THE EFFECT OF A TREMATODE PARASITE (MICROPHALLUS SP.) ON THE RESPONSE OF THE FRESHWATER SNAIL POTAMOPYRGUS ANTIPODARUM TO LIGHT AND GRAVITY

by

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Summary

Parasites often influence the behavior of their hosts in ways that increase the probability of transmission of the parasite. The digenetic trematode *Microphallus* sp. has been demonstrated to alter the behavior of the New Zealand freshwater snail *Potamopyrgus antipodarum* in a way that increases the probability that infected snails will be eaten by the final host (waterfowl). Infected snails are found foraging on top of rocks more often in the early morning when waterfowl are feeding and less often in the afternoon when unsuitable hosts (fish) are feeding. The mechanism(s) that the parasite utilizes to produce this behavioral change is not known. The present study investigated three possible behaviors (phototaxis, geotaxis, and photokinesis) that the parasite could alter that may account for the behavioral change seen in the field. Infected and uninfected snails were assessed in terms of their orientation to light (phototaxis), orientation to gravity (geotaxis), and movement in response to light (photokinesis). There was no evidence of phototactic behaviors in either infected or uninfected snails. However, uninfected snails were found to positively orient towards gravity, while infected snails did not. Also, both infected and uninfected snails were found to be positively photokinetic (they move faster in the light than in the dark), but *Microphallus*-infected snails were found to move more slowly than uninfected snails. The differences found between infected and uninfected snails may be part of the manipulative effort of the parasite, but by themselves the differences are not sufficient to explain the patterns observed in the field.

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Introduction

The alteration of host behavior due to parasitism can be an adaptation of the host or parasite, or the behavioral change can be a non-adaptive byproduct of the host-parasite relationship (Holmes & Bethel, 1972; Dobson, 1988; Moore & Gotelli, 1990). Often behaviors that increase the chance of parasite transmission are caused by relatively simple changes in aspects of the host's behavior, such as geotactic, phototactic (Carney, 1969; Hindsbo, 1972; Bethel & Holmes, 1973; Kennedy *et al.*, 1978; Demont & Corkum, 1982; Moore, 1983; 1984; Moore & Lasswell, 1986; Lefcort & Durden 1996; Bakker *et al.*, 1997) or photokinetic responses (Moore & Gotelli, 1992). The majority of these tactic or kinetic responses have been found associated with acanthocephalan parasites.

The digenetic trematode *Microphallus* sp. appears to induce behaviors in its intermediate host, the snail *Potamopyrgus antipodarum*, that are advantageous for the parasite's transmission. Previous studies have demonstrated that *Microphallus*-infected snails are more likely to be found on top of rocks in the early morning when the final host, waterfowl, feeds most often (Levri & Lively, 1996). This behavior appears to increase the susceptibility of infected snails to predation by the final host (which is necessary for transmission to occur). Later in the morning, infected snails move to the bottom of the rocks. This time-specific shift in behavior corresponds with the increased feeding of fish which cannot serve as a final host (Levri, 1998a). Only snails infected with mature transmissible metacercaria move to the top of the rocks in the early morning when waterfowl are feeding (Levri & Lively, 1996). Further, other castrating trematode parasites do not induce the same behavior (Levri, 1999). Thus *Microphallus* appears to induce a time-specific change in the behavior of its host. Previous studies have examined the result of the snail's behavior (being on the top or bottom of a rock). This previous work has not fully investigated the actual behavioral components that determine the location of the snail.

The present study asks the question: What environmental stimuli influence the behavior of *P. antipodarum*? The study examines the influence of light and gravity on the behavior of infected and uninfected snails. The experiments were conducted in an effort to determine if the parasite may alter the phototactic, geotactic, and/or photokinetic responses of the snail in a way that is consistent with the change in foraging behavior observed in the field. Specifically, we were looking for effects of the parasite on responses to light and/or gravity that change from the early to late morning.

The system of study

Potamopyrgus antipodarum is a small prosobranch snail found in New Zealand lakes and streams. It feeds predominantly on green algae, diatoms, and detritus (Winterbourn, 1970; Haynes & Taylor, 1984; Winterbourn & Fegley, 1989), and in areas with rocky substrates it forages on the tops of rocks, predominantly at night (Levri & Lively, 1996). The snail is commonly eaten by fish (McCarter, 1986; Jellyman, 1989; McDowall, 1990; Levri, 1999) and waterfowl.

The predominant parasite of *Potamopyrgus* is an undescribed species of *Microphallus* (Trematoda: Microphallidae; Lively, 1987). Mature *Microphallus* produces eggs in waterfowl, which pass out of the bird with the feces. The eggs ingested by *Potamopyrgus* develop and encyst in the snail. *Microphallus* castrates the snail as the parasite matures. The cysts hatch upon ingestion by waterfowl, where they mature in the intestine to complete the life cycle. *Potamopyrgus* is also the first intermediate host to at least a dozen other castrating trematode parasites (Winterbourn 1973), that occur in low frequencies (Lively, 1987; Jokela & Lively, 1995).

Methods

A single aerated 40 l tank containing flat rocks and tap water was placed in direct sunlight on a window sill in a room with a constant temperature of 23°C on the campus of Indiana University. The rocks were arranged so as to allow the snails to freely move from the top to the bottom. The water was treated with amquel and novaqua to condition the water. Approximately 500 snails taken earlier in the year from Lake Alexandrina on the South Island of New Zealand were added to the tank after two days. The density of snails was similar to that found in the field which ranges from tens to tens of thousands per square meter (Dorgelo, 1987; Schreiber *et al.*, 1998; Dybdahl, pers. comm.). The snails were maintained relatively undisturbed for a period of one month. The snails were fed ground-up trout chow every other day during this time and throughout the experiments. After the one month acclimation period, snails were randomly removed from this tank daily to perform the following experiments. The phototaxis experiments were performed first, which took approximately 10 days. The geotaxis and photokenesis experiments were performed on the same days for the next two months. The data from the first half of the trials were compared to the data from the second half of the trials, and the results did not vary with the duration of the experiment.

Phototaxis:

A petri dish filled with water was set on top of paper that had a circle drawn on it showing degree marks from 0° to 360°. Individual snails were placed in the center of the petri dish. A box was set over the dish to block out light. A hole in the box allowed a beam of light from a fiber optic light source to shine in the box from the 0° direction. The snail was left alone for three minutes in the petri dish. The box was then removed, and the angular orientation of the snail was recorded. The trials were conducted between 0600 and 0800 hours and between 1100 and 1300 hours. The experimental set up was rotated randomly in different directions *i.e.* north, south, east, west, to make sure the snails were not orienting themselves to a specific direction rather than toward or away from the light source. The snails were then dissected to determine size, sex, reproductive condition, and infection status. The phototactic response data was analyzed using Circstat resulting in r-statistics that measured the significance of the orientation.

Geotaxis:

A 40 l tank with 30 l of water was set up with an overhead lamp attached to it. Glass tubing approximately 260 mm long and 6 mm wide was marked showing each centimeter. The snails were put individually into the glass tubing at the center mark. The tube was then put into the water. After the snail attached itself to the center mark in the tube, the tube was turned vertical for five-minute trials in both light and dark conditions. A cardboard box was used to block light during the dark trials. After each trial, the position of the snail to the nearest centimeter mark was noted. Snails that failed to move at least one centimeter were excluded from the analysis. The snails were then dissected to determine size, sex, reproductive condition, and infection status. The trials were carried out from 0600 to 0800 hours and 1100 to 1300 hours. The geotactic response was analyzed using log-linear analyses to determine if each group of snails moved up or down more than 50% of the time. Orientation (up or down) was used as the dependent variable, and time of day, class of snail, and light or dark condition were used as independent variables.

Photokinesis:

In the same tanks and using the same glass tubing described above, individual snails were put into the glass tube at the center mark, and the tube was placed horizontally into the water. The trials lasted for five minutes at which time the position of the snail to the nearest centimeter mark was recorded. The snails were then dissected to determine size, sex, reproductive condition, and infection status. Both light and dark conditions were tested using a cardboard box to block light in the dark trials. The trials took place from 0600 to 0800 hours and 1100 to 1300 hours. The photokinetic response was analyzed using repeated measures of analysis of variance with class of snail and time of day as between-subjects effects and distance travelled in light and dark conditions as a within-subjects effect.

Results

Phototaxis:

A total of 150 snails were tested for their orientation to light: 80 uninfected snails and 70 *Microphallus*-infected snails. No snail class, including infected snails, oriented significantly in any single direction ($p > 0.10$ in all cases) (Table 1; Fig. 1). There was no effect of time of day on the phototactic response in infected and uninfected snails.

Geotaxis:

A total of 417 snails were tested for their orientation to gravity: 325 uninfected snails and 92 *Microphallus*-infected snails. Uninfected snails significantly oriented themselves toward gravity compared to the null hypothesis of no orientation ($p < 0.03$ in all cases). There were no significant differences between the uninfected classes of snails in their orientation. *Microphallus*-infected snails did not orient significantly in either direction ($p = 0.38$) (Fig. 2). Further, infected snails were significantly different from all uninfected snail classes (brooding females, non-brooding females, and males) ($p < 0.002$ in all cases). There was no effect of time of day on the geotactic response in infected and uninfected snails ($p = 0.47$). Also, no difference was found between light and dark trials ($p = 0.23$ at least in all cases).

TABLE 1. *The orientation of snails to light (phototaxis)*

Class	*N*	Vector angle	*r*-statistic	*p*-value
Non-brooding females (morning)	5	156	0.46	0.37
Non-brooding females (afternoon)	8	190	0.077	0.90
Brooding females (morning)	16	213	0.117	0.86
Brooding females (afternoon)	18	267	0.368	0.10
Males (morning)	16	81	0.335	0.20
Males (afternoon)	17	208	0.157	0.65
Microphallus-infected (morning)	38	77	0.045	0.87
Microphallus-infected (afternoon)	32	177	0.201	0.30

No class of snail showed a significant orientation toward or away from light.

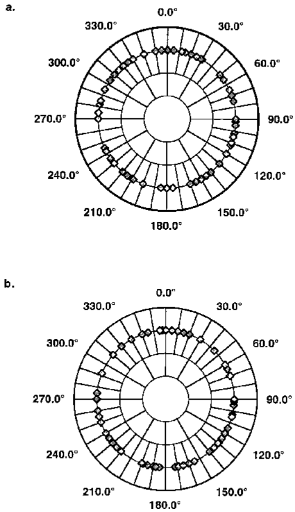

Fig. 1. Phototaxis in infected (a) and uninfected snails (b). Neither infected nor uninfected snails significantly oriented toward or away from light (see Table 1).

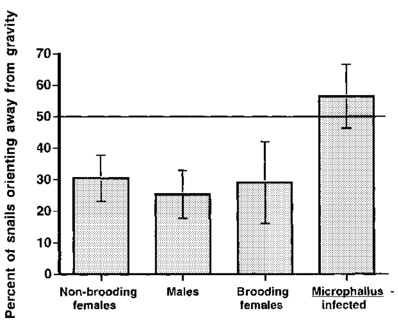

Fig. 2. Geotaxis in infected and uninfected snails. All uninfected classes of snails significantly moved toward gravity, while infected snails did not significantly move in either direction. *Microphallus*-infected snails were significantly different from all of the uninfected snail classes. Error bars are 95% confidence intervals.

Photokinesis:

A total of 329 snails were tested for their photokinetic response: 261 uninfected snails and 68 *Microphallus*-infected snails. There was no effect of time of day on the photokinetic response in infected and uninfected snails ($p = 0.58$), thus time was not used as a factor in subsequent analyses. All classes of snails were found to be positively photokinetic; they moved faster

TABLE 2. *Results of repeated measures analysis of variance of photokinetic data*

Source of variation	SS	DF	MS	*F*	*p*
Between-subjects effects:					
Within + Residual	7112.77	325	21.89		
Class	666.92	3	22.31	10.16	< 0.001
Within-subject effects:					
Within + Residual	5096.24	325	15.68		
Distance	200.78	1	200.78	12.80	< 0.001
Class by Distance	7.79	3	2.60	0.17	0.920

Distance represents the difference between the distance travelled in light and dark conditions. Class refers to infected *vs* uninfected snails.

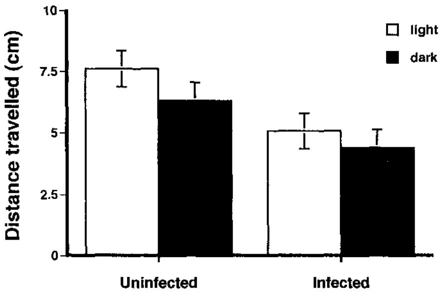

Fig. 3. Photokinesis in infected and uninfected snails. Both infected and uninfected snails moved further in light than in dark conditions. *Microphallus*-infected snails moved significantly slower than uninfected snails regardless of time of day or light conditions. Error bars are standard errors of the mean.

in the light than in the dark (Table 2; Fig. 3). Uninfected snails moved further than *Microphallus*-infected snails ($p < 0.001$) in all conditions.

Discussion

The goals of these experiments were to determine if *Microphallus* altered the phototactic, geotactic, or photokinetic response of *P. antipodarum*. If these behaviors were altered, were they altered in a time-specific way that was consistent with patterns observed in the field? Natural time-dependent changes in orientation related behaviors have been previously demonstrated in gastropods (Warburton, 1973; Lederhendler *et al.*, 1980). In this case, however, no time-dependent effect of the parasite on the snail's behavior was found.

Phototaxis:

Phototaxis appears to be a common method of orientation in gastropods (*e.g.* Burdon-Jones & Charles, 1958; Charles, 1961a, b; Warburton, 1973; van Duivenboden, 1982). However, neither infected nor uninfected snails in the present study were found to be phototactic (Fig. 1). Thus, the snails do not appear to use light to aid them in their orientation. Since uninfected snails do not orient towards or away from light, it is not surprising that infected snails fail to do so as well.

Geotaxis:

Geotactic behaviors have been demonstrated numerous times in gastropods (Wolff, 1975; Janse, 1982), and they have been found in all major gastropod sub-classes (Wolff, 1975). In the present study, uninfected individuals tended to be positively geotactic, orientating towards gravity. In the field, uninfected individuals are found underneath rocks during mid-morning and afternoon in a likely effort to avoid predators (Levri, 1998b, 1999). The positive geotactic response may in part explain the behavior of uninfected snails in the field. *Microphallus*-infected snails did not seem to react in any way to gravity at any time of the day. The lack of a geotactic response may partly explain why *Microphallus*-infected snails are on top of the rocks in the early morning. However, the persistence of the lack of a geotactic response in the afternoon in infected snails is not consistent with field results. In the field, *Microphallus*-infected snails move to the bottom of the rocks in the late morning, and this response decreased the probability of the snail being eaten by unsuitable fish hosts (Levri, 1998b). The behavior of non-brooding female snails in the field is also inconsistent with a purely geotactic explanation of behavior. Non-brooding female snails were more likely to be found on the tops of rocks during the middle of the day than any other snail group, including *Microphallus*-infected snails (Levri & Lively, 1996). In the present study, however non-brooding females were found to be positively geotactic at all times. Thus, some other factor must be important in determining the behavior of these snails.

Photokinesis:

Both infected and uninfected snails were found to be positively photokinetic; moving faster in the light than in the dark. This behavior should lead to the snails spending more time on the bottom of rocks than on the top, which, overall, is what is found in the field. A difference was found between infected and uninfected snails in that infected snails were significantly slower than uninfected snails in both light and dark conditions. This result was unexpected, and it may be a non-adaptive byproduct of the host-parasite relationship. Infected snails may suffer from a nutrient depletion that reduces their rate of movement. No difference was found between any of the uninfected snail classes. Thus, photokinesis in not a likely explanation of the differences in behavior observed between uninfected snail classes in the field (Levri & Lively, 1996). The difference in rate of movement between infected and uninfected snails was found both in the morning and in the afternoon. Since, there was no effect of time of day or infection, photokinesis does not likely play a role in the manipulation of the snail. Differences in the rate of movement in light and dark conditions have been demonstrated in gastropods previously (*e.g.* Lederhendler *et al.*, 1980).

Conclusion

The results presented here fail to fully explain how the parasite *Microphallus* sp. alters the behavior of its host in a time-specific manner. Infected and uninfected individuals did differ in their speed and geotactic responses. These differences may be part of an adaptive manipulative effort on the part of the parasite, or they may be non-adaptive byproducts of the host-parasite relationship. The present experiment was performed in the lab. It is possible that certain crucial cues from the natural environment are necessary for the time-specific behaviors to occur. It appears that other variables that influence behavior may be important in generating the differences seen between infected and uninfected snails as well as the differences observed between uninfected snail classes. Other variables such as cues from predators or competitors (Levri, 1998b) may interact with the cues of light and gravity to influence the behavior of the snails. Further research in the field and into the interaction between multiple cues would be beneficial and potentially enlightening.

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