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Swimming behavior of marine cercariae: Effects of gravity and hydrostatic pressure

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ABSTRACT

Many species of marine larvae use exogenous cues, such as gravity and pressure, for orientation and dispersal, but it is unclear if parasitic larvae exhibit comparable geotactic and barokinetic behaviors. Using two species of marine cercariae, *Euhaplorchis* sp. and *Probolocoryphe lanceolata*, which utilize the same first intermediate host, the mangrove snail *Cerithidea scalariformis*, the hypothesis that they use gravity and pressure to position themselves in the water column to increase the probability of contacting their respective second intermediate hosts was tested. In a test chamber under dark laboratory conditions, *Euhaplorchis* sp. cercariae ascended, and therefore displayed negative geotaxis, increasing the probability of finding their second intermediate host, salt marsh killifish (*Fundulus* spp.). In contrast, *P. lanceoloata* cercariae swam downwards towards the bottom of the chamber, and this positive geotactic behavior would place them in a benthic microhabitat consistent with their second intermediate host, fiddler crabs (*Uca* spp.). In a subsequent experiment testing swimming activity in response to step increases in pressure, no effect of pressure changes over the range tested (0.3–30 kPa) was observed for either species. Collectively, these results suggest that while cercariae are unresponsive to changes in pressure, they are able to utilize gravity for orientation, placing them in the preferred microhabitat of the second intermediate host. This finding is consistent with published photobehavioral studies in which *Euhaplorchis* swam upwards towards light and *P. lanceolata* swam downward towards the bottom upon light stimulation. These complementary phototactic and geotactic behaviors optimize host contact, and thus completion of their complex life cycle.

1. Introduction

For parasites, transmission from one host to another is essential to maintaining their life cycle and prevalence in host populations (Zimmer et al., 2009). For example, trematodes use multiple hosts to complete their life cycle and successful transmission depends upon the ability of their free-swimming larvae (cercariae) to quickly find a suitable host within the host-space environment, a process which is tightly coupled to environmental cues (Sukhdeo and Sukhedo, 2004; Zimmer et al., 2009). Cercariae may rely on a variety of exogenous cues to aid in orientation and distribution (Combes et al., 1994; Haas, 1994). Studies have shown that marine cercariae use light intensity, temperature, and water flow to position themselves in the water column where they are more likely to encounter an appropriate host (Combes et al., 1994; Sukhdeo and Sukhedo, 2004; Fingerut et al., 2003b; Smith and Cohen, 2012). Such behavioral and physiological adaptations are especially important because for most swimming larvae, dispersal can be costly, resulting in high mortality rates (Morgan, 1995). By exhibiting some control over their abbreviated dispersal phase, cercariae can increase the likelihood of successful recruitment to their intended hosts.

Given the ephemeral nature of cercariae dispersal and the limited opportunity to be transmitted to a second intermediate host, identifying the factors that influence cercarial distribution or position within the water column is central to understanding transmission processes. Perhaps one of the most important exogenous factors that can influence vertical distribution is gravity, a factor well known to determine the orientation of a variety of marine organisms, including larval invertebrates and fish (Huebert, 2008; Forward and Wellins, 1989; McCarthy et al., 2002a, 2002b; Mogami et al., 1988; Young, 1995). Gravity can serve as an orienting cue for swimming behavior since it is a constant and predictable factor (Forward, 1988). More specifically, organisms may display active movement or taxis towards the center of the Earth (positive geotaxis) or away from it (negative geotaxis) (Sulkin, 1984). For cercariae, gravity could evoke an ascent or descent response to guide their swimming to a particular host habitat, however, swimming activity could also be a kinetic response, involving non-directional changes in speed or locomotory activity (Sulkin, 1984). Tactic orientation behaviors have been best studied in zooplankton, which use gravity to regulate depth during diel vertical migration and/or export from

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estuaries during early larval development (Sulkin, 1984; Forward and Wellins, 1989; Cohen et al., 2015). Under dark laboratory conditions, freshwater cercariae have also been shown to use gravity for orientation (Platt et al., 2009). Second intermediate hosts (pulmonate snails) that were located higher in the water column experienced significantly greater rates of infection by the trematode, *Echinostoma caproni*, indicating that the cercariae are negatively geotactic (Platt et al., 2009). In a subsequent study, Platt et al. (2010) found that when an overhead light source was added, there was a significant increase in infection rates relative to trials conducted under dark conditions. However, they found no increase in infection rates when light was placed at the bottom, indicating that the cercariae only responded to the light source when it was complimentary to their negative geotactic behavior (Platt et al., 2010). Thus, depth regulation may involve multiple exogenous cues in which one cue is reinforced by another (e.g. negative geotaxis reinforced with positive phototaxis resulting in upward swimming), however, some species may exhibit behavioral responses that work in opposition to each other (e.g. negative geotaxis and negative phototaxis) where one cue may be more dominant under certain conditions (Young, 1995).

Vertical distribution of zooplankton can also be influenced by barokinesis, or change in swimming activity due to changes in hydrostatic pressure (Sulkin, 1984; Forward, 1988). Such pressure-mediated swimming behavior commonly aids in depth regulation, where increased hydrostatic pressure generally evokes increased swimming activity (high barokinesis) and ascent towards lower pressures, while decreased hydrostatic pressure evokes decreased swimming (low barokinesis) and descent towards higher pressure in negatively buoyant animals. While high barokinesis commonly aids in ascent behavior in negatively geotactic animals such as crab larvae (Wheeler and Epifanio, 1978; Forward and Wellins, 1989; Forward et al., 1989), high barokinesis could alternatively result in descent behavior of positively geotactic animals. Pressure and light are the only consistent environmental indicators of depth, and with daily changes in light levels, hydrostatic pressure becomes the only indicator that can be relied upon at night (Rice, 1964). This may help explain why barokinesis is so prevalent in crustacean zoea larvae, particularly those that live in estuaries where daily tidal fluctuations result in more frequent need to readjust vertical position (Wheeler and Epifanio, 1978). Since cercariae are also found in shallow tidal environments and are likely to experience frequent pressure changes, they may be able to utilize barokinesis to maintain their position within the water column, optimizing contact and transmission to their next host.

This study focused on two common species of trematodes which utilize the same first-intermediate host (*Cerithidea scalariformis*, Say, 1925), but different second intermediate hosts. *Euhaplorchis* sp. (similar to *E. californiensis*, Martin, 1950) and *Probolocoryphe lanceolata* (synonym *Cercariae lanceolata*, Holliman, 1961, and *Probolocoryphe glandulosa*, Heard and Sikora, 1969) are commonly found in snails inhabiting mangrove marshes on the Atlantic and Gulf coasts of Florida (Smith, 2001; Smith and Cohen, 2012). Both species possess a complex life cycle involving three hosts (Holliman, 1961; McNeff, 1978), including *C. scalariformis*. The emergence of marine cercariae from snail hosts has been linked to a variety of environmental factors such as light, temperature, and water level (Fingerut et al., 2003a; Koprivnikar and Poulin, 2009), and these cues tend to coincide with periods when the second intermediate host is present and active within the aquatic environment (Sukhdeo and Sukhedo, 2004). This temporal and spatial overlap is important because after emerging from the first intermediate host, cercariae usually have less than 24 h to find and infect the appropriate second intermediate host (Holliman, 1961), and infectivity decreases as cercariae age (Pechenik and Fried, 1995).

Using their subterminally attached tail, *Euhaplorchis* cercariae swim rapidly upward followed by a slow period of passive sinking, and while at rest, their bodies are orientated downward with their tails upwards (personal observation). Because they typically infect estuarine fishes as secondary-intermediate hosts, cercariae would be expected to have evolved behaviors that would place them in the pelagic waters that are the habitat of a common secondary intermediate host, salt marsh killifish of the genus *Fundulus* (Martin, 1950; Lafferty and Morris, 1996; Shaw et al., 2010). Previous work has shown that *Euhaplorchis* cercariae exhibit positive phototaxis (directional swimming towards the light) when stimulated with light intensities greater than 4.0×10^{15} photons $\mathrm{m}^{-2}\,\mathrm{s}^{-1}$ 1 (Smith and Cohen, 2012). Complementary to this established behavior, *Euhaplorchis* cercariae would be predicted to exhibit negative geotaxis and may respond to changes in hydrostatic pressure, especially when they have descended too far and need to swim upwards to stay closer to the killifish habitat.

In contrast, *P. lanceolata* cercariae display a distinctive circular swimming pattern in which they are rarely in a state of rest unless anesthetized (personal observation). They possess a stylet which is used for penetrating their second intermediate host, fiddler crabs (*Uca* spp.) (Holliman, 1961; Heard, 1976). *P. lanceolata* cercariae have been shown to be negatively phototactic (swim away from light when stimulated) in a simulated natural angular light field, enhancing the probability that a released larva would descend and encounter a burrowing crab (Smith and Cohen, 2012). However, additional experiments by Smith and Cohen (2012) using a horizontal trough suggested that light did not serve as the orienting cue for vertical swimming in *P. lanceolata* cercariae. While *Euhaplorchis* exhibited directional swimming in a horizontal trough (i.e. oriented towards the light in a horizontal plane), *P. lanceolata* did not orient to a directional light source at all light intensities when tested in this way (Smith and Cohen, 2012). This indicates that while *P. lanceolata* responds to light, it utilizes an exogenous cue apart from light for orientation. Their descent may therefore be directed by gravity (positive geotaxis) or increasing hydrostatic pressure (positive barotaxis) to optimize contact with their burrowing secondary hosts.

Accordingly, the present study measured the behavioral responses of *Euhaplorchis* and *P. lanceolata* cercariae under laboratory conditions to determine the role of two exogenous cues — gravity and pressure, on their swimming behavior. To complement earlier work on phototaxis in these species (Smith and Cohen, 2012), geotactic responses were measured by quantifying cercarial vertical distribution under dark conditions, and barokinetic responses were measured by tracking their swimming direction and speed upon changes in hydrostatic pressure. Between trematode species, contrasting geotactic and barokinetic behavioral responses that would facilitate their movement towards microhabitats frequented by their secondary hosts were expected. Specifically, *Euhaplorchis* and *P. lanceolata* cercariae were predicted to display negative and positive geotaxis, respectively. Likewise, positive barokinesis upon step increases in pressure were predicted as increased frequency of ascent responses for the negatively geotactic species (*Euhaplorchis*) and as increased frequency of descent responses for the positively geotactic species (*P. lanceolata*).

2. Materials and methods

2.1. Study organisms, field collections, and laboratory apparatus

Cercariae of *Euhaplorchis* and *P. lanceolata* were collected from infected *Cerithidea scalariformis* from a mangrove marsh on Weedon Island, St. Petersburg, Florida, USA (N 27°50.699′ W 82°36.663′) in June 2014. The marsh and mudflat are inundated daily by semidiurnal tides, with a maximum tidal range of 0.5 m (Belgrad and Smith, 2014). Infection prevalence (the proportion of hosts that are parasitized, Margolis et al., 1982) at Weedon Island ranges from 19 to 25% (Belgrad and Smith, 2014), and *Euhaplorchis* and *P. lanceolata* are among the most abundant trematode species that infect the snail population. To determine if snails were parasitized (without sacrificing them), individuals were placed in plastic containers and inundated in 0.45 μm-filtered seawater (FSW) to shed parasites. FSW was examined for the presence of swimming *Euhaplorchis* and *P. lanceolata* cercariae after 24 h. Once infected snails were identified, they were kept alive in the lab (1–5 days)