Longevity of *Trichobilharzia stagnicolae* miracidia in various aqueous solutions and during desiccation

Alison Clune, Jennifer Foster, Harry Kuo, Selcuk Uremek

University of Michigan Biological Station

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ABSTRACT

Swimmer's itch is caused by nonhuman schistosomes, including *Trichobilharzia stagnicolae*, which have complex indirect life cycles. Miracidia are a free living life cycle stage of these schistosomes that are transmitted from either an avian or mammalian host to a specific snail intermediate, depending on the species. The goals of this study are to determine the life span of the miracidia of *T. stagnicolae* in various solutions and to examine the effect of desiccation on the hatching ability of miracidia. Miracidia were hatched and placed in dishes of five different solutions (filtered lake water, well water, distilled water, distilled water with sucrose and avian ringers) to determine their life span. Samples of host fecal material were dried and miracidia were hatched from these samples to test the effects of desiccation. Longevity of miracidia was greatest in filtered lake water and distilled water. Hatching ability decreased with the amount of water evaporated from the fecal samples ($p = 4.03 \times 10^{-10}$). These results suggest that *T. stagnicolae* miracidia are sensitive to hyperosmotic solutions and that once they hatch, they lose many protective functions provided by the egg.

Swimmer's itch (cercarial dermatitis) is a common problem in freshwater lakes worldwide (Verbrugge et al., 2004; Rao et al., 2007; Valdovinos and Balboa, 2008; Levesque et al., 2002). Although the disease is primarily a nuisance, the occurrence of swimmer's itch infections can deter tourists from recreational lakes, triggering economic losses (Verbrugge et al., 2004), and cause health problems near lakes that people in the developing world depend on for washing and food (Rao et al., 2007).

Swimmer's itch is caused by numerous species of digenetic trematodes in the family Schistosomatidae that normally infect nonhuman animals. These parasites have an indirect life cycle involving two hosts. Adult schistosomes usually reside in the mesentery veins of the small intestine of the definitive host, usually an aquatic bird or mammal. Eggs pass from the female schistosome, burrow through the mucosal layer of the small intestine to the lumen and are passed with the feces. Inside the egg, the miracidium completely develops before hatching into the water. Miracidia are a free-living, non-feeding life cycle stage with the purpose of infecting a specific species of snail intermediate host. Upon entering a snail, the miracidium develops through two generations of sporocysts, which then shed cercariae into the water. Cercariae are another free-living stage with the purpose of infecting the definitive host where the cercariae will develop into an adult schistosomes. It is the cercariae that cause swimmer's itch when they mistakenly burrow into human skin and die, causing an allergic reaction.

Miracidia are the focus of this study. Light and dilution are essential for hatching schistosome miracidia from their eggs. Miracidia are short-lived, surviving only about 24 hours unless a snail intermediate is encountered. Oliver and Short (1956) demonstrated this life span for *Schistosomatium douthitti* in filtered river and spring water at room temperature. However most miracidia in this study died long before the 24th hour (90% died by the end of 19 hours).

Short (1952, in Oliver and Short, 1956), using *S. douthitti*, Najim (1951, in Oliver and Short, 1956), using *Gigantobilharzia huronensis*, and Wu (1953, in Oliver and Short, 1956), using *Trichobilharzia cameroni* obtained similar results in previous studies. Singh (1950, in Oliver and Short, 1956) examined the effects of water from various sources on miracidia longevity for *Schistosoma indicum*, finding that the miracidia survived longest (20-25 hours) in pond water, then tap water (12-14 hours), and shortest in distilled water (less than 5 hours).

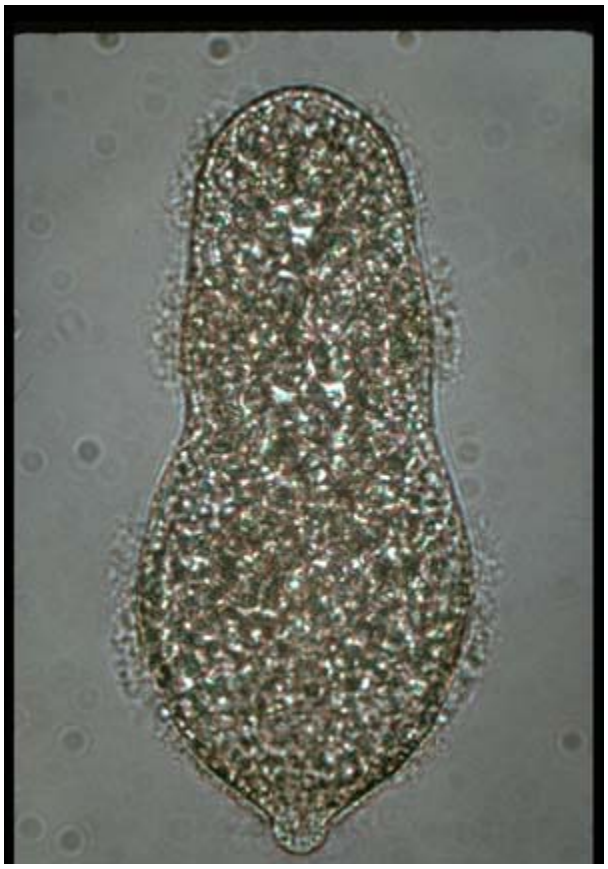

Figure 1. Microphotograph of a *Gigantobilharzia huronensis* miracidium showing plates of cilia.

During their short life span, miracidia actively swim forward until they encounter a host or run out of energy and die. Miracidia do not feed and lack any digestive organs, so energy cannot be replenished. Plates of cilia on the tegument of the miracidia allow them to move through the water (Figure 1). Positive geotactic and negative phototactic behavioral adaptations aid the miracidia in reaching the lake bottom where the intermediate hosts are found. Compounds released by host snails also serve to attract miracidia to the host by causing a chemokinetic response, as has been demonstrated for *T. ocellata* miracidia. This reaction is very host-specific, and many miracidia only respond to chemicals released by the single host snail species that they normally infect (Hertel et al., 2006; Haas, 2003).

This study examines the miracidia of *Trichobilharzia stagnicolae*, one species of avian schistosome that is present in Douglas Lake, Michigan. The common merganser (*Mergus merganser*) is the definitive host, and the freshwater snail *Stagnicola emanginata* is the intermediate host for this species.

This study has two goals. The first is to determine how long miracidia of *T. stagnicolae* survive in solutions other than their natural lake water (the longevity experiment). The second is to examine the effect of desiccation on the hatching ability of these miracidia (the desiccation experiment). It is expected that miracidia will survive longest in lake water, then well water, avian ringers solution, distilled water with sucrose, and finally distilled water. Similar results would be consistent with Singh's (1950, in Oliver and Short, 1956) study of the longevity of *S. indicum*. In the desiccation experiment, it is expected that fewer miracidia will hatch as the eggs dry out.

MATERIALS AND METHODS

Longevity Experiment

Fecal samples, containing *T. stagnicolae*, were collected from a flock of common mergansers on a dock on Marl Bay on Douglas Lake, Michigan. The flock, consisting of six adults, was monitored but undisturbed for 45 minutes to allow time for defecation. At this time the flock began to leave the dock. Fecal material was collected from the surface of the dock using flexible forceps and temporarily stored in small plastic Petri dishes lined with moist filter paper and covered to prevent desiccation. These Petri dishes were stored in a cooler with an ice pack until they arrived in the lab. Samples from a brood consisting of one adult female and several young were collected from another dock in the same manner 60 minutes later.

Fecal material was diluted in filtered lake water to hatch the miracidia. To increase the sample dilution and facilitate hatching, excess sediment was removed by carefully pouring off the top half of the water in the Petri dish after a short settling period. This process was repeated three times for each dish. Fully diluted samples were placed in front of a fluorescent light on a surface of white paper towels and left undisturbed for 50 minutes. At this time, 30 miracidia were individually transferred with a Pasteur pipette to each of five uncovered glass Petri dishes filled with one of the following solutions: filtered lake water from Douglas Lake, well water from the University of Michigan Biological Station, distilled water, distilled water with 1% sucrose (0.0295 M), or avian ringers solution (7 g NaCl, 0.42 g KCl, 0.25 g $CaCl_2$ and 0.1 g $NaHCO_3$ dissolved in 1 L distilled water, 0.1289 mol solute/L). Every two hours after this initial count, the number of surviving miracidia was counted in the same manner, moving the miracidia to a Petri dish of fresh solution. This process was repeated until all miracidia died in each solution.

Desiccation Experiment

Fecal samples were collected on a separate day from the brood of common mergansers sighted for the longevity experiment on the same dock on Marl Bay. The brood was allowed 25 minutes to defecate at which point they left the dock and fecal material was collected in the same manner as before.

Fecal material was combined and thoroughly homogenized with a metal spatula. Approximately 0.2 g fecal material was placed in each of 25 uncovered glass Petri dishes. Mass of the empty Petri dish and mass of the sample were measured and recorded. Twenty-four samples were placed in a Percival environmental control chamber at 25°C in the dark. One sample was retained as a control.

Samples were removed from the environmental control chamber once every ten minutes for the first two hours, once every 20 minutes for the second two hours, and once every 30 minutes for the last three hours. Upon removal, the Petri dish and partially dried sample were weighed and recorded. Miracidia in the sample were then hatched using the same method of dilution and light exposure as the longevity experiment. Samples were allowed two hours to hatch. During the ten minutes prior to the end of the hatching period the hatched miracidia in each Petri dish were counted by individual removal with a Pasteur pipette.

Statistical Analysis

For the longevity experiment, Chi-square tests were used to compare the distribution of surviving miracidia in each experimental solution (well water, distilled water, distilled water with sucrose, and avian ringers) to the distribution in the control solution (filtered lake water). For the desiccation experiment, the correlation between the number of hatched miracidia per

gram of fecal material and the mass of water lost per gram of fecal material was examined using a least-squares linear regression model.

RESULTS

Longevity Experiment

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 ($\chi^2 = 68.9$, $p = 1.53 \times 10^{-8}$; $\chi^2 = 90.2$, $p = 2.32 \times 10^{-12}$; $\chi^2 = 141$, $p = 4.50 \times 10^{-22}$; respectively), but not when placed in distilled water ($\chi^2 = 19$, $p = 0.271$).

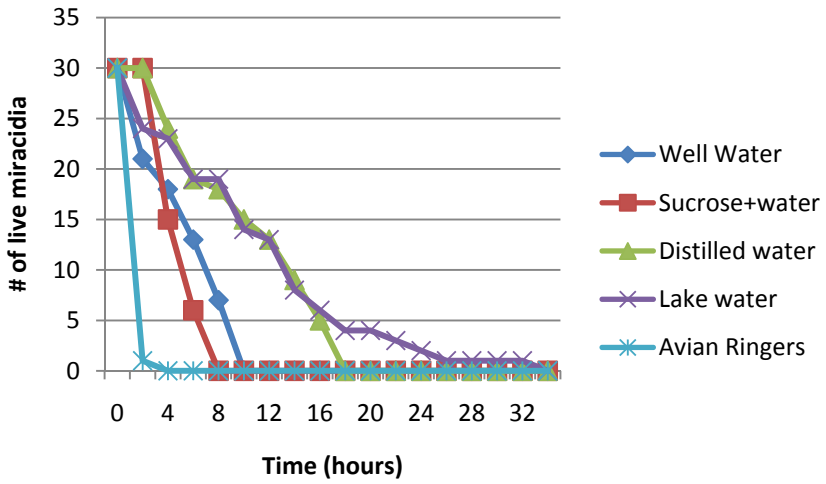

Figure 2. Longevity of *T. stagnicolae* miracidia placed in various aqueous solutions.

Desiccation Experiment

The number of hatched miracidia and the amount of water loss per gram of fecal material in each sample are shown in Figure 3. Linear regression analysis indicated that the observed decrease in the number of hatched miracidia with increased water loss was significant ($r^2 = 0.8108$, $p = 4.03 \times 10^{-10}$).

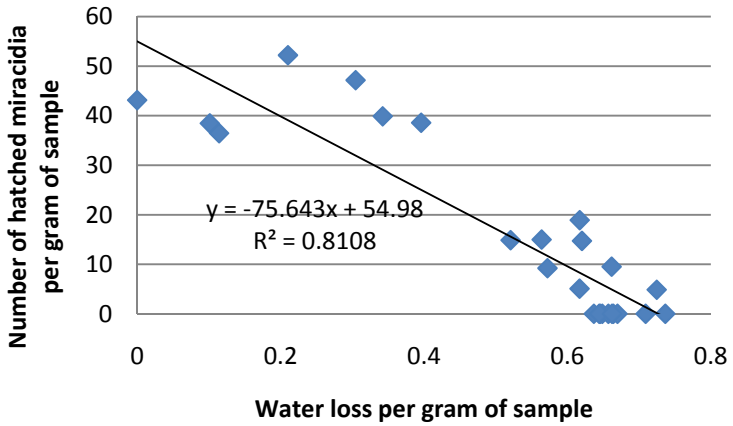

Figure 3. Number of hatched *T. stagnicolae* miracidia per gram of fecal sample compared to the amount of water loss per gram of sample.

DISCUSSION

Longevity Experiment

The data from the longevity experiment were inconsistent with the hypothesized results and those found by Singh (1950, in Oliver and Short, 1956). Lake water longevity was in fact the longest, as expected, but miracidia survived much longer in the present study (up to 34 hours) than in Singh's pond water experiment (20-25 hours). Distilled water longevity showed no significant difference from lake water longevity, in sharp contrast to Singh's results in which miracidia survived less than five hours in distilled water. In well water, miracidia did not survive as long in this experiment as in Singh's tap water (up to 10 hours in this study compared to 12-14 hours previously). No previous data could be found for solutions comparable to the distilled water with sucrose or avian ringers solution. The differences in results between these two studies could be due to physiological differences between the two species studied (*T. stagnicolae* in this study, and *S. indicum* previously). Based only on these two studies, it seems that *T. stagnicolae* may have more stored energy, but a lower tolerance for hyperosmotic environments than *S. indicum*.

Survivorship of *T. stagnicolae* miracidia seemed to increase with decreasing solute concentration. Osmolarity of the filtered lake water and well water solutions was not measured, so it is difficult to compare the results of these solutions with the others. For the three solutions with known solute concentration, however, survivorship was longest in distilled water (0 M), then in distilled water with sucrose (0.0295 M), and finally in avian ringers solution (0.1289 M). These results suggest that these miracidia have a limited ability to regulate solute concentrations across their tegument, however further physiological studies would be required to verify this. Survivorship in avian ringers solution is of particular interest because this solution is meant to

closely resemble the solute concentrations inside the common merganser from which *T. stagnicolae* eggs are released. Miracidia survived no more than four hours in avian ringers solution, far less time than in any other solution. This suggests that the egg shell from which the miracidium hatches plays an essential role in protecting the miracidium from the host's environment until the external environment is reached. Once the egg shell is lost, the miracidia seem to be much more vulnerable to changes in solute concentration, as well as desiccation, as discussed below.

The results of this experiment should be considered when working with miracidia under laboratory conditions. Miracidia are best kept alive in filtered lake water, but must be used quickly if they are needed in large quantities.

Dessication Experiment

The outcome of the desiccation experiment was as expected, with miracidia having decreased ability to hatch from the egg after longer periods of drying. This was probably because *T. stagnicolae* eggs are adapted to an aquatic environment in which desiccation is not normally a problem. The egg shell offered some protection from the terrestrial conditions of our environmental chamber, but these defenses were exhausted by the end of three hours.

These results may have implications for residents of riparian (shoreline) households along lakes infected with swimmer's itch. Because the common merganser spends approximately 50% of its time on boat docks or other stationary platforms near the shore, much of its feces is deposited on these surfaces. Dock owners may aid in the control of swimmer's itch by waiting at least three hours after finding fresh merganser droppings on their docks before they rinse these droppings into the water. Researchers working with *T. stagnicolae* miracidia or

other related miracidia should also be advised not to allow their fecal samples to dry significantly before using them to achieve maximum hatching.

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