

Behavioral analysis of *Microphallus turgidus* cercariae in relation to microhabitat of two host grass shrimp species (*Palaemonetes* spp.)

Patricia A. O’Leary^{1,2,*}, Oscar J. Pung¹

¹Department of Biology, Georgia Southern University, Statesboro, Georgia 30458, USA

²Present address: Department of Aquatic Health Sciences, Virginia Institute of Marine Science, PO Box 1346, State Route 1208, Gloucester Point, Virginia 23062, USA

ABSTRACT: The behavior of *Microphallus turgidus* cercariae was examined and compared to microhabitat selection of the second intermediate hosts of the parasite, *Palaemonetes* spp. grass shrimp. Cercariae were tested for photokinetic and geotactic responses, and a behavioral ethogram was established for cercariae in control and grass shrimp-conditioned brackish water. Photokinesis trials were performed using a half-covered Petri dish, and geotaxis trials used a graduated cylinder. Both photokinesis and geotaxis trials were performed in lighted and unlighted conditions. In 9 of 10 photokinesis experiments, over half of the cercariae swam horizontally under the covered half of a Petri dish in both the lighted and the unlighted trials. However, movement of the cercariae to the covered half of the dish was highest (81.4 %) when the parasites were exposed to light. In the geotaxis study, most cercariae were found in the bottom third of a graduated cylinder water column in both the lighted and unlighted trials. The most frequently observed activity of individual cercariae in a lighted Petri dish was swimming on the bottom of the dish. Activity patterns of the cercariae were not affected by shrimp-conditioned water. Movement of the cercariae away from light into dark, active swimming at or near the bottom of the water column, and a lack of response to host odors suggest that the cercariae utilize search patterns that place the parasite in the preferred microhabitat of the principle second intermediate host, the grass shrimp *P. pugio*.

KEY WORDS: *Palaemonetes pugio* · *Palaemonetes vulgaris* · Photokinesis · Geotaxis · Ethogram

Resale or republication not permitted without written consent of the publisher

INTRODUCTION

The cercariae of many trematode species have host-finding strategies that allow them to locate the preferred microhabitat of the host. Behavioral adaptations of cercariae are related to both ‘host-space’ and ‘host-time’, which are the locations where and time periods during which cercariae are most likely to encounter their intended host (Combes et al. 1994). These strategies include photoreception, geotaxis, and response to chemical cues (reviewed by Combes et al. 2002, Sukhdeo & Sukhdeo 2004). Examples of light sensitive trematodes include *Cryptocotyle lingua* (Heterophyidae), *Trichobilhar-*

zia ocellata (Schistosomatidae), and the echinostomatidae *Himasthla rhigedana* (Rees 1975, Feiler & Haas 1988, Fingerut et al. 2003). There are cercariae without apparent photoreceptors that respond to light as well, including the microphallids *Maritrema arenaria* and *Microphallus similis* (McCarthy et al. 2002). When cercariae have pigmented photoreceptors with directed movement towards or away from light, this is referred to as phototaxis. However, when cercariae have unpigmented photoreceptors, they respond to light intensity (undirected), which is referred to as photokinesis (Fraenkel & Gunn 1961, Combes et al. 1994). Cercariae of *C. lingua* have both pigmented and unpigmented photoreceptors (Rees

1975). In order to differentiate phototaxis and geotaxis, geotaxis studies are done in both the light and dark, while phototaxis studies are done in a shallow dish bifurcated between light and dark regions. For example, the swimming behavior of *Maritrema subdolum* (Microphallidae) cercariae can be attributed to geotaxis, because the species does not respond to light (Mouritsen 2001). Similarly, geotactic and barokinetic behaviors can be differentiated through studies utilizing increases in hydrostatic pressure (Fitzpatrick et al. 2016).

The host-finding strategies of the trematode *Microphallus turgidus* (Microphallidae) have not been fully explored. (*M. choanophallus* is a junior synonym of *M. turgidus*, Deblock 1971). *M. turgidus* is a digenean found in salt marshes from the coast of New Jersey to Louisiana, USA (Heard 1970). The adult worm matures in the small intestine of a bird or mammal (Leigh 1958, Heard 1970). The trematode eggs in the host feces are dispersed into the water, settle into marsh sediments and are consumed by the first intermediate host, hydrobiid snails (Heard & Overstreet 1983, Pung et al. 2008). In Georgia salt marshes, the hydrobiid snail *Spurwinkia salsa* is most abundant in the high marsh, both in sediment and on the stems of the cord grass *Spartina alterniflora*. The snails are less common in low marsh and creek bed sediments (Pung et al. 2008). The trematode eggs hatch in the gut of the snail, releasing miracidia that develop into cercariae-producing sporocysts (Pung et al. 2009).

The cercariae emerge from the snail and swim through the water column to find and infect the second intermediate host, *Palaemonetes* spp. grass shrimp. The cercariae of *M. turgidus* do not have apparent photoreceptors, i.e. eyespots (Heard & Overstreet 1983), but this does not necessarily mean they are not photokinetic. For example, the cercaria of *Cryptocotyle lingua* have a third unpigmented rhabdomeric photoreceptor visible only with electron microscopy (Rees 1975). *M. turgidus* cercariae swim fast and erratically, stopping if disturbed by respiratory currents of a potential host (Heard & Overstreet 1983). The life cycle of the parasite is completed when the infected grass shrimp is consumed by a bird or mammal (Heard 1970, Heard & Overstreet 1983).

In Georgia, 2 species of brackish water grass shrimp, *P. pugio* and *P. vulgaris*, are second intermediate hosts of the trematode *M. turgidus* (Pung et al. 2002). The prevalence and abundance of *M. turgidus* metacercariae in *P. pugio* are usually high, while the parasite is only found occasionally in *P. vulgaris* and always at low abundance (Pung et al. 2002, 2006). Though sympatric and demersal, these 2 species of

grass shrimp have different microhabitat preferences (Heard 1982, Khan et al. 1995, 1997, Pung et al. 2006), as do the hydrobiid first intermediate hosts (Pung et al. 2008).

The abundance of adult *P. pugio* varies temporally, i.e. emerging in the late afternoon, active throughout the night and early morning (Shenker & Dean 1979). Those authors hypothesized that *P. pugio* buried during the day, explaining its absence in midday collections. Although the activity levels of *P. pugio* vary by photoperiod, they can be collected during the day (authors' pers. obs.). Newly hatched *P. pugio* larvae have negative phototaxis, which may aid in predator avoidance (Douglass et al. 1992). Negative phototaxis occurred before or during low tide, while positive phototaxis occurred before high tide. The larvae were shown to respond to a light stimulus of 500 nm, at an intensity of 4.6×10^{13} quanta $m^{-2} s^{-1}$. *P. pugio* can be collected from areas with scattered beds of *Ulva* on muddy substrate (Khan et al. 1997).

P. vulgaris, on the other hand, are often associated with oyster reefs and fouling organisms on docks and pilings (Heard 1982) and are found only occasionally in the marsh (Kneib 1991). Both *P. pugio* and *P. vulgaris* prefer areas of macrophytic cover, and the wood pilings and floats where *P. vulgaris* are found have the sponge algae *Codium* (Khan et al. 1997). Also, both *P. pugio* and *P. vulgaris* are demersal but differ in their specific substrate preferences. In the laboratory, *P. pugio* chooses mud over wood, shell and sand, while *P. vulgaris* prefers wood (Khan et al. 1995). Adult *P. vulgaris* can perceive polarized light at 500 nm and an intensity of 2.5×10^{17} quanta $m^{-2} s^{-1}$ (Ritz 1991). *P. vulgaris* also has a very strong preference for white backgrounds (85.2%) over black backgrounds (Ritz 1991, Khan et al. 1995); those authors hypothesized, based on their results and Goddard & Forward (1989), that *P. vulgaris* uses this light–dark cue to swim away from shore during escape responses and that the darker shore vegetation might be the darker area they are swimming away from.

The host-seeking characteristics of *M. turgidus* cercariae have not been examined and may partially explain the differences in prevalence and intensity of the parasite in *P. pugio* and *P. vulgaris*. In this study, we hypothesized that the behaviors of *M. turgidus* cercariae would place them in close proximity to the known preferred microhabitat of the primary intermediate host. To do this, we examined the response of the parasite to light and gravity. We also constructed an ethogram of the behavior of the cercariae in the presence and absence of host chemical cues.

MATERIALS AND METHODS

Laboratory-infected hydrobiid snails

Hydrobiid snails *Spurwinkia salsa* were infected in the laboratory with the parasite *Microphallus turgidus* and used as the source of cercariae. We did this because accurate identification of microphallid cercariae in naturally infected snails is problematic whereas cercariae produced by laboratory-infected snails are of known identity (Pung et al. 2009). Snails were collected in salt marsh sediments along the Skidaway River, Georgia, USA, and screened for natural trematode infection as described previously (Pung et al. 2009). *S. salsa* represents greater than 90% of the hydrobiids in this locality, which is why this species was chosen (Pung et al. 2008). Individual snails were placed in the wells of 24-well tissue culture plates containing 2 ml of artificial brackish water (Instant Ocean®, Aquarium Systems; prepared using dechlorinated tap water, salinity adjusted to 23 practical salinity units [psu]) and incubated at 30°C on a 12 h light: 12 h dark cycle. An inverted microscope was used to examine each well twice a week for the presence of trematode cercariae, and snails shedding cercariae from natural infections were discarded. Snails not producing cercariae within the 4 wk time period were assumed uninfected, retained, and then infected with the parasite *M. turgidus* as described previously (Pung et al. 2011).

Briefly, individual snails were fed 80 *M. turgidus* eggs from worms cultured *in vitro* and were then incubated and monitored as above. Laboratory-infected snails that produced cercariae 4–8 wk later were maintained at room temperature in brackish water until needed. Neutral red staining was used to check the morphology of the cercariae, which were confirmed to be *M. turgidus*. On the morning of each experimental trial, laboratory-infected snails were placed in Petri dishes (60 × 15 mm, Falcon polystyrene, Becton Dickinson) containing 10 ml of 30°C brackish water (1 animal per dish). The Petri dishes were incubated at 30°C and checked 2 h later for the presence of cercariae.

For all experiments, cercariae used were no older than 12 h post shedding, used once, then discarded. Based on our observations, cercariae from naturally infected snails survive at least 15 h at room temperature (data not shown). Morning and afternoon experiments were also compared by approximate cercarial age. Cercariae from each snail were transferred to separate clean dishes and used for experimental trials. To prevent nonspecific adherence of cercariae to

plastic and glassware, all Petri dishes and graduated cylinders were coated for 5 min with 0.45% bovine calf serum (GIBCO) and then rinsed 3 times with brackish water. Pipettes and pipette tips used to transfer cercariae were coated with undiluted serum.

Cercaria photokinesis experiment

The response of cercariae to light was tested as follows. For each experimental trial a 60 × 15 mm Petri dish was filled with 10 ml artificial brackish water (20 psu), and 20 shed cercariae from a single snail were placed at multiple locations over the bottom of the dish. The Petri dish was carefully transferred to a dissecting microscope stage and covered with a light tight cardboard box, the room lights were turned off, and the cercariae were allowed to adjust for 30 min. Next, the box was removed, a red plastic-filtered white light-emitting diode (LED) was turned on, and a piece of black construction paper was aligned to block light to the bottom half of the dish. The outside wall of the same half of the dish was covered with a second piece of black paper held in place by folding so that no glue or tape was needed. A small piece of solid black plastic was set over half of the Petri dish in the same orientation as the black paper covering the dish bottom and side wall. Two white LEDs (Mega Flex LED flashlight Item #5874b, 1.5 W) were placed on each side of the microscope stage to illuminate the Petri dish from above so that the shadow of the plastic cover was cast straight down. LED lights were chosen to prevent the addition of heat. Individually, the lights each had an intensity of 19.47 $\mu\text{mol s}^{-1} \text{m}^{-2}$ at a 3 inch distance in a dark room (PAR, 400–700 nm, Quantum LiCor sensor Serial #Q37821).

For comparison, another study in a mangrove marsh habitat in full sunlight had surface light intensities of 13.3 $\mu\text{mol photons s}^{-1} \text{m}^{-2}$, and the minimum light intensity for a phototactic response was $6.64 \times 10^{-3} \mu\text{mol photons s}^{-1} \text{m}^{-2}$ (Smith & Cohen 2012). The red-filtered LED was then turned off, and the white LEDs, the only light source for the next 30 min, were turned on. After 30 min the number of cercariae in the uncovered half of the dish was counted. The orientation of the masked half of the dish was rotated 90° from trial to trial to ensure that the cercariae were not swimming directionally prior to exposure. The unlighted trials were performed as above except that cercariae were exposed to light only when the dish was prepared and when the cercariae in the uncovered half of the dish were counted. The light tight box was retained throughout the unlighted trials to

ensure total darkness except during viewing. The lighted and the unlighted photokinesis trials were each repeated 10 times. Thirteen different snails were used to produce the cercariae.

Cercaria geotaxis experiment

The response of the cercariae to gravity was tested in a 10 ml graduated glass cylinder (external diameter = 17 mm, height = 140 mm, Pyrex) filled with 9 ml of artificial brackish water (20 psu). The total height of the water column in the cylinder was 81 mm. A micropipette was used to transfer 30 freshly shed cercariae to the cylinder, which was then fitted with a tube of black construction paper and a black paper cover with a 1 cm² opening. The room lights were turned off, and the top of the cylinder was either illuminated with a white LED (lighted trials, 19.47 μmol s⁻¹ m⁻²) or not (unlighted trials). After 60 min, a 10 ml pipette was used to transfer the top, middle, and bottom third portions of the water column (3 ml each) to separate Petri dishes. The cercariae in each dish were counted while removing them one at a time using a micropipette. The lighted and the unlighted geotaxis trials were each repeated 6 times. Six different snails were used to produce the cercariae.

Ethogram of cercariae behavior

A behavioral activity catalog of the cercariae in shrimp-conditioned and control artificial brackish water (20 psu) was constructed. The behaviors were identified based on preliminary observations. Shrimp-conditioned water was prepared by maintaining 10 *P. pugio*, in 10 l of brackish water for 72 h. In each trial, 1 cercaria was transferred to a 60 × 15 mm Petri dish containing 10 ml of water. After a 30 s acclimation period, the cercaria was viewed for 9.5 min using a mirrored dissecting microscope illuminated from above by 2 LED lights, and the activities of the cercaria were recorded. This time period was chosen, because it was sufficient to observe many behavioral changes (e.g. 80). To determine the distance travelled by the cercaria, a grid consisting of 1 cm² boxes was drawn on the underside of the Petri dish, and the number of boxes entered by the cercaria during the observation period was recorded. Eighteen trials were performed for cercariae in both control and shrimp-conditioned water. All trials were performed blind (i.e. the observer did not know

whether the cercaria was in the control or the shrimp-conditioned water). Six different snails were used to produce the cercariae.

Statistical analysis

Power analysis was done on initial data to determine the necessary number of replicates, and all statistical analysis was completed using JMP 8 software. For the photokinesis experiment, a chi-square analysis was used to test the null hypothesis that the ratio of cercaria in the uncovered and covered half of the Petri dish was 50:50. Because the total number of cercariae varied slightly between trials (Dark: actual n ranged from 18 to 22, Light: actual n ranged from 19 to 25), significance was evaluated using a method for combining probabilities from independent tests (Sokal & Rohlf 1998). This variation was caused in rare instances when the cercaria was not ejected from the pipette or more than one was drawn up. Statistical significance was based on the $-2\sum \ln p$ and a statistical table used to determine critical values of 37.6 (Rohlf & Sokal 1981). A Wilcoxon rank-sum test was also used in the photokinesis experiment to determine if there was a difference between lighted and unlighted trials with respect to the percentage of cercariae in the uncovered side of the Petri dish. The geotaxis experiment was analyzed using replicated goodness-of-fit *G*-tests. The degrees of freedom (df) and *G*-values were used to calculate *p* for each geotaxis trial. After the sums were calculated, the number of cercariae in the bottom, middle, and top thirds of the water column were compared (Sokal & Rohlf 1998). Behavioral activities of cercariae in control and shrimp-conditioned water were compared using the Wilcoxon rank-sum test. To compare distances travelled by cercariae, a roaming index based on the number of 1 cm² boxes travelled was analyzed with a Wilcoxon rank-sum test. To test if cercariae age affected light–dark selection or the behavioral ethogram, Wilcoxon rank-sum tests were performed comparing morning experiments to experiments done later in the day.

RESULTS

Cercaria photokinesis experiment

Whether exposed to white light or in unlighted conditions, the ratio of cercariae in the covered and uncovered halves of a Petri dish was not 50:50

(lighted trials, $-2\sum \ln p$ of 122.4 > critical value 37.6; unlighted trials, $-2\sum \ln p$ of 55.0 > critical value 37.6). In both of the treatment groups, on average fewer cercariae were found in the uncovered half of the dish than in the covered half. However, the percentage of cercariae in the uncovered half of the Petri dish was lower in the dishes exposed to light than in those kept in the dark (Fig. 1, Wilcoxon rank-sum, $p = 0.007$). The age of the cercaria did not affect the light-dark choice for either the experiments done in the dark (Wilcoxon rank-sum, $p = 0.359$) or light (Wilcoxon rank-sum, $p = 0.520$). The average cercarial age in the morning treatments was 4 h 30 min and 9 h 10 min for the afternoons. For all experiments, to determine the cercarial age, we used the time the cercaria was viewed at the completion of the experiment minus the time that the snails were placed in the incubator. Since the snails were in the incubator for 2 h, the actual age post shedding could be 0–2 h less than the above (Dark/Morning: $n = 7$, Dark/Afternoon: $n = 3$, Light/Morning: $n = 4$, Light/Afternoon: $n = 6$).

Cercaria geotaxis experiment

In the geotaxis trials, regardless of whether or not the graduated cylinder was lighted from above or unlighted, the cercariae were not evenly distributed

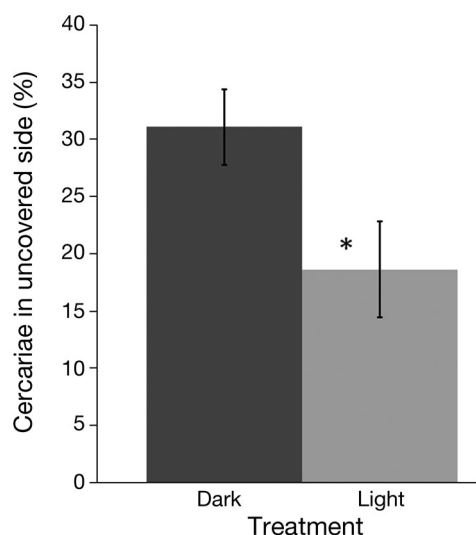


Fig. 1. Photokinetic response of *Microphallus turgidus* cercariae. When exposed to white light, fewer (18.6%) cercariae were viewed in the uncovered half of a Petri dish than during unlighted trials (31.1%), i.e. more cercariae sought cover when exposed to light. Asterisk indicates significant difference between lighted and unlighted dishes ($p = 0.007$). Bars represent mean value of 10 trials ± 1 SEM

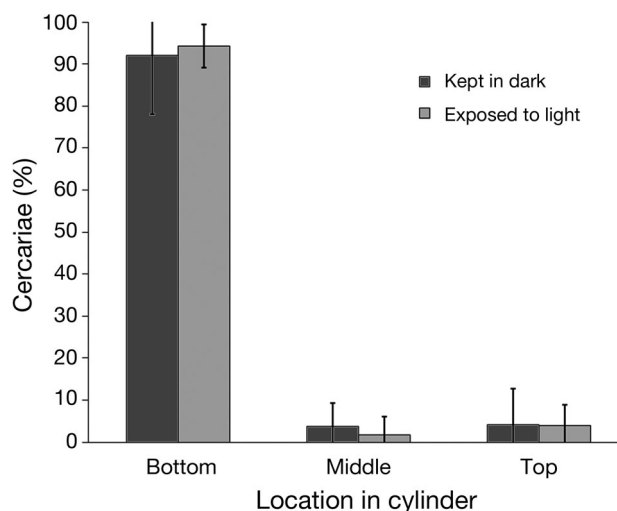


Fig. 2. Geotactic response of *Microphallus turgidus* cercariae. When placed in a 10 ml graduated cylinder filled with 9 ml of water (location in 27 mm increments), most cercariae remained in the bottom third of the water column ($p < 0.001$) regardless of whether the cylinder was illuminated from above or kept in the dark. Bars represent mean of 6 trials ± 1 SD

through the top, middle, and bottom thirds of the 9 ml water column (lighted trials, replicated goodness-of-fit G -test, summed $G = 317.9$, 12 df, $p < 0.001$; unlighted trials, replicated goodness-of-fit G -test, summed $G = 278.6$, 12 df, $p < 0.001$). In both lighted and unlighted conditions, the majority of cercariae were found in the bottom third of the water column (Fig. 2).

Ethogram of cercariae behavior

Eleven distinct behavioral activities were recorded during the ethogram observations. Frequently observed activities of the cercariae were swimming along the bottom of the Petri dish, resting motionless on the bottom of the dish, resting near the top of the water column, swimming up or swimming down, and sinking toward the bottom of the dish. On average, swimming along the bottom of the dish accounted for over 60% of the time spent by cercariae in both control and shrimp-conditioned water (Fig. 3). Less frequently observed activities, i.e. viewed less than 3% of the time, included swimming at the top or in the middle of the water column, resting motionless in the middle of the water column, crawling on the bottom of the dish, and wriggling but attached to the bottom of the dish. Cercariae favored 1 of 2 distinct behavioral activity patterns. Most cercariae swam on the bottom throughout the observation period. A small

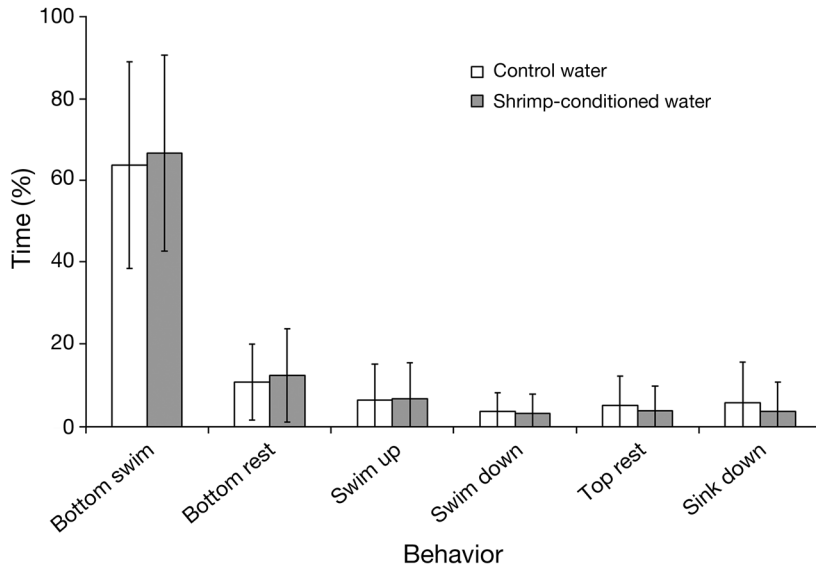


Fig. 3. Most frequently observed behavioral activities of *Microphallus turgidus* cercariae in control and grass shrimp-conditioned brackish water. There was no significant difference between treatments with respect to the amount of time cercariae spent in any activity. Bars represent mean of 18 trials \pm 1 SD. Behaviors that occupied less than 3% of time not shown, i.e. swimming at the top or in the middle of the water column, resting motionless in the middle of the water column, crawling on the bottom of the dish, and wriggling but stuck to the bottom of the dish

percentage of the cercariae spent most of their time swimming on the bottom of the dish but regularly swam up to the surface, rested at the surface then sank to the bottom and repeated this pattern of activities throughout the observation period. There was no difference between the cercariae in control and shrimp-conditioned water with respect to the amount of time spent in any of the 11 activities ($p > 0.05$). Additionally, there was no difference between cercariae in either control or shrimp-conditioned water with respect to distance travelled (Fig. 4). The cercarial age did not statistically affect the time spent in behaviors that would be associated with depletion of energy reserves (Bottom Swim, Wilcoxon rank-sum $p = 0.120$; Bottom Rest, $p = 0.399$; Stuck, $p = 0.285$). The average age of cercariae for the morning control behavior experiments was 3 h 27 min and 6 h 11 min for the afternoons (Morning $n = 10$, Afternoon $n = 8$).

DISCUSSION

Many species of cercariae are sensitive to light. Whether a photokinetic response directs the parasite toward or away from light appears to depend on the microhabitat of the next host. For example, *Micro-*

phallus similis cercariae, which infect a species of shore crab that hides under rocks during the daytime, move under the black-painted cover of a Petri dish when exposed to light (McCarthy et al. 2002). On the other hand, cercariae of the genus *Euhaplorchis* infect fish and ascend toward a light source in the laboratory (Smith & Cohen 2012). Like *M. similis*, the grass shrimp hosts of the trematode *M. turgidus* prefer areas with macrophytic cover (Khan et al. 1997), where they may seek shelter to avoid visual predators (Kunz et al. 2006). Also like *M. similis*, more *M. turgidus* cercariae move under the covered half of a Petri dish when exposed to light than when kept in the dark. This behavior would increase the likelihood of the parasite encountering a shelter-seeking grass shrimp. Since *Palaemonetes vulgaris* has an avoidance of dark-colored backgrounds (Ritz 1991), they might not encounter a negatively photokinetic cercaria as frequently.

Even though the dishes were turned 90° from one photokinesis trial to the next, we observed that on average over half of the *M. turgidus* cercariae in the unlighted trials consistently moved to the covered

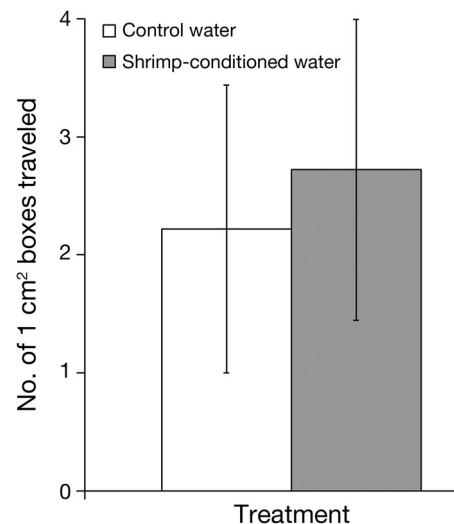


Fig. 4. Average number of 1 cm² boxes traveled during 9.5 min by *Microphallus turgidus* cercariae in control and grass shrimp-conditioned brackish water. No significant difference between treatments. Bars represent mean of 18 trials \pm 1 SD

side of the dish. It may be that the phenomenon is due to the brief but necessary exposure of the cercariae to light during the initial set up of the experiment. In a study on host-finding behavior in the cercariae of the trematode *Trichobilharzia ocellata*, the intensity and duration of light was found to be an important factor in the percentage of cercariae that responded (Feiler & Haas 1988). Perhaps *M. turgidus* cercariae have a residual memory of light that would cause them to move towards the covered half of the dish, even after even a brief exposure. If so, an increase in the length of the adjustment time period prior to initiation of the experiment may result in the expected 50:50 ratio of cercariae in the covered/uncovered halves of the dish for the unlighted trials. This study addresses a light vs. dark response; however, future studies could vary the intensity of the light source. There are few articles, including Smith & Cohen (2012) and McCarthy et al. (2002), showing photokinesis in cercariae without apparent photoreceptors. These studies and ours highlight the need for transmission electron microscopy work on microphallid cercariae, including *M. turgidus*.

We observed during our ethogram study that the most frequent activity of cercariae in a Petri dish was swimming at the bottom of the dish. The ethogram observations were, of necessity, performed in the light, so it is not possible to differentiate the effect of light from the effect of gravity on this observation. However, *M. turgidus* cercariae were more likely to be found in the bottom third of a water column during our geotaxis experiments regardless of whether the cercariae were exposed to overhead light or kept in the dark. These findings suggest that in the marsh, the cercariae stay in the demersal microhabitat favored by *P. pugio*. *P. vulgaris* can be collected from floats and pilings near the surface and may be less likely to encounter the parasite. Also, we observed during the behavioral activity observations that, in addition to spending most of their time bottom swimming, some of the cercariae combined bottom swimming with cyclic swimming from the bottom of the water column up to the top and back. In contrast, the cercariae of the trematode *Bunodera mediovitellata* (Allocreadiidae), which also have a benthic second intermediate host (caddisfly larva), do not move more than 3 to 5 mm from the bottom (Kennedy 1979).

Crustaceans produce chemicals that could act as attractants or recognition factors to cercariae. These include dispersed, water-soluble chemicals such as urine (Shabani et al. 2009) and insoluble, undispersed compounds such as contact sex pheromones (Caskey & Bauer 2005). Though many of the cer-

cariae that infect motile hosts do not respond to chemical cues (Haas 1992, Combes et al. 2002), there are some exceptions. For example, the cercariae of *Schistosoma mansoni* (Schistosomatidae) orient to linoleic acid (Shiff & Graczyk 1994) and cercariae of the trematode *Pseudechinoparyphium echinatum* (Echinostomatidae) reverse course if the chemical components in snail-conditioned water are diluted (Haas 1992). We investigated the possibility of a chemical response by *M. turgidus* cercariae because the grass shrimp hosts of the parasite are motile, even though they have a tendency to cling to macrophytic vegetation and other objects (Khan et al. 1995, 1997). However, we observed that the frequencies of behavioral activities for cercariae in the shrimp-conditioned water were not different from those of the cercariae in control water. This suggests that *M. turgidus* cercariae do not use host chemical cues to find grass shrimp. Other chemical cues, such as from aquatic vegetation, might elicit a cercarial response and merit further exploration. Since cercariae change behavior in response to respiratory currents in close host proximity, future studies could test a tactile chemical stimuli such as *P. pugio* exuviae.

The microhabitat preferences of *P. pugio*, the principle second intermediate host of the trematode *M. turgidus* (Heard & Overstreet 1983, Pung et al. 2002), may explain some of the behaviors of the cercariae observed in the present study. The location of *P. pugio* is also influenced by the tide. Juvenile *P. pugio* characteristically reside in salt marshes throughout the day (Kneib 1987a,b); however, adult *P. pugio* leave the marsh at low tide to reside in subtidal creeks and return to the marsh when it is reflooded at high tide (Kneib 1987a,b). The behaviors of *P. pugio*, combined with the habitat preferences of the first intermediate hosts, may also offer clues as to why the other sympatric, brackish water grass shrimp species in Georgia, *P. vulgaris*, is less frequently and less heavily infected with the parasite. Since the second intermediate host species differ in behavior, perhaps the bird and mammal definitive hosts consume one species more than the other. For example, if the definitive hosts primarily selected *P. pugio*, this could potentially impact the prevalence of *M. turgidus*. The cumulative results of our present taxes and activity studies indicate that cercariae of the trematode *M. turgidus* would most likely be found swimming at the bottom of the water column in salt marsh sediment and possibly move under macrophytic cover away from light. Juvenile *P. pugio* could encounter the parasite in shallow pools of standing water in the high

marsh at low tide. Those adult *P. pugio* seeking shelter under macrophytic cover and resting or feeding in high marsh sediments during high tide would also have a greater probability of infection by cercariae. Shrimp clinging to *Spartina* stalks above the sediment, though less likely to encounter the parasite, could be targets of the occasional upward/downward swimming cercaria. *P. vulgaris*, on the other hand, are less likely to be exposed to the cercariae because they are less apt to frequent the marsh at high tide and, when they do, may avoid bottom sediment where the parasite is found. If *M. turgidus* cercariae had positive photokinesis or were oriented toward shrimp-secreted chemicals, *P. vulgaris* shrimp might have a higher prevalence and intensity of infection.

Acknowledgements. This project was partially funded by a grant to O.J.P. from the Georgia Southern University Faculty Research Committee through the Office of Research Services and Sponsored Programs. We thank Ashley Burger, Kyona Jarrett, and Michael Walker for assistance in the laboratory and the field. We thank Sophie George, Stephen Vives, and C. Ray Chandler for their advice and encouragement.

LITERATURE CITED

- Caskey JL, Bauer RT (2005) Behavioral tests for a possible contact sex pheromone in the caridean shrimp *Palaemonetes pugio*. *J Crustac Biol* 25:571–576
- Combes C, Fournier A, Theron A (1994) Behaviours in trematode cercariae that enhance parasite transmission: patterns and processes. *Parasitology* 109:S3–S13
- Combes C, Bartoli P, Theron A (2002) Trematode transmission strategies. In: Lewis EE, Campbell JF, Sukhdeo MVK (eds) *The behavioural ecology of parasites*. CABI Publishing, New York, NY, p 1–12
- Deblock, S (1971) Contribution à l'étude des Microphallidae Travassos, 1920. XXIV. Tentative de phylogénie et de taxonomie. *Bull Mus Nat Hist Paris* 7:353–468
- Douglass JK, Wilson JH, Forward RB Jr (1992) A tidal rhythm in phototaxis of larval grass shrimp (*Palaemonetes pugio*). *Mar Behav Physiol* 19:159–173
- Feiler W, Haas W (1988) Host-finding in *Trichobilharzia ocellata* cercariae: swimming and attachment to host. *Parasitology* 96:493–505
- Fingerut JT, Zimmer CA, Zimmer RK (2003) Patterns and processes of larval emergence in an estuarine parasite system. *Biol Bull* 205:110–120
- Fitzpatrick KB, Smith NF, Cohen JH (2016) Swimming behavior of marine cercariae: effects of gravity and hydrostatic pressure. *J Exp Mar Biol Ecol* 476:8–14
- Fraenkel GS, Gunn DL (1961) *The orientation of animals; kinesis, taxes, and compass reactions* (New enl. edn.) Dover, New York, NY
- Goddard SM, Forward RB Jr (1989) The use of celestial cues in the offshore escape response of the shrimp, *Palaemonetes vulgaris*. *Mar Behav Physiol* 16:11–18
- Haas W (1992) Physiological analysis of cercarial behavior. *J Parasitol* 78:243–255
- Heard RW (1970) Parasites of the clapper rail, *Rallus longirostris* Boddaert. II. Some trematodes and cestodes from *Spartina* marshes of the eastern United States. *Proc Helm Soc Wash* 37:147–153
- Heard RW (1982) Guide to common tidal marsh invertebrates of the northeastern Gulf of Mexico, MASGP-79-004. Mississippi–Alabama Sea Grant Consortium, Ocean Springs, MS
- Heard RW, Overstreet RM (1983) Taxonomy and life histories of two North American species of 'carneophallus' (= microphallus) (Digenea: Microphallidae). *Proc Helm Soc Wash* 50:170–174
- Kennedy MJ (1979) The response of miracidia and cercariae of *Bunodera mediovitellata* (Trematoda: Allocreadiidae) to light and gravity. *Can J Zool* 57:603–609
- Khan RN, Knowlton RE, Merchant HC (1995) Distribution of two sympatric species of grass shrimp, *Palaemonetes pugio* and *Palaemonetes vulgaris*, in relation to homogeneous and heterogeneous aquarium substrates. *J Elisha Mitchell Sci Soc* 111:83–95
- Khan RN, Merchant HC, Knowlton RE (1997) Effects of macrophytic cover on the distribution of grass shrimps, *Palaemonetes pugio* and *P. vulgaris*. *Invertebr Biol* 116:243–247
- Kneib RT (1987a) Predation risk and use of intertidal habitats by young fishes and shrimp. *Ecology* 68:379–386
- Kneib RT (1987b) Seasonal abundance, distribution and growth of postlarval and juvenile grass shrimp (*Palaemonetes pugio*) in a Georgia, USA, salt marsh. *Mar Biol* 96:215–223
- Kneib RT (1991) Flume weir for quantitative collection of nekton from vegetated intertidal habitats. *Mar Ecol Prog Ser* 75:29–38
- Kunz AK, Ford M, Pung OJ (2006) Behavior of the grass shrimp *Palaemonetes pugio* and its response to the presence of the predatory fish *Fundulus heteroclitus*. *Am Midl Nat* 155:286–294
- Leigh WH (1958) *Carneophallus turgidus* sp. nov. (Trematoda: Microphallidae) from the raccoon, *Procyon lotor*, in south Florida. *J Parasitol* 44:100–102
- McCarthy HO, Fitzpatrick S, Irwin SWB (2002) Life history and life cycles: production and behavior of trematode cercariae in relation to host exploitation and next host characteristics. *J Parasitol* 88:910–918
- Mouritsen KN (2001) Hitch-hiking parasite: a dark horse may be the real rider. *Int J Parasitol* 31:1417–1420
- Pung OJ, Khan RN, Vives SP, Walker CB (2002) Prevalence, geographic distribution, and fitness effects of *Microphallus turgidus* (Trematoda: Microphallidae) in grass shrimp (*Palaemonetes* spp.) from coastal Georgia. *J Parasitol* 88:89–92
- Pung OJ, Grinstead CB, Vives SP (2006) Variation in the geographic and temporal and distribution of *Microphallus turgidus* (Trematoda: Microphallidae) in grass shrimp (*Palaemonetes* spp.) on tidal rivers in subtropical southeast Georgia, USA. *Comp Parasitol* 73:172–178
- Pung OJ, Grinstead CB, Kersten K, Edenfield CL (2008) Spatial distribution of hydrobiid snails in salt marsh along the Skidaway River in southeastern Georgia with notes on their larval trematodes. *Southeast Nat* 7:717–728
- Pung OJ, Burger AR, Walker MF, Barfield WL, Lancaster MH, Jarrous CE (2009) *In vitro* cultivation of *Microphallus turgidus* (Trematoda: Microphallidae) from metacercaria to ovigerous adult with continuation of the life cycle in the laboratory. *J Parasitol* 95:913–919
- Pung OJ, Lester T Jr, Burger AR, Alyanak E, O'Leary PA

- (2011) Optimization of culture conditions for *in vitro* fertilization and reproduction of *Microphallus turgidus* (Trematoda: Microphallidae). *J Parasitol* 97:1–7
- ✦ Rees FG (1975) Studies on the pigmented and unpigmented photoreceptors of the cercaria of *Cryptocotyle lingua* (Creplin) from *Littorina littorina* (L.). *Proc R Soc Lond B Biol Sci* 188:121–138
- ✦ Ritz DA (1991) Polarised light responses in the shrimp *Palaeomonetes vulgaris* (Say). *J Exp Mar Biol Ecol* 154:245–250
- Rohlf FJ, Sokal RR (1981) *Statistical tables*, 2nd edn. WH Freeman and Company, San Francisco, CA
- ✦ Shabani S, Kamio M, Derby CD (2009) Spiny lobsters use urine-borne olfactory signaling and physical aggressive behaviors to influence social status of conspecifics. *J Exp Biol* 212:2464–2474
- ✦ Shenker JM, Dean JM (1979) The utilization of an intertidal salt marsh creek by larval and juvenile fishes: abundance, diversity, and temporal variation. *Estuaries* 2: 154–163
- ✦ Shiff CJ, Graczyk TK (1994) A chemokinetic response in *Schistosoma mansoni* cercariae. *J Parasitol* 80:879–883
- ✦ Smith NF, Cohen JH (2012) Comparative photobehavior of cercariae with differing secondary host preferences. *Biol Bull* 222:74–83
- Sokal RR, Rohlf FJ (1998) *Biometry: the principles and practice of statistics in biological research*, 3rd edn. Freeman and Company, New York, NY
- ✦ Sukhdeo MVK, Sukhdeo SC (2004) Trematode behaviours and perceptual worlds of parasites. *Can J Zool* 82: 292–315

*Editorial responsibility: Bernd Sures,
Essen, Germany*

*Submitted: June 1, 2016; Accepted: October 21, 2016
Proofs received from author(s): December 17, 2016*