The effects of various solutions and levels of desiccation on the survival of *Trichobilharzia stagnicolae* miracidia

Selçuk Üremek, Alison Clune, Jennifer Foster, and Harry Kuo

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University of Michigan Biological Station, 9133 East State St, Pellston, Michigan 49769-9133.
email: uremesel@umich.edu

ABSTRACT

Miracidia of *Trichobilharzia stagnicolae* were placed under two tests: one test examined the longevity of miracidia in various aqueous solutions; the other test examined the effects of desiccation on the hatching of miracidia from eggs. For the solutions test, we used five different solutions: filtered lake water, distilled water, well water, distilled water with 10% sucrose, and avian ringers. Longevity of miracidia was the greatest in filtered lake water and distilled water respectively. For the desiccation test, the number of miracidia hatching from eggs decreased as time allowed for desiccation increased. Further analysis suggested that longevity of miracidia is greatly decreased in hyperosmotic solutions.

INTRODUCTION

In humans, cercarial dermatitis (colloquially known as swimmer's itch) is caused by the penetration of the skin by cercaria of avian schistosomes (Narain et al., 1998). Swimmer's itch is an emerging health problem worldwide (Knight and Worms, 1972; Kullavanijana and Wongwaisayawak, 1993; Narain et al., 1994; Thune, 1994; De Gentile et al., 1996; Kolářová et al., 1999; Verbrugge et al., 2004; Rao et al., 2007). Communities at the greatest general risk are those in the developing world that depend on cercariae-harboring lakes, ponds, and streams for most or all of their water needs (Rao et al., 2007). In the developed world, swimmer's itch is common in recreational, fresh-water sites (Chamot et al., 1998; Caumes et al., 2003).

Cercarial dermatitis can be caused by various avian schistosomes – our primary focus is one that is harbored in Douglas Lake, Michigan. Common mergansers (*Megus merganser*) in Douglas Lake can serve as a definitive host for two species of *Trichobilharzia*. The one we studied was *T. stagnicolae*.

Adults of these digenetic trematodes usually reside in the tissues and veins of the gastrointestinal tract of their avian host. The adult female produces boomerang-shaped eggs that are passed through the intestinal tract with feces during host defecation. Miracidia can take from one to two weeks to fully develop inside the eggs. Once fully immersed in water, miracidia hatch and become free-living, using their sensory package to detect the specific snail intermediate host they need to infect. After entering the snail, via ingestion or penetration of snail tegument, the miracidia develop into mother sporocysts, which asexually produce daughter sporocysts, which asexually produce another free-living stage called cercariae. In order to complete the life cycle, cercarieae must enter their avian definitive host. Though cercariae cannot complete their life-cycle in mammals, they can accidentally enter human skin and cause a histamine reaction leading to a maculopapular response in situ – this is know as cercarial dermatitis (swimmer's itch) (Baird, 1987).

For our study we will be testing the miracidia of *T. stagnicolae*. Certain levels of light and dilution are factors that have been shown to optimize conditions of hatching miracidia from eggs (Blankespoor, personal communication). Generally, miracidia are thought to survive up to 24 hr at room temperature unless a snail intermediate is found. A study by Oliver and Short (1956), which tested for longevity of *Schistosomatium douthitti* miracidia in spring water and filtered river water at room temperature, showed that most miracidia (out of 1217 miracidia) died before 24 hr; however, there were a few that survived up to the 25th hr. Previous studies by Singh (1950), using *Schistosoma indicum*, Najim (1951), using *Gigantobilharzia huronensis*, Short (1952), using *S. douthitti*, and Wu (1953), using *Trichobilharzia cameroni*, showed similar results.

For the purpose of this experiment, longevity is measured from the time that miracidia hatch to the time they die. While inside the egg, a fully developed miracidium will use an enzymatic reaction that will cause the miracidium to rapidly spin inside the egg before bursting out. Free from the egg, the miracidium will swim constantly forward, never backward, until its energy packet is depleted or until they enter their specific snail host. Miracida have no head or digestive system; only some have eyespots. All miracidia have dermal plates of cilia (Figure 1), and a well-developed sensory package sensitive to excretory waste and other chemical cues from their host, aiding in host penetration (Hertel et al, 2006; Haas, 2003). Postive geotaxis and negative phototaxis aid miracidia in reaching host snails located at the bottom of the lake.

For our project, we conducted two tests. One was to examine miracidia longevity in various aqueous solutions (solutions test), the other was to examine the effects of desiccation on the hatching of miracidia from eggs (desiccation test). We hypothesized that miracidia would survive the longest in lake water, well water, avian ringers solution, distilled water with 10% sucrose, and distilled water respectively. In the desiccation experiment, we expected fewer miracidia to hatch the longer fecal samples were allowed to desiccate.

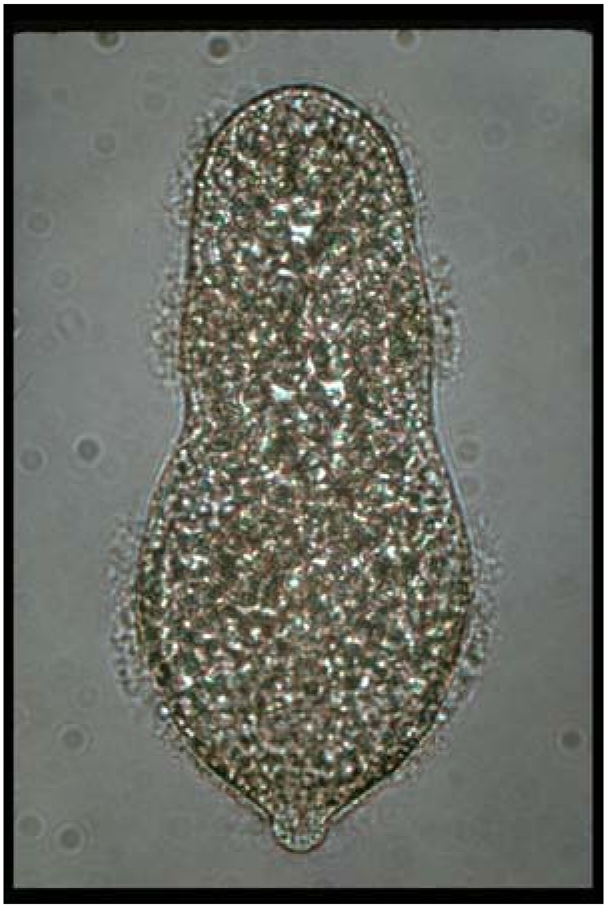

Figure 1. Microphotograph of a *Gigantobilharzia huronensis* miracium showing dermal plates of cilia. Courtesy of *swimmeritch.org.*

MATERIALS AND METHODS

Solutions Test

We collected fecal samples containing *T. stagnicolae* from a flock of common mergansers on a dock on Marl Bay on Douglas Lake, Michigan. The flock of six adults was monitored from a distance for 45min as we waited for them to defecate. Fecal material was collected from the dock using flexible forceps and stored in disposable plastic Petri dishes, each lined with a moist filter paper, and covered to prevent desiccation, and stored in a cooler with an ice pack until we arrived at the lab (Lakeside Lab, University of Michigan Biological Station (UMBS), Pellston, MI). Approximately an hour later, a second sample collection was taken from a flock of one adult female and several young in a similar manner.

In order to homogenize the distribution of *T. stagnicolae* eggs in the feces, collected samples were placed in a glass vial and manually stirred with a spatula for about three minutes. Some of the mixture was transferred to tall Petri dishes and diluted with lake water to hatch the miracidia (the other portion was used for the desiccation test). The dilution procedure was as follows: we poured lake water on top of the fecal matter until Petri dishes were filled to the top; we waited for fecal matter to settle then carefully poured the top half of the water – this technique was repeated three more times for each dish. Diluted samples were then placed on a white surface under fluorescent light and left undisturbed for at least 45min. ~ 200 healthy miracidia were then transferred from the dishes with fecal matter into Petri dishes with filtered lake water using a Pasteur pipette. 30 miracidia were then individually transferred into five Petri dishes, each containing one of the five solutions: filtered lake water from Douglas Lake, well water from the UMBS, distilled water, distilled water with 10% sucrose (0.0295M), and avian ringers (a solution of 7g NaCl, 0.42g KCl, 0.25g $CaCl_2$, and 0.1g $NaHCO_3$ dissolved in 1L distilled water, 0.1289 mol solute/L). Number of surviving miracidia was counted and recorded every two hr after initial count by individually transferring miracidia into Petri dishes containing the same solutions as the previous Petri dishes. This process was repeated until all of the miracidia died.

Chi-square tests were used to compare the distribution of surviving miracidia in each of the five solutions to the distribution in the control solution (filtered lake water).

Desiccation Test

We used the same fecal samples that were homogenized in the Solutions Test. 25 glass Petri dishes were each filled with ~0.2g of fecal matter; weights in each Petri dish were recorded. 24 samples were placed in a Percival© incubator set at 25°C with no light; one sample was kept outside the incubator at regular room temperature and lighting as a control.

Samples were removed from the incubator once every 10min for the first 2hr, once every 20min for the second 2hr, and once every 30min for the last 3hr. Shortly after removal from the incubator, each sample was weighed and recorded. We used the same technique as in the Solutions Test to hatch the miracidia. After 1.83hr, hatched miracidia were counted and recorded using the same counting technique as in the Solutions Test.

A least-squares regression model was used to examine correlation between the number of hatched miracidia per gram of fecal matter and the mass of water lost per gram of fecal matter.

RESULTS

Solutions Test

The number of surviving miracidia in each solution is shown in Figure 2. Chi-square analysis indicated that survivorship of miracidia decreased significantly from lake water when miracidia were placed in well water, distilled water with sucrose or avian ringers solution (χ^2=68.9, p = 1.53x10^{-8}; χ^2=90.2, p = 2.32x10^{-12}; χ^2=141, p = 4.50x10^{-22}; respectively), but not when placed in distilled water (χ^2 = 19, p = 0.271).

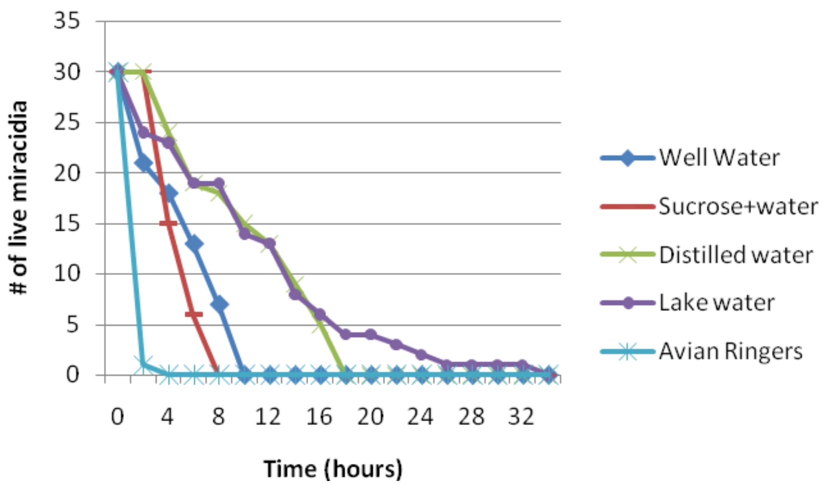

Figure 2. Longevity of *T. stagnicolae* miracidia placed in various aqueous solutions.

Desiccation Test

The number of hatched miracidia and the amount of water loss per gram of fecal material in each sample are shown in Figure3. Linear regression analysis indicated that the observed decrease in the number of hatched miracidia with increased water loss was significant (r^2=0.8108,p=4.03x10^{-10}).

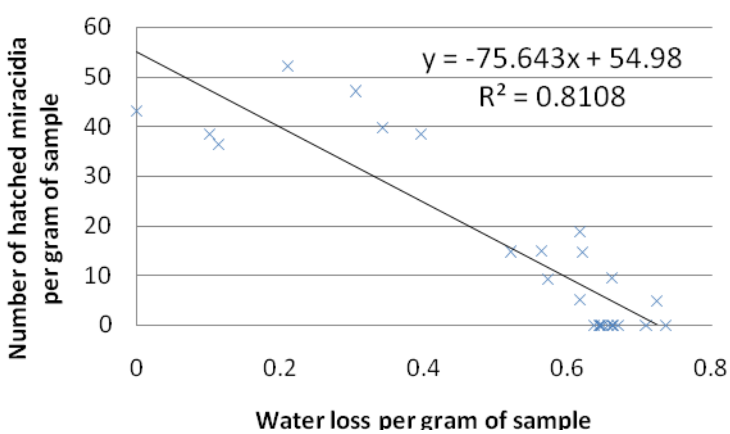

Figure 3. Number of hatched *T. stagnicolae* miracidia per gram of fecal sample compared to the amount of water loss per gram of sample.

DISCUSSION

Solutions Test

Our predictions seemed to be supported in that lake water had the greatest longevity of miracidia compared to the other four solutions. However, we did not expect miracidia to last more than 32hr in lake water. Although only a few survived past 24hr, and only one survived more than 32hr out of the initial 30 miracidia in lake water, this was still much a greater longevity than previous studies have recorded in various other solutions (Singh, 1950; Najim, 1951; Short 1952; Wu 1953; Oliver and Short, 1956). Our study seemed most comparable to Singh's study (1950) which showed that miracidia of *Schistosoma indicum* survived 20-25hr in pond water, less than 5hr in distilled water, 12-14hr in tap water. The closest solution we had to pond water was lake water, which showed longevity past 32hr; the closest solution we had to tap water was well water, which showed longevity up to 10hr. The greatest contrast, though, was in distilled water: while Singh found that his miracidia survived less than 5hr in distilled water, our miracidia survived up 14-16hr, showing longevity that was similar to that in lake water. However, we find it difficult to compare these results since Singh used a different species of miracidia; differences in longevity may be due to biological differences between *T. stagnicolae* and *S. indicum*. Perhaps, based on the survival in distilled water, *T. stagnicolae* have a higher tolerance to hypoosmotic solutions than *S. indicum* – further studies are needed to confirm this.

Longevity of *T. stagnicolae* miracidia seemed to increase with decreasing solute concentration. Since osmolarity of filtered lake water and well water solutions was not measured, our basis for the correlation depends on the other three solutions with known concentrations. Longevity was the greatest in distilled water (0M), distilled water with 10% sucrose (0.0295M), and avian ringers (0.1289M) respectively. These results suggest that the miracidia have a greater survivorship in hyposomotic solutions than hyperosmotic solutions;

however, further studies are needed to confirm this. The solution of avian ringers is of particular interest to students of the UMBS parasitology class since avian ringers is often used when preparing fecal smears of dissected avian specimens. However, miracidia had the shortest longevity in this solution (no more than four hours) out of the five tested.

Our results should be considered for when working with miracidia in lab conditions. As our results suggest, miracidia survive the best in filtered lake water; however, most of them die after 8-10hr.

Dessication Test

The results from this experiment support our hypothesis, with fewer miracidia hatching the longer we allowed samples to desiccate. The correlation is understandable since eggs of *T. stagnicolae* normally inhabit aquatic environments without the stress of desiccation. Miracidia were able to hatch up to 3hr after desiccation in our incubator. This may be relevant to residents of riparian households on the shores of Douglass Lake since they are at risk for swimmer's itch. The common merganser in this lake spends about 50% of its time on boat docks or stationary platforms near shore, often times defecating in these areas (Blankespoor, personal communication). Owners of these docks or platforms are recommended to wait at least 3hr after finding droppings before rinsing them off into the lake. In order to maximize the amount of eggs that hatch, we advise researchers working with *T. stagnicolae* allow little time for desiccation.

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