1996). The presence of calcified
lesions may protect the parasite from the drug. Increasing the drug dose would not be practical
on a large hatchery system, so using praziquantel would not be an effective treatment for encysted
metacercariae of *C. formosanus*.

The intention of the sand filtration test was to expose the June suckers to 1000 cercariae/fish
for each exposure. However, the cercariae harvest from the 16 infected *M. tuberculata*
significantly varied between snails as well as within snails. On a given day, one infected snail
would shed 56 cercariae in 2 hrs one day and 1733 cercariae the next. The June suckers from the
sand filter treatment were not infected, but unfortunately positive controls were not infected
either, indicating our exposure regimen was ineffective. Using the same sand filtration design,
Arndt and Wagner (2004) found 98.3% fish infected with whirling disease from the positive
control. Cercariae of *C. formosanus* are much larger in size (362 x 197 µm; Chen,1942) than
triactionomyxons (146 x 12 µm; Arndt and Wagner, 2004). Therefore the 250-300 µm sand
should have theoretically filtered out the parasite. Our hypothesis may have worked but we are
unable make any conclusions due to 0% infection rate in the positive control.

Reasons beyond the experimental design should explain the lack of infected fish in the
positive control. The coke rings used to degas the water in the 6 inch PVC pipe may have caused
the cercariae to loose their tails, eliminating the possibility of infection. Without their tails they
are incapable of motility and penetration into gills of a fish. Another reason may be due to their
tendency to become motionless in moving water. Velez-Hernández et al. (1998) found cercariae
to freeze in response to water current, perhaps in hopes to be siphoned through the fish’s gills.
The positive phototaxis behavior of *C. formosanus* may have also contributed to the 0% infection
rate in the positive control. The June suckers in this experiment tended to aggregate on the
bottom of the tank where a negative phototaxis behavior would be advantageous to a parasite.
Benthic fish would not be infected if the cercariae remained near the surface.

Taxis is an important factor for cercariae in ensuring contact with its second intermediate
host. Having a positive or negative phot-, geo-, thigmo-, chemo-,and/or rheotaxis concentrates
the cercariae in the area of the water column in which the secondary host may be found (Ginetsinskaya, 1988). For example, cercariae of *Cercaria pseudarmata* exhibit a negative photo- and positive geotaxis, which increases the probability of encountering chironomidae larvae, the second host for this species. Other cercariae, such as *Cercaria limbifera* have a positive photo- and negative geotaxis that prompts them to leave the first mollusk host during daylight hours and congregate near the surface. The second host is a bottom dwelling mollusk that surfaces to breath atmospheric air during the day, at which time are attacked by cercariae. Yet other species of cercariae exhibit a positive geo, thermo and phototaxis. This species, *Opisthorchis felineus*, emerges from the mollusk host during daylight hours when fish (the second host) are most active. The positive geotaxis then prompts the cercariae to migrate to deeper water where benthophagous fish (the second host) lives.

Generally, cercariae tend to follow a circadian rhythm often subject to photoperiod, thermoperiod, or tidal depth, in the case of trematodes found in intertidal marshes (Fingerut et al. 2003). Many species emerge diurnally with a single peak in 24 hrs, while other trematode cercariae show 2 peaks, or emerge nocturnally (Umadevi and Madhavi 1997). In many climates, temperature coincides with the photoperiod, cooling off during the night. Any significant change in thermoperiod from dusk to dawn enforces the photoperiod.

*Centrocestus formosanus* cercariae do not follow a distinct photoperiod, as demonstrated by Lo and Lee (1996). Cercariae were released in 1-5 peaks in 24 hrs, although they do tend to emerge in elevated numbers if subjected to light and higher temperatures. When exposed to dark vs. light at 25°C, *M. tuberculata* shed 1,390 verses 6,025 cercariae, respectively. A change in temperature at a constant 500 lux produced 11,990 at 35°C and only 2 at 15°C. If temperatures were reverted back to normal (25°C), shedding resumed to approximately 150 cercariae.

The first obligate host of *C. formosanus*, *M. tuberculata*, tends to bury in the mud or bottom substrate of a pond during daylight hours and then emerge nocturnally to feed (Rader et al. 2003). If *M. tuberculata* are buried below the pond substrate during daylight hours, how have cercariae developed a preference for emerging during daylight? Studies have demonstrated snails infected with trematodes change their behavior in favor of the parasite. *Stagnicola elodes*, mollusk host of *Plagiorchis elegans*, migrates to the top of the water column within 15 minutes of light reduction. This is not a typical behavior of *S. elodes* but it triggers cercarial shedding. The snail remains at the top of the water column for up to 3 hrs, during which time 79% of all cercariae emerge (Lowenberger and Rau 1994; McCarthy 1999).

Cercarial emergence of digenetic trematodes often corresponds with the location and ecology of the second intermediate host (Ginetsinskaya, 1988). A positive phototaxis behavior
increases the probability of *C. formosanus* to intercept their secondary host, typically a warm water fish, by swimming from the pond bottom towards surface light allowing cercariae to encounter fish throughout the water column. Surface migration of cercariae would also increase the probability of infecting top dwelling fish, which would in turn increase the probability of completing their complex life cycle via bird or mammal. Again, 100% of top dwelling *Gambusia* from FSNWR were infected.

The short life span exhibited by cercariae (50 hrs) increases the importance of a specific emergence pattern. Cercariae survive by the glycogen reserves in their body, which is directly related to their body size (Ginetsinskaya 1988; Karvonen et al. 2002; Lo and Lee 1996). As the glycogen diminishes, the probability of finding a secondary host decreases. Although cercariae of *C. formosanus* can swim, they are not strong swimmers and might benefit from being released near the surface of the water. The positive phototaxis behavior would keep the cercariae from sinking to the pond floor, and ultimately decreasing the probability of completing the life cycle. Indeed, *C. formosanus* cercariae swim upwards after sinking for a short time (Salgado-Maldonado et al. 1995).

The random diurnal peaks of emergence may explain why infected snails from the current study inconsistently shed *C. formosanus* cercariae. Although snail behavior was not the focus of this study, it may be an important factor in understanding this parasite’s cercarial shedding pattern. Understanding the phototaxis behavior of *C. formosanus* cercariae could prove to be useful in eradicating cercariae from hatchery water by concentrating them into a small area and applying a chemical or mechanical treatment for eradication. Their tendency to become static in response to moving water (Velez-Hernandez et al. 1998) may limit use of this method to static water systems although; a large holding tank to treat infected water might alleviate this problem.

Ultraviolet light may inhibit *C. formosanus* cercariae from parasitizing fish. Although cercaria in this study died at an irradiation between 28,340 and 283,400 mW/cm², even the lowest rate of 28,340 mW/cm² is orders of magnitude higher than some research has suggested is needed to kill several species of parasites. *Trichomonas vaginalis* had 99% mortality at a dose of 401.7 mW/cm², while *Schistosoma mansoni* cercariae attenuated with a dose of 18mW/cm². Using the small 25-Watt lamp we conducted the experiment with, and assuming a dose of 283,400 would be necessary to kill *C. formosanus*, it would take 7,857 years or 2,872,320 lamps to treat one day’s worth of water at 0.113 m³/sec (4 cfs) in a hatchery system. Obviously, this is unreasonable. Larger lamps with higher wattage are available and may cut the number of lamps and/or time down dramatically, possibly making UV a feasible treatment to eradicate *C. formosanus*. Further
research may also show that viability as determined by vital staining may not equate with infectibility, i.e., lower doses of UV may prevent infection although the cercariae are still alive.

Other measures of control for *C. formosanus* focus on the snail host, either killing them directly with chemicals such as copper sulfate (Hoffman and Bauer 1971) or Bayluscide (Francis-Floyd et al. 1997), or using molluscivorous predators (Stauffer et al. 1997). Research with other trematodes such as *Diplostomum* has shown that mechanical filtration with 32 μm mesh filters eliminated 99% of the cercariae (Larsen et al. 2005). Treatment of water with greater than 20 mg/l of sodium percarbonate was effective in killing infective cercariae as well, although it required at least 34 h (Larsen et al. 2005). An additional control strategy has been the treatment of the fish host with anti-helminthic drugs such as praziquantel (Bylund and Sumari 1981; Székely and Molnár 1991). Our research indicated that this drug is not useful for treating encysted metacercariae of *Centrocestus formosanus*. Ultrasonic waves have been used to kill trematode cercariae, but energy and equipment costs may preclude the large-scale implementation of this technology (Wolber and Pietrock 2004).

For any June sucker facility constructed in the infected springs, we recommend treatment of the water prior to use of the water for aquaculture. Filtration technology is costly, but would help alleviate problems with *C. formosanus* and other pathogens as well.

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