

PARASITE-INDUCED ALTERATION OF DIURNAL RHYTHMS IN A FRESHWATER SNAIL

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ABSTRACT: The trematode *Microphallus* sp. alters the behavior of its snail intermediate host, *Potamopyrgus antipodarum*, in ways that seem to increase transmission to its final host, e.g., waterfowl, and decrease the probability of being eaten by other predators, e.g., fish. The parasite seems to cause the snail to move from the top to the bottom of rocks at about 0900 hr. Waterfowl feed predominantly before 0900 hr, and fish feed predominantly after 0900 hr. In the present study, we tested the hypothesis that *Microphallus* sp.-infected snails exhibit a change in behavior at around 0900 hr by examining their response to light and vertical orientation before and after 0900 hr. Results demonstrated that uninfected snails generally move toward light, oriented downward, and move a greater distance in the light compared with the dark at all times of day. *Microphallus* sp.-infected snails behaved differently from uninfected snails in the early morning but similarly to uninfected snails in the late morning with regard to downward orientation and distance moved in response to light. Snails infected with parasites other than *Microphallus* sp. behaved similarly to uninfected snails during both time periods. These results suggest that *Microphallus* sp. manipulates the behavior of *Potamopyrgus* sp. by altering rates of movement in response to light and vertical orientation in a manner consistent with the hypothesized 0900-hr shift.

Parasites often influence the behavior of their hosts (for review, see Moore, 2002). These changes may be adaptive for the host (Boorstein and Ewald, 1987; Hart, 1988) or the parasite (for review, see Combes, 1991; Moore, 2002) or produce non-adaptive effects in the host–parasite relationship (Levri, 1999; Gourbal et al., 2001). Although some examples of behavioral change are most likely adaptive for the parasite (e.g., Moore, 1983; Lafferty and Morris, 1996), others are less obvious but still beneficial to the host–parasite interaction (e.g., Gourbal et al., 2001). Nonetheless, it is often difficult to infer adaptation when there is a parasite-induced change in behavior (Poulin, 1995).

The New Zealand mud snail (*Potamopyrgus antipodarum*) shows altered behavior when infected with the trematode *Microphallus* sp. (Levri and Lively, 1996; Levri, 1998a, 1999; Levri and Fisher, 2000). In Lake Alexandrina, New Zealand, snails forage on algae in rocky areas. The algae provide obvious energetic and nutritive benefits to the snails; however, foraging on the top of rocks where algae grow comes at the cost of increasing the probability of predation by fish and waterfowl. Thus, most snails feed predominantly at night when predation risk is lower (Levri and Lively, 1996; Levri, 1998b). *Microphallus* sp. is unusual in that it possesses a 2-host life cycle where transmission from the intermediate host (*Potamopyrgus* sp.) occurs when metacercariae are ingested by waterfowl during predation. *Microphallus* sp.-infected snails spend more time exposed to predators by foraging on the tops of rocks during early morning hours (0600–0900) than their uninfected counterparts (Levri and Lively, 1996). This behavior seems to increase the probability of being eaten by waterfowl final hosts, which have been shown to feed more extensively in the morning than other times of day (Levri and Lively, 1996). However, *Microphallus* sp.-infected snails seem to move to the bottom of the rocks in the morning hours at around 0900 hr (Levri and Lively, 1996). This movement also coincides with an increase in the feeding intensity of fishes, primarily *Gobiomorphus cotidianus*, in the late morning and early afternoon. Moreover, infected snails make up a smaller proportion of the diet of the fishes than expected by chance (Levri, 1999). Thus, the behav-

ior induced by *Microphallus* sp. seems to increase the probability of being eaten by the final avian definitive host and to decrease the probability of being eaten by predators that cannot serve as the final host, i.e., fishes (Levri, 1999). These behavioral changes are not caused by other castrating trematodes, and they are not the result of differing nutritional demands of infected and uninfected snails (Levri, 1998b). *Microphallus* sp.-infected individuals stay on the top of the rocks in the early morning only when the parasite has reached a level of maturity at which the parasite can be transmitted to the final host (Levri and Lively, 1996). These observations suggest that *Microphallus* sp. manipulates the behavior of *Potamopyrgus* sp. to increase the likelihood of transmission.

In the present study, we tested the hypothesis that the behavioral change occurs at around 0900 hr by examining infected and uninfected snail before and after this time. We examined directional movement in response to light, distance traveled in response to light, and vertical orientation.

MATERIALS AND METHODS

System of study

Potamopyrgus antipodarum is commonly found in New Zealand lakes and streams where it is a host to at least 10 castrating trematode parasites (Winterbourn, 1970; Lively, 1987). Among these species is *Microphallus* sp., which has a 2-host life cycle. Adult worms reproduce sexually in the intestines of waterfowl, and eggs are passed in the feces. *Potamopyrgus antipodarum* becomes infected when eggs are accidentally ingested. On hatching, the emerging larval stage leaves the snail's intestine and proceeds through a series of developmental stages in the gonads, eventually producing cercariae that encyst in the snail as metacercariae. The life cycle is completed when infected snails are eaten by waterfowl. In Lake Alexandrina, *P. antipodarum* also is infected by several other species of castrating trematodes. These other parasites included *Telogaster opistorchis*, 2 *Gymnocephalous* species, and 2 undescribed species of monostomes (listed in Winterbourn, 1974).

Snail collection and maintenance

Snails were collected from shallow water habitats in Lake Alexandrina on 15 December 2002 and kept in 2-L plastic containers. The water was changed 4 times per day, and the containers were kept in the shade. In each experiment, a relatively large number of snails were used (>900). The densities of the snails used in the experiments (ranging from 400 to 800/m²) did not exceed the densities naturally found in the lake. Vertical orientation and distance moved in response to light experiments were conducted outdoors adjacent to Lake Alexandrina a few days after the snails were collected. These 2 experiments were per-

formed in ambient light. Thus, light changed in direction and intensity over the course of the experiments. We did not attempt to control light direction and intensity, because we wanted the trials to be as natural as possible. The experiments measuring directional movement in response to light were conducted in the laboratory 6 days after snail collection. The early experiments began at 0600 hr, which was soon after sunrise.

Snails were preserved in 70% ethanol immediately after each experiment. The length of each snail was measured. The snail was then dissected, and the gender, brooding condition, and presence and type of parasite were noted. The snail data were then divided into 6 mutually exclusive classes: uninfected nonbrooding females, uninfected brooding females, uninfected males, *Microphallus* sp.-infected females, *Microphallus* sp.-infected males, and snails infected with other parasites. Snails infected with multiple parasites were rare and were not included in the analysis. Parasites were identified in part by using the descriptions provided by Winterbourn (1974).

Vertical orientation

Vertical orientation experiments were conducted on 18 December 2002 in full sunlight. Two, 70-L opaque tanks were filled with approximately 65 L of lake water. Plexiglas trays measuring 32 × 32 cm with 5-cm-high walls on each edge were placed on the floor of the tanks. Each tray had a horizontal line in the center. Approximately 100 snails were placed along the centerline. After the snails had emerged from their shells (approximately 15 sec) the trays were oriented vertically. Any snails that initially fell from the trays were removed for 30 sec after the chamber was vertically oriented. Each trial lasted 7 min, after which snails above or below the centerline were separated. Half of the trials were performed using ambient light; the other half were performed in the dark by wrapping the tanks in opaque black plastic sheeting. A light meter verified that the plastic sheeting did not allow a detectable level of light into the experimental chambers. Multiple trials of each type (in light and dark) were performed between 0600 and 0800 hr (10 trials), and another set was performed between 1000 and 1200 hr (11 trials). The water in the large tanks was periodically changed with water taken directly from the lake to avoid variation in temperature. In total, 2,518 snails were used in this experiment.

Hierarchical log-linear analyses were used to compare the ratios of snails within each class that moved up or down in the light and dark in both the early and late morning. A significant direction × light interaction indicated significant directional movement in response to light. The procedure also was used to compare different classes of snails within the same time period. In this case, a significant class × direction × light interaction indicated a significant difference in the directional movement between the 2 groups. Log-linear analysis also was used to compare late and early morning behavior within classes of snails. Here, a significant time × light × direction interaction indicated significantly different directional movement at different times.

Directional movement in response to light

The experiments to determine whether snails directionally move in response to light were conducted in the laboratory at the Edward Percival Field Station in Kaikoura, New Zealand, on 21 December 2002. Snails from the shallow water habitats of Lake Alexandrina were transported to Kaikoura and maintained in 60-L plastic containers out-of-doors in a shady environment for 2 days before the experiment. The experiment was conducted in 32 × 32-cm Plexiglas containers with 5-cm-high walls on each edge (Fig. 1A). Etched onto the bottom of the containers were concentric circles with lines radiating from the center corresponding to 15, 45, 75, 105, 135, 165, 195, 225, 255, 285, 315, and 345°. This created 12 zones of orientation (sectors) around the center of the chamber. Sector 1 ranged from 345 to 15°, sector 2 ranged from 45 to 75°, and so on. Approximately 75 snails were placed in the center of the chamber filled with aged tap water. The chamber was covered with a large opaque plastic container that had a 3-cm-diameter hole in the side of the chamber at 1 end. The hole was 8 cm off of the ground when the container was placed over the chamber (Fig. 1B). A light with a 100-W incandescent light bulb was used to shine through the hole. The orientation of light was at 0° in the chamber. The direction of light was rotated at least 90° for each trial. The snails were allowed to disperse from the center of the chamber for 5 min. At this point, the plastic covering was removed, and the snails were separated using a

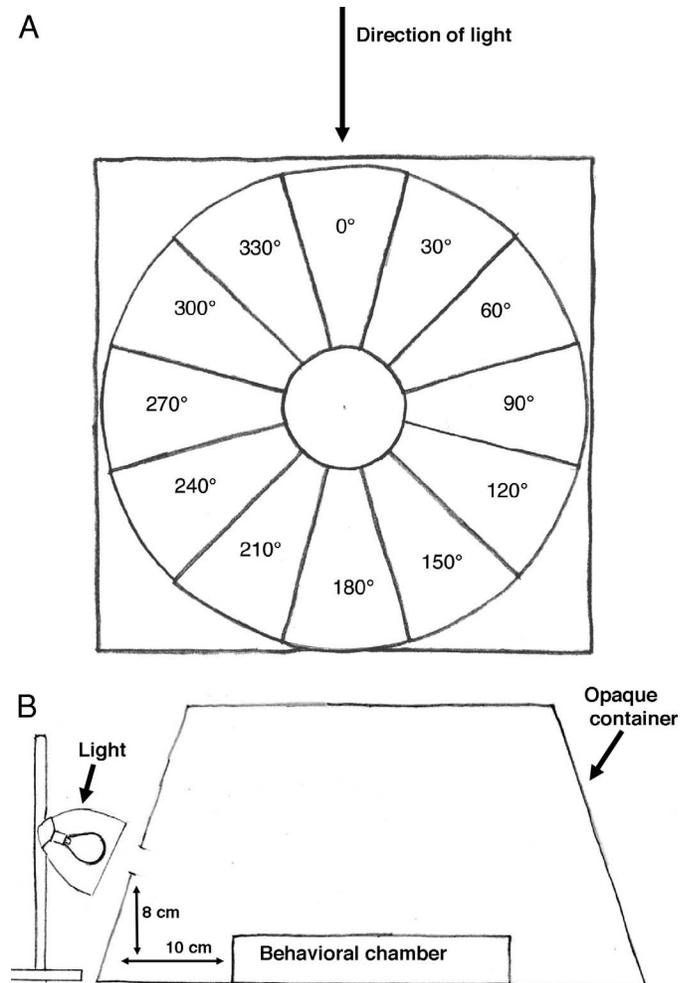


FIGURE 1. Behavioral chamber used in the directional movement in response to light experiment (A) and a side view of the experimental setup (B).

plastic divider that corresponded to the degree lines mentioned above. The snails were then separated according to which sector the snails were in (Fig. 1A). Any snails found in the 6-cm-diameter circle in the center were not included in the data. Trials were conducted between 0600 and 0800 hr and then again from 1000 and 1200. Eight trials were conducted during each time period. The water in the chamber was changed between each trial to avoid changes in temperature and effects of previous snails. In total, 914 snails were used in this experiment.

The data from within a snail class from different time periods were compared with a multisample chi-square test by using the Oriana statistical program (Kovach Computing Services, 2003). To test for a significant orientation, a Rayleigh's uniformity test was used to calculate the Rayleigh's Z-test statistic to test for a significant mean vector (Kovach Computing Services, 2003). A significance value of less than 0.05 indicates a nonuniform distribution of the data points. If a significant mean vector was found, a chi-square test was conducted to determine whether the snails oriented toward, or away from, light. This was done by dividing the behavioral chamber into 4 quadrats, 1 quadrat pointing directly at the light source (330, 0, and 30° sectors [Fig. 1]), another quadrat directly away, and 2 quadrats at right angles. If significantly more snails were found in the quadrat that contained the significant mean vector, then it was concluded that the snails oriented significantly in that quadrat's direction.

Distance traveled in response to light

The experiments to examine the effect of light on the distance the snails travel were conducted on 17 December 2002 on flat ground next

to Lake Alexandrina in full sunlight. A large, rectangular, 70-L opaque plastic tank was filled with approximately 65 L of lake water. In the tank was placed a 32- × 32-cm Plexiglas container with 5-cm sides. Two sets of experiments were run, 1 set from 0600 to 0800 hr and 1 set from 1000 to 1200 hr. Approximately 75 snails were placed in the center of a Plexiglas chamber. The snails then dispersed from the center over the next 7 min. Seven minutes was selected after preliminary trials revealed that it took the fastest snails approximately 7 min to travel to the edge of the chamber. A plastic ring that was 16 cm in diameter was placed over the center of the chamber. The ring separated snails that had moved >8 cm from the center from snails that moved <8 cm during the trial. Half of the trials were conducted in daylight; the other half were conducted by covering the container in opaque black plastic. Ten trials were conducted between 0600 and 0800 hr, and 11 trials were conducted from 1000 and 1200 hr. The water in the large tanks was periodically changed with water taken directly from the lake to avoid variation in temperature. In total, 5,087 snails were used in this experiment.

Hierarchical log-linear analyses were used to compare the ratios of snails within each class that moved more and less than 8 cm in the light and dark in both the early and late morning. A significant distance × light interaction indicated significant effect of light on distance traveled. The procedure also was used to compare different classes of snails within the same time period. In this case, a significant class × distance × light interaction indicated a significant difference in the response to light between the 2 groups. Log-linear analysis also was used to compare late and early morning behavior within classes of snails. Here, a significant time × light × distance interaction indicated significantly different responses at different times.

RESULTS

Vertical orientation

Of the 2,518 snails used in the experiment, 901 were found to be nonbrooding uninfected females, 228 were uninfected brooding females, 164 were uninfected males, 1,081 were infected by *Microphallus* sp. (926 females and 155 males), and 144 were infected by castrating trematode species other than *Microphallus* sp. All uninfected snail groups were found to be more likely to move downward when exposed to light. No differences were found between uninfected snail classes with regard to vertical orientation ($P > 0.49$ in all cases). Thus, uninfected classes were grouped together in Figure 2 and in subsequent analyses. No significant difference in orientation was found between *Microphallus* sp.-infected males and females ($P > 0.79$); thus, they were grouped together in subsequent analyses. The downward orientation in all snail groups was weak or absent when snails were not exposed to light at either time of day (Fig. 2). Thus, movement up or down seemed to be light dependent. Uninfected snails demonstrated more movement downward in the light than they did in the dark during both the early morning ($P < 0.001$) and late morning ($P < 0.001$). Unlike uninfected snails, *Microphallus* sp.-infected snails did not exhibit a strong directional response in the early morning (Fig. 2). Moreover, there was no difference between *Microphallus* sp.-infected snails in light or dark conditions in the early morning ($P = 0.88$). However, in the late morning, *Microphallus* sp.-infected snails did move up more in the dark than in the light ($P < 0.001$). *Microphallus* sp.-infected snails behaved significantly differently between the early and late morning trials ($\chi^2 = 14.68$, $P = 0.0001$ [Fig. 2]). Uninfected snails also behaved significantly differently between early and late morning trials ($\chi^2 = 22.69$, $P < 0.0001$ [Fig. 2]); however, as stated above, they moved up more in the dark than in the light during both times. Snails infected with parasites other than *Micro-*

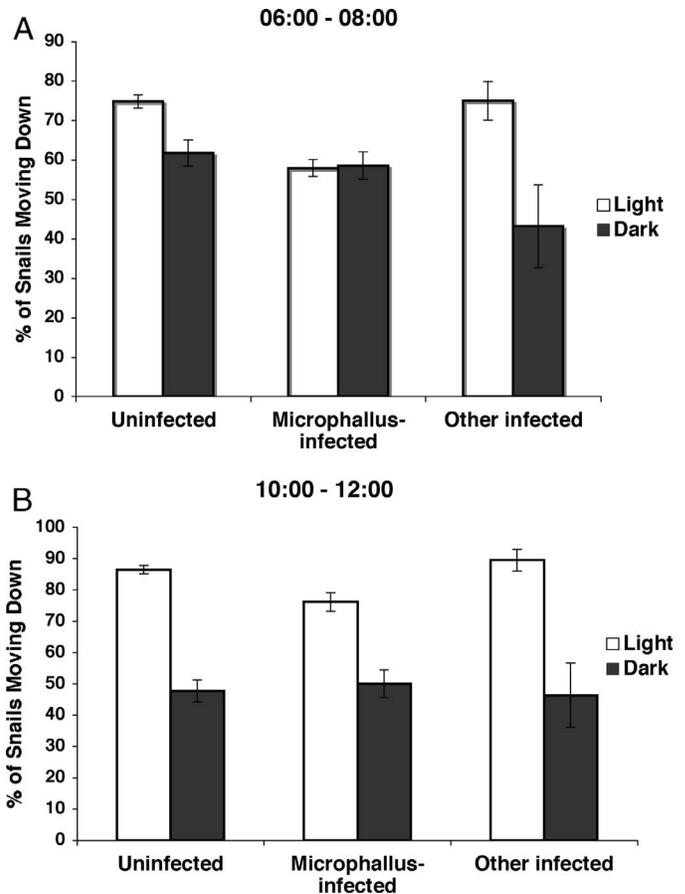


FIGURE 2. Percentage of snails moving downward in light and dark conditions for uninfected snails, *Microphallus* sp.-infected snails, and snails infected with parasites other than *Microphallus* sp. The results from trials conducted between 0600 and 0800 hr are shown in A, and the results from trials conducted between 1000 and 1200 hr are shown in B. Error bars are 95% confidence intervals.

phallus sp. did not show a difference in behavior between early and late morning trials ($\chi^2 = 1.02$, $P = 0.31$ [Fig. 2]). *Microphallus* sp.-infected snails behaved significantly differently from uninfected snails and snails infected with other castrating parasites in the early morning (Table I; Fig. 2). Snails infected with castrating parasites other than *Microphallus* sp. behave similar to uninfected snails during both the early morning and the late morning (Table I; Fig. 2).

Directional movement in response to light

Of the 914 snails used in this experiment, 497 were found to be nonbrooding uninfected females, 257 were uninfected brooding females, 122 were uninfected males, 21 were infected by *Microphallus* (19 females and 2 males), and 17 were infected by castrating trematode species other than *Microphallus*. Because only 2 *Microphallus* sp.-infected males were found, they were grouped with *Microphallus* sp.-infected females. However, the results did not change when *Microphallus* sp.-infected males were removed from the analyses. In the early morning, *Microphallus* sp.-infected snails showed a significant orientation toward light (Rayleigh $Z = 5.275$, $P = 0.003$). In the late morning, *Microphallus* sp.-infected snails did not sig-

TABLE I. Pairwise comparisons of each snail group in their vertical orientations for early morning and late morning trials by using log-linear analysis. Significant values are in bold. 1 df in each case.

Trial time	Chi-square	P
0600–0800 hr		
Uninfected vs. <i>Microphallus</i> sp.-infected	6.54	=0.011
Uninfected vs. other parasite-infected	1.85	=0.17
<i>Microphallus</i> sp.-infected vs. other parasite-infected	6.24	=0.013
1000–1200 hr		
Uninfected vs. <i>Microphallus</i> sp.-infected	5.15	=0.023
Uninfected vs. other parasite-infected	0.20	=0.66
<i>Microphallus</i> sp.-infected vs. other parasite-infected	2.12	=0.14

nificantly orient toward light (Rayleigh $Z = 1.67$, $P = 0.191$). However, the sample size was low here, and the trend was in the direction of light. Because of small sample sizes and a high number of zero values in the sectors of snails infected with parasites other than *Microphallus* sp., statistical comparisons with other classes or times could not be made. However, there did not seem to be an obvious difference between times on examination of the data. No snail groups, that had large enough sample sizes to be compared, oriented differently depending upon time of day ($P > 0.13$ in all cases). Thus, snail classes were grouped across times. All classes of snails, including *Microphallus* sp.-infected snails (when grouped across times), demonstrated significant movement toward light except for brooding females and snails infected with castrating parasites other than *Microphallus* sp., both of which did not significantly orient in any direction (Table II). Nonbrooding females, brooding females, and *Microphallus* sp.-infected snails all showed similar orientation vectors (Fig. 3), but in the case of uninfected brooding female snails, the orientation was not significant ($P = 0.49$).

Distance traveled in response to light

Of the 5,087 snails used in the experiments, 1,879 were found to be nonbrooding, uninfected females; 319 were uninfected brooding females; 230 were uninfected males; 2,456 were infected by *Microphallus* sp. (2,178 females and 278 males); and 203 were infected by castrating trematode species other than *Microphallus* sp. No snails in any of the trials seemed to orient in any particular direction. All uninfected snail classes tended to move farther in the light than in the dark in both the early morning and late morning ($P < 0.0005$ in all cases). *Microphallus* sp.-infected males and females did not ex-

hibit a significant difference in their movement ($P = 0.53$); thus, they were grouped together in subsequent analyses. *Microphallus* sp.-infected snails did not significantly move farther in the light than in the dark in the early morning ($P = 0.16$), but they did move farther in the light in the late morning ($P < 0.0005$; Fig. 4). Snails infected with castrating parasites other than *Microphallus* were similar to uninfected snails in that they moved farther in the light at both times ($P = 0.002$ in early morning and $P < 0.001$ in the late morning; Fig. 4). *Microphallus* sp.-infected snails were found to be significantly different from all other snail classes in the early morning but only different from nonbrooding females in the late morning (Table III; Fig. 4). Both *Microphallus* sp.-infected snails and nonbrooding females were found to behave differently between the early and late mornings (Table IV). Other classes showed no differences between early and late morning.

DISCUSSION

Vertical orientation

Typically, vertical orientation experiments measure geotactic behavior. However, in the way that these experiments were conducted geotaxis cannot be concluded due to confounding light variables. The goal of this experiment was not to test for geotaxis per se, but to look for any aspect of the snail's behavior that may be altered in a time-dependent manner. The results from the vertical orientation experiments showed that uninfected snails and snails infected with castrating parasites other than *Microphallus* sp. were much more likely to move downward in light than in the dark at all times of day. *Microphallus* sp.-infected snails, however, only exhibited a strong downward directional response in the late morning (Fig. 2). This response

TABLE II. Results of directional movement in response to light experiments for each group of snail. A significant P value (<0.05) for the Rayleigh test indicates a nonuniform distribution of data points and a significant vector of orientation. If the Rayleigh test was significant, a chi-square test was performed to determine whether the snails significantly oriented toward the direction of light. Data were grouped across times for each group, because no significant differences between times of day were found. Significant values are in bold.

Class	Rayleigh test (Z)	P	Mean vector ($^{\circ}$)	Chi-square	P
Nonbrooding females	24.63	<0.001	14.0	60.73	<0.001
Brooding females	0.72	=0.489	32.0		
Males	6.29	=0.002	331.6	19.46	<0.0001
<i>Microphallus</i> sp.-infected	4.24	=0.013	31.4	11.95	=0.008
Other-infected	2.35	=0.09	42.6		

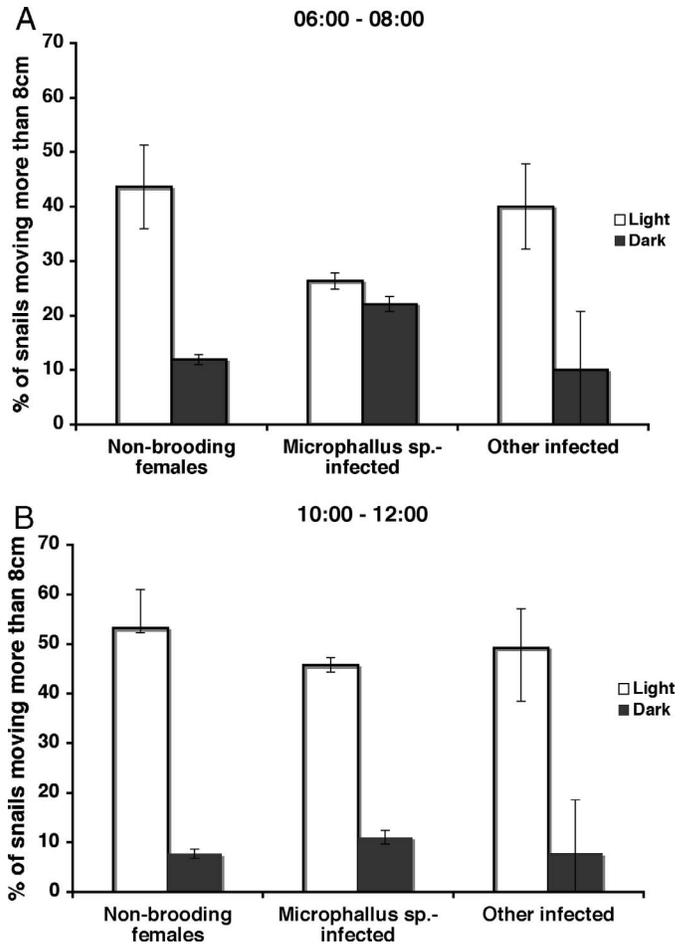
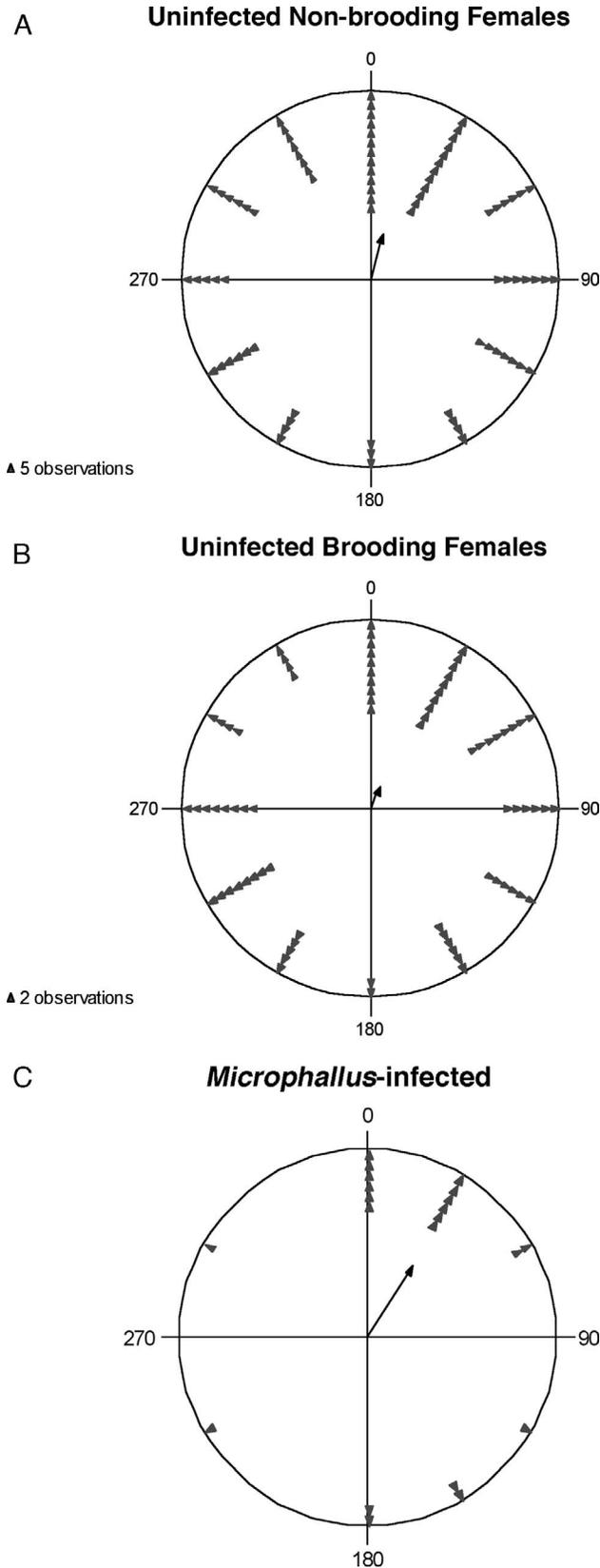


FIGURE 4. Proportion of snails moving a net distance of greater than 8 cm in 7 min for uninfected nonbrooding female snails, *Microphallus* sp.-infected snails, and snails infected with parasites other than *Microphallus* sp. in the early morning (A) and late morning (B). Error bars are 95% confidence intervals.

by *Microphallus* sp.-infected snails is consistent with the hypothesis that the parasite induces a behavioral change at approximately 0900 hr. In the early morning, the light-dependent downward movement in *Microphallus* sp.-infected snails seems to be “turned off.” The effect of this behavioral change should leave more of these snails exposed on top of rocks in the early morning when the final hosts, waterfowl, are feeding (Levri and Lively, 1996).

The light-dependent downward movement in *Microphallus* sp.-infected snails returned for the 1000–1200 hr trials (Fig. 2). This is consistent with results from the field, which show that *Microphallus* sp.-infected snails retreat to the bottom of the rocks at about 0900 hr when fish begin to feed (Levri, 1998b). Because fish cannot serve as the final host, and they are the

FIGURE 3. Orientation of snails with respect to the direction of light for uninfected non-brooding female snails (A), uninfected brooding females snails (B), and *Microphallus* sp.-infected snails (C). The arrow in each graph indicates the mean vector, and its length indicates the relative strength of the vector.

TABLE III. Pairwise comparisons of each snail group in their distance traveled in light and dark responses for early morning and late morning trials by using log-linear analysis. Significant values are in bold. 1 df in each case.

Trial time	Brooding females	Males	<i>Microphallus</i> -infected	Other-infected
0600–0800 hr				
Nonbrooding females	$\chi^2 = 0.26, P = 0.61$	$\chi^2 = 0.27, P = 0.60$	$\chi^2 = \mathbf{46.7}, P < \mathbf{0.0001}$	$\chi^2 = 0.01, P = 0.91$
Brooding females		$\chi^2 = 0.08, P = 0.78$	$\chi^2 = \mathbf{6.84}, P = \mathbf{0.009}$	$\chi^2 = 0.14, P = 0.71$
Males			$\chi^2 = \mathbf{7.50}, P = \mathbf{0.006}$	$\chi^2 = 0.11, P = 0.74$
<i>Microphallus</i> sp.-infected				$\chi^2 = \mathbf{6.34}, P = \mathbf{0.012}$
1000–1200 hr				
Nonbrooding females	$\chi^2 = 0.82, P = 0.36$	$\chi^2 = 0.39, P = 0.53$	$\chi^2 = \mathbf{8.52}, P = \mathbf{0.004}$	$\chi^2 = 0.10, P = 0.75$
Brooding females		$\chi^2 = 0.16, P = 0.69$	$\chi^2 = 0.47, P = 0.49$	$\chi^2 = 0.14, P = 0.71$
Males			$\chi^2 = 0.62, P = 0.43$	$\chi^2 = 0.05, P = 0.83$
<i>Microphallus</i> sp.-infected				$\chi^2 = 0.82, P = 0.36$

most abundant and predominant predator in the lake (data not shown), it would seem that the return of the downward movement may increase the fitness of the parasite by decreasing the probability of predation by animals that cannot serve as final hosts (fishes). Snails infected by parasites other than *Microphallus* behaved similar to uninfected snails (Fig. 2). This is consistent with the idea that the parasite-induced behavioral change is *Microphallus* sp.-specific.

In the current experiment, uninfected snails exhibited a stronger directional response in the late morning than in the early morning (Fig. 2). This trend is consistent with the idea that the behavior is influenced by the intensity of sunlight, which increases through the morning.

It is not clear whether the vertical orientation of the snails is completely absent in the dark. The late morning trials showed that roughly 50% of the snails moved up, whereas that percentage was lower in the early morning (Fig. 2). We have no explanation for this pattern.

Directional movement in response to light

We found that all snail classes with reasonable sample sizes, except for brooding females, moved significantly toward light (Table II; Fig. 3). *Microphallus* sp.-infected snails significantly oriented toward light in the early morning, but showed no significant orientation toward light in the late morning. This is consistent with the hypothesized shift in behavior at around 0900 hr, but the sample sizes of infected snails in the experiment were low; thus, the lack of significant orientation in the late morning by *Microphallus* sp.-infected snails may be an artifact of low sample size. Also, the late morning trials, even though not significant, resulted in a mean vector directed to-

ward light. Therefore, we are reluctant to conclude that *Microphallus* sp. induces a change in this behavior at around 0900 hr. There were no differences in orientation of any other snail group between early and late morning trials. In the vertical orientation and distance traveled in response to light experiments, the effect of the parasite was to eliminate the response in the early morning. This did not occur with directional movement in response to light. Thus, the parasite does not seem to affect the directional movement response of the snail. It is also possible that change in behavior around 0900 hr was not detected in *Microphallus* sp.-infected snails because these experiments were conducted under laboratory conditions, and a crucial environmental signal was absent.

Distance traveled in response to light

Influencing the distance traveled in response to light in the snail by *Microphallus* sp. could lead to increased exposure to predators by infected snails. Snails that move a greater distance in the light than in the dark would spend more time in dark, less exposed areas, keeping them at less risk of predation by visual predators. The results for these experiments were similar to the vertical orientation experiments. Snails generally moved a greater distance in the light than in the dark. *Microphallus* sp. seemed to “turn off” the response of the snails in the early morning when waterfowl feed and the chance of successful transmission is greater. By “turning off” this response, the snails may spend more time in exposed areas than they would otherwise. The greater distance moved in light returns in the late morning when predatory fishes become active (Fig. 4). Snails infected by parasites other than *Microphallus* sp. behaved similarly to uninfected snails (Fig. 4). This is consistent with the idea that the 0900-hr change in behavior is *Microphallus* sp.-specific.

The data also showed that *Microphallus* sp.-infected snails behaved significantly different from nonbrooding females in the late morning (Table III). This difference was detectable due to the very large sample sizes of both uninfected nonbrooding females and *Microphallus* sp.-infected snails. However, both groups were significantly more likely to move a greater distance in the light in the late morning (Fig. 4).

Nonbrooding females were significantly more likely to move a greater distance in the light in the late morning compared with the early morning. This is likely to due to the greater light

TABLE IV. Comparisons of the distance traveled in light and dark of each snail class in the early morning to the late morning by using log-linear analysis. Significant values are in bold. 1 df in each case.

Class of snail	Chi square	P
Nonbrooding females	12.51	=0.0004
Brooding females	1.16	=0.28
Males	1.37	=0.24
<i>Microphallus</i> sp.-infected	51.51	<0.0001
Other infected	0.53	=0.47

intensity in the late morning and that the larger sample size of nonbrooding females compared with other uninfected classes.

The experiments presented here are similar to those described by Levri and Fisher (2000). The results of Levri and Fisher (2000) differed from the present results in that, in the previous experiments (1) *Microphallus*-infected snails exhibited no change in vertical orientation behavior at around 0900 hr, and there was no effect of light on the response; (2) there was no evidence for phototaxis in infected or uninfected snails; and (3) no difference in response with time of day was found in infected snails in distance moved in response to light. These differences are most likely due to the experiments performed by Levri and Fisher (2000) being conducted in the laboratory under artificial light in the United States, whereas the present results were obtained from field trials in New Zealand, with a larger sample size and under natural light where possible.

The stimulus causing the parasite-induced change in behavior is not known. The parasite-induced changes in behavior occurring at around 0900 hr could be stimulated by (1) a change in light intensity over the course of the morning; (2) a change in the angle of light; or (3) a time-specific circadian response by the parasite. Light intensity seems to be an unlikely stimulant for the parasite because the 0900-hr shift in behavior observed in Levri and Lively (1996) was observed at 0900 hr on several different sampling days. Each of these days had different ambient light intensities, including cloudy days, where light intensities were an order of magnitude lower than on sunny days (data not shown). Future experiments will attempt to distinguish among the aforementioned hypotheses.

In summary, *Microphallus* sp. seems to eliminate the light-dependent distance traveled response and up or down directional response of the snail in the early morning and restores the behaviors in the late morning. This pattern is consistent with the foraging patterns of final host (waterfowl) and nonfinal host predators (fishes) in Lake Alexandrina; thus, it strengthens the conclusion of adaptive parasite-induced behavioral change in this system. This study also demonstrates the importance of field experiments as opposed to laboratory experiments in determining the nature of complex ecological interactions.

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