Parasite-induced change in host behavior of a freshwater snail: parasitic manipulation or byproduct of infection?

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Host behavioral changes due to parasitism are often assumed to be adaptations of the parasite. However, behavioral effects of parasites may be a generalized response to parasitism and only coincidentally beneficial for parasite transmission. For this reason, alternatives to the manipulation hypothesis should be tested. Previous work demonstrated that the trematode parasite *Microphallus* sp. influences the behavior of the snail *Potamopyrgus antipodarum* in a way that may increase the probability of transmission. Here I report work conducted to test alternatives to the manipulation hypothesis. In a field study, the effect of *Microphallus* on behavior was compared to that of two other castrating parasite groups to determine if the behavioral change is simply a byproduct of parasitism. Also, the foraging behaviors of infected and uninfected snails were examined in the presence and absence of food resources to determine if the hunger level of *Microphallus*-infected snails could account for the parasite-induced behavioral change. First, *Microphallus*-infected snails were found on top of rocks during the day less often than the two other parasite groups. This evidence suggests that the behavioral change caused by *Microphallus* is specific to *Microphallus*-infected snails. Second, *Microphallus*-infected snails responded to the lack of food differently from uninfected snails. Uninfected snails retreated to safer positions under rocks when the food source was removed from the top of the rocks, while *Microphallus*-infected snails remained on top of the rocks where the risk of consumption by the final host is greater. Taken together with previous studies, these results suggest that infection by *Microphallus* results in behavior that enhances parasite transmission. *Key words:* foraging, host behavior, *Microphallus*, parasitism, *Potamopyrgus antipodarum*, snails, trematodes. *[Behav Ecol 10:234–241 (1999)]*

Parasites often have striking effects on the behavior of their hosts (Dobson, 1988; Holmes and Bethel, 1972; Moore and Gotelli, 1990). These behavioral changes can be classed as adaptations on the part of the host, on the part of the parasite, or as nonadaptive byproducts of infection. In many cases it seems that these changes in behavior are adaptive for the parasite because they appear to facilitate transmission of the parasite to the next host in the parasite's life cycle. However, caution must be used in inferring that adaptive behavioral manipulation is occurring because simple byproducts of infection can have transmission-enhancing effects (Holmes and Zohar, 1990; Moore and Gotelli, 1990). In fact, in instances where host behaviors are altered, parasites that appear to benefit from behavioral changes often have less dramatic effects on host behavior than parasites that do not appear to benefit from altered behaviors (Poulin, 1994).

In cases where parasitic manipulation is likely, the manipulation hypothesis can be strengthened in one of two general ways: supporting predictions of the manipulation hypothesis, and rejecting alternatives to the manipulation hypothesis. Evidence supporting the manipulation hypothesis often includes demonstrations of increased overlap in time and/or space between infected intermediate hosts and the next host in the life cycle as a result of the behavioral change (Dobson, 1988; Holmes and Bethel, 1972; Moore and Gotelli, 1990). Furthermore, the onset of transmission-enhancing behavioral changes should occur only when parasite is transmissible—i.e., mature enough for transmission to occur (Bethel and Holmes, 1974; Hurd and Fogo, 1991; Levri and Lively, 1996; Poulin et al., 1992; Tierney et al., 1993).

Rejecting alternatives to the manipulation hypothesis seems to be less common (Moore and Gotelli, 1990). This may be because it is often difficult to predict exactly what the behavioral byproducts of infection should be. One possible way to assess the effects of byproducts of infection is to examine the effects of different parasites on the behavior of infected hosts, especially parasites whose transmission is not necessarily enhanced by altered host behavior. The manipulation hypothesis would likely have different predictions for parasites with different life cycles. However, few studies have used this approach (but see Bethel and Holmes, 1973; Moore and Lasswell, 1986).

In a previous study, the trematode parasite *Microphallus* sp. was found to influence the behavior of its intermediate host, the snail *Potamopyrgus antipodarum* (Levri and Lively, 1996). *P. antipodarum* is a small prosobranch snail found in New Zealand lakes and streams (Talbot and Ward, 1987). It feeds predominantly on green algae, diatoms, and detritus (Haynes and Taylor, 1984; Winterbourn, 1970; Winterbourn and Fegley, 1989), and in areas with rocky substrates it forages on the tops of rocks, predominantly at night (Levri and Lively, 1996). Fish (Jellyman, 1989; Levri, 1998; McCarter, 1986; McDowall, 1990) and waterfowl are common predators of the snail.

The predominant parasite of *P. antipodarum* is an undescribed species of *Microphallus* (Trematoda: Microphallidae; Lively, 1987). Mature *Microphallus* produce eggs in waterfowl, which pass out of the bird with the feces. The eggs ingested by *P. antipodarum* develop and encyst within the snail, castrating the snail as the parasite larvae develop. The cysts hatch upon ingestion by waterfowl, where they mature in the bird's intestine to complete the life cycle. *P. antipodarum* is also the first intermediate host to at least a dozen other castrating trematode parasites (Winterbourn, 1973) that occur at low frequencies (Lively, 1987; Jokela and Lively, 1995). Any change in behavior of the snails due to *Microphallus* infection is unlikely to be adaptive for the host due to the irreversible castration caused by the parasite.

Microphallus-infected snails foraged on top of rocks in a similar frequency to nonbrooding females in the early morning and foraged similarly to brooding females for the remainder of the day (Levri and Lively, 1996). Nonbrooding females were found on top of the rocks more than any other class of snail throughout the day and thus were likely to be at the greatest risk of predation. Brooding females were on the bottom of the rocks more than any other class of snail. Hence, *Microphallus*-infected snails exposed themselves to predation in the early morning and moved to a safer environment in the late morning and afternoon. The final hosts of *Microphallus* (waterfowl) were found to feed most often in the early morning (Levri and Lively, 1996), and other predators (fish) that cannot serve as a host for the parasite were found to feed more in the late morning and afternoon (Levri, 1998). Thus, *Microphallus* seems to induce foraging behavior that increases the probability of the snail being eaten when the final host is feeding and decrease the probability of being eaten when other predators are active. Also, only transmissible *Microphallus* (those parasites that are mature enough for transmission to occur) induce the transmission-enhancing behavior in the early morning (Levri and Lively, 1996). Nontransmissible *Microphallus*-infected snails were more likely to be found on the bottom of the rocks.

These results support the manipulation hypothesis, but it is still possible that the effect of *Microphallus* could simply be a byproduct of parasitism. I consider a byproduct of infection to be nonadaptive, and it may result from damage caused by the parasite or from the host response to infection. I tested two alternatives to the manipulation hypothesis in this system. First, I compared the behavior of snails infected by *Microphallus* to snails infected with two other independent groups of castrating trematode parasites. If the daily foraging pattern of *Microphallus*-infected snails is simply a byproduct of infection, then snails infected with other parasites are likely to forage in a similar way. In stating the above, I am explicitly assuming that behavioral byproducts of infection are similar from one species of parasite to another in the same host. In this case, each parasite assessed is a castrating digenetic trematode, but it is possible that the behavioral effects of different parasites (or genotypes of the same parasite) may vary without regard to behavioral manipulation.

Second, I examined the effect of food availability on the foraging behavior of the snail. The snails face a trade-off while foraging. Being on top of the rocks allows them to feed on algae, but it also makes them more vulnerable to avian and piscine predators. I conducted several experiments in which I removed the benefit (food) of being on top of the rocks in one treatment. Uninfected snails were predicted to move to the bottom of the rocks when food was not present; infected snails were predicted to remain on the top of rocks in the early morning when waterfowl feed most intensively regardless of the presence of food. Later in the day, when waterfowl foraging declines and fish foraging increases, *Microphallus*-infected snails were expected to retreat to the bottom of the rocks if food is no longer present.

METHODS

The effect of non-Microphallid parasites on foraging behavior

I undertook a survey to determine the effects of size, gender, brooding condition, and parasitism on the foraging behavior of *P. antipodarum*. The data were collected as described in Levri and Lively (1996) from shallow, rocky areas (approximately 0.5 m deep) on the margin of Lake Alexandrina on the South Island of New Zealand (170°27′ E, 43°56′ S). Lake

Alexandrina is a clear-water, mesotrophic lake where *P. antipodarum* is the most abundant mollusk species. I took a total of 27 samples in, 1993,, 1994, and, 1995. In, 1993, I collected snails nine times over a 24-h period beginning on 10 February at 1530 h. In, 1994, snails were sampled 10 times over a 24-h period beginning on 23 January at 0800 h. Five smaller samples were also taken on 25 January (1000 h), 5 February (1500 h), 6 February (0600, 0900, and 1500 h), and 27 February (0600 and 0900 h). In 1995 a sample was collected at 1000 h on 9 January. Each sample from each year was collected from the same site on the lake. During each collection, I selected at least eight rocks and carefully lifted them to the surface to minimize the number of snails that became detached. I separated the snails based on whether they were collected from the top or bottom of the rocks (I excluded snails judged to be on the sides of rocks) and preserved them in 70% ethanol. I recorded the size (distance from the apex to the basal lip of the shell), sex, brooding status, infection status, and parasite type of all collected snails. I measured snail size (length) using an ocular micrometer. Males can be distinguished from females by the presence of a verge behind the right tentacle at sizes >2.1 mm. All snails <2.1 mm in length were labeled juveniles. Parasites were identified using the descriptions provided by Winterbourn (1973).

I sampled 12,411 snails during the study. When analyzing the data, I separated the snails into seven mutually exclusive classes based on their reproductive status and whether or not they were infected: (1) uninfected, adult, nonbrooding females, (2) uninfected adult males, (3) uninfected brooding females, (4) juveniles, (5) *Microphallus*-infected individuals, (6) snails infected by externally encysting metacercariae, and (7) snails infected by unencysted cercariae. *Microphallus*-infected males were grouped together with *Microphallus*-infected females because there was no difference between the two groups in their behavior patterns found in a previous study (Levri and Lively, 1996). Infected juveniles and brooding females are rare (<0.1% of each class), and when encountered, they were classified as parasitized (classes 5, 6, or 7). Only snails infected with encysted (transmissible) *Microphallus* metacercariae were counted as *Microphallus* infected. If the snail was infected with an earlier stage of the parasite or if the snail was infected by multiple parasites (both of these occurrences were rare), it was not counted in this study.

Classes 6 and 7 include multiple species of trematode parasites. This grouping was necessary because of the rarity of each parasite species. Each of the classes of parasites have different life cycles. Class 6 includes what appear to be two or three undescribed species of monostome cercariae with similar life cycles (Winterbourn, 1973). These monostome species are similar to each other in that they castrate the snail, mature as swimming cercariae, and then swim out of the snail and encyst on the shell and operculum of the snail as well as other nearby surfaces. *Microphallus* never passes through the swimming cercariae stage and encysts internally within the snail. Thus, like *Microphallus,* externally encysting metacercariae are transmitted trophically, through the food chain, to their final host (probably waterfowl). Hence, if manipulation is occurring with these parasites, it is expected that they will exhibit behavior that increases the probability of the infected snails being eaten by waterfowl. I hereafter refer to all of the snails infected by trophically transmitted parasites besides *Microphallus* as externally encysting metacercariae infected. The other parasite group (unencysted cercariae) include six species that castrate and mature as cercariae that swim to the next host in the life cycle (Winterbourn, 1974); i.e., they are not transmitted trophically. Thus, host manipulation, if it occurs, by these parasites would be expected to have different behavioral effects from those caused by trophically transmitted par-

Table 1

Description of differences between the four separate experiments to test the effect of food availability on the behavior of infected and uninfected snails

Experiment	Design	Snails sampled from	No. of tanks	No. of snails added to each tank	Date(s) sampled	Time(s) sampled (h)
1	Each treatment in separate tanks	Top and bottom of rocks	6	300	11 Jan 1995	0700–0900
2	Each tank contains both treatments	Top and bottom of rocks	4	1000	12 Feb 1995	0630–0830
3	Each treatment in separate tanks	Top and bottom of rocks	8	1500	21 Jan 1996	0630–0900
4	Each tank contains both treatments	Top of rocks only	8	500	29–30 Jan 1995	0500–0600, 0700–0800, 1200–1300, 2000–2100

asites. This nontrophically transmitted group includes the *Telogaster opisthorchis,* two undescribed species of furcocercariae, one undescribed species of xiphidiocercariae, and two undescribed *Gymnocephalous* spp. cercariae (Winterbourn, 1973).

I made comparisons between groups by calculating the percentage of each group on top of the rocks over the course of the sampling period. I assume that snails are foraging while on tops of rocks because algal food sources are abundant there. I analyzed the data using SAS software (SAS Institute, 1990) using a modified multiple logistic regression procedure described in Levri and Lively (1996) to determine whether the daily foraging patterns differed between the snail classes. The logistic regression was modified by using sine and cosine functions to account for the daily cycle. The resulting regression curve, therefore, results in sine waves rather than the typical logistic regression sigmoidal functions. The analysis was performed identically to that in Levri and Lively (1996), except that day of sampling was not used in the analysis. This was done to increase sample sizes in the rare parasite-infected classes. Thus data were combined across day and year. I used the variables time of day and snail class to estimate the probability of finding a snail on top of the rocks. A Bonferroni correction was used to adjust the alpha level for the number of tests.

The effect of food availability on foraging behavior

I conducted four separate experiments to determine the effect of food availability on behavior. The first three were nearly identical. The differences between the experiments are presented in Table 1. In each experiment, snails were collected using a dip net from Lake Alexandrina. The snails were taken from the shore bank habitat of the lake, where they could easily be collected. The snails from this subpopulation of the lake yielded infection rates of *Microphallus* from 4% to 9% from various collections (Levri, unpublished data). I set up 40-l opaque plastic tanks along the lake shore and filled them with lake water. In half of the tanks used in two of the experiments, enough rocks from the lake were placed in the tanks (food tanks) to cover the entire bottom of the tanks. The snails fed upon algae that coated the tops of the rocks. The remaining tanks (nonfood) contained rocks that were found above the water level of the lake (no algae had been growing on them). Thus there were two treatments: the snails were exposed to food or nonfood rocks. The tanks were arranged by alternating treatments.

In the other two experiments both treatments were represented in each tank with each treatment occupying half of the tank space (Table 1). The number of snails added to each tank varied depending on the experiment (Table 1). I controlled infection rate by mixing the snails together before adding them to the tanks and by adding approximately the same number of snails to each tank. Shade cloth was put over the tanks to minimize the temperature increase during the daylight hours. The snails were allowed to acclimate for 3 days. Snails were sampled from the rocks at the times indicated in Table 1, and they were separated by whether they were found on the top or bottom of the rocks. I continued the sampling until all the snails were removed from the top and bottom of the rocks (approximately 15–25 min per tank). In experiment 4, only snails from the tops of the rocks were sampled. This was done to speed up the sampling so that samples could be taken at several times over the course of the day. The snails were taken back to the laboratory and dissected in the manner described previously. The mature snails were separated into one of four mutually exclusive classes: (1) uninfected, nonbrooding females, (2) uninfected, brooding females, (3) uninfected males, and (4) *Microphallus*-infected snails infected with transmissible parasites [some snails were infected by nontransmissible (immature) *Microphallus,* but too few were found for analysis].

I analyzed the data for the first three experiments using chi-square tests. Pairwise comparisons were made by pooling data across replicates within treatment for each class of snail. The data for the fourth experiment were analyzed using a logistic regression analysis. Once again, to increase sample sizes, the data were pooled across replicates and grouped by whether the snails were collected in the morning (0500 and 0700 h), afternoon (1200 h), or evening (2000 h). The dependent variable in the analysis was the ratio of the number of snails of each class found on top of the nonfood rocks to the number of snails of each class found on top of the food rocks. The independent variables in the analysis were time of day (morning or afternoon) and class of snail. I chose nonbrooding females as the reference uninfected class because they were found to be similar to *Microphallus*-infected snails in the early morning in examining the effect of infection in behavior (present study) and in a previous study (Levri and Lively, 1996). The morning sample was compared to the afternoon and then the evening sample. A third comparison was made by analyzing the morning sample versus the pooled afternoon and evening samples. I did this to increase the sample sizes in the *Microphallus*-infected classes in the afternoon + evening sample. Afternoon and evening samples were pooled only after I determined that there was no significant difference between the two samples in sample size or ratio of snails on food to nonfood for either class (nonbrooding females and *Microphallus*-infected snails).

Table 2

Pairwise comparisons of the daily behavior patterns all snail classes using multiple logistic regression analysis (*p* values shown)

Class	Brooding females	Males	Juveniles	*Microphallus* infected	Unencysted cercariae infected	Externally encysting metacercariae infected
Nonbrooding females	<.001	<.001	<.001	<.001	.03	.01
Brooding females		<.001	<.001	<.001	<.001	<.001
Males			<.001	<.001	<.001	<.001
Juveniles				<.001	<.001	<.001
Microphallus infected					<.001	<.001
Unencysted cercariae infected						.56

A significant *p* value indicates a significant difference in the intercept, ''slope'' of the curve, or both. A Bonferroni correction sets the 5% risk level at 0.002.

RESULTS

The effect of non-Microphallid parasites on foraging behavior

Of the 12,412 snails collected in the study, 3699 were non-brooding females, 2993 were brooding females, 2209 were males, 1669 were juveniles, 1428 were *Microphallus*-infected snails, 265 were snails infected by unencysted cercariae, and 149 were infected by externally encysting metacercariae. Nine different castrating parasite species were identified from the collections.

All snail classes were more likely to be found on top of the rocks at night than during the day. *Microphallus*-infected snails foraged significantly different from any other snail group, including the other two infected classes (Table 2). The resulting logistic regression curves are presented graphically in Figure 1. Unencysted-cercariae–infected and externally encysting-metacercariae–infected snails did not forage significantly different from each other (hence they were grouped together in Figure 1) or each from nonbrooding females (al-

though the difference is marginally significant; see Table 2; a Bonferroni correction set the critical alpha level to 0.002). These three groups foraged on top of rocks more than other classes in the late morning and early afternoon (Figure 1). The patterns shown by uninfected adult males and juveniles were similar to brooding females, except that males and juveniles foraged more frequently during the day.

The effect of food availability on foraging behavior

The first three experiments yielded similar results to each other (Figure 2). In each experiment, uninfected, nonbrooding females moved to the bottom of the rocks significantly more in the nonfood treatment than did nonbrooding females in the food treatment. *Microphallus*-infected snails did not exhibit significant movement to the bottom of the rocks in the nonfood treatment compared to the food treatment, except in experiment 3. Pairwise comparisons using chi-square analysis of *Microphallus*-infected snails and nonbrooding females within each treatment are indicated in Figure 2. A Bonferroni
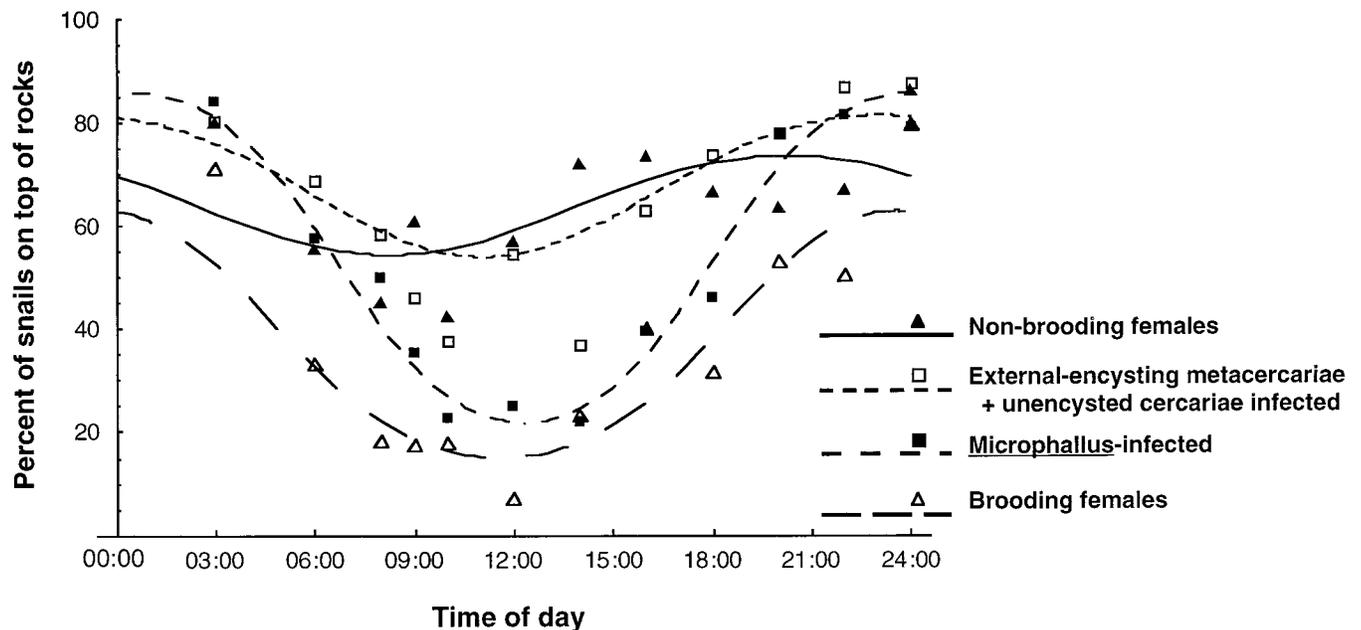

Figure 1
Behavior patterns of infected and uninfected snails predicted from modified multiple logistic regression analysis over time. *Microphallus*-infected snails were significantly different from all other classes, including the two other infected classes (see Table 2). Externally encysted-metacercariae–infected and unencysted-cercariae–infected snails did not behave significantly different from each other (hence they are grouped in the graph) or from nonbrooding females.
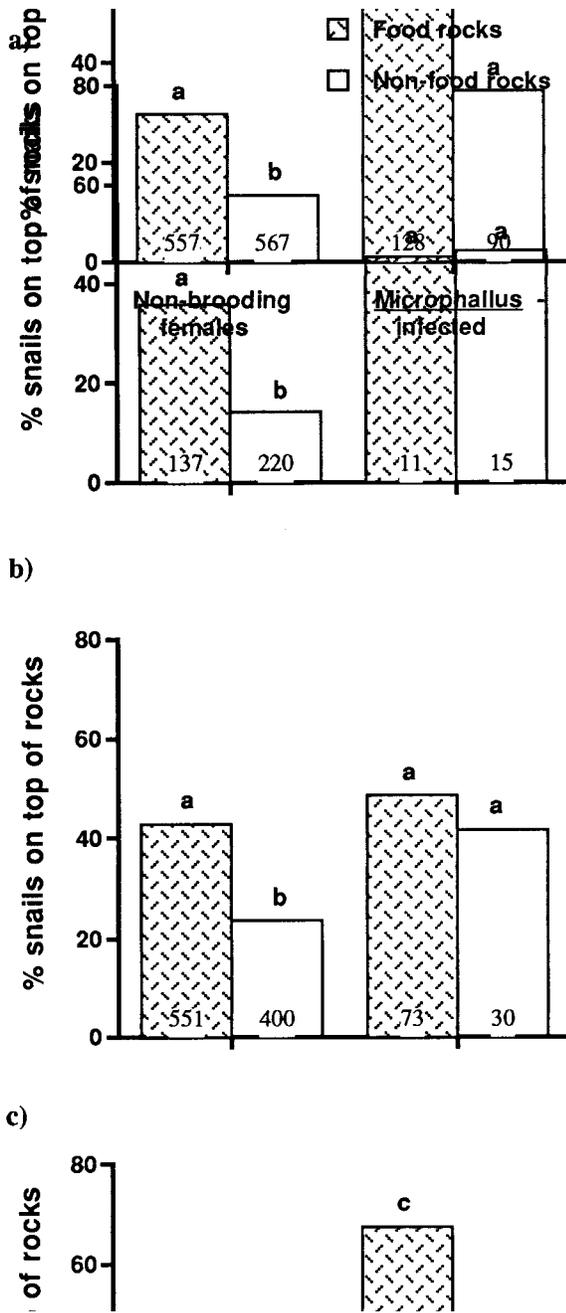
b)

c)

Figure 2
Results from three separate experiments—(a) experiment 1, (b) experiment 2, and (c) experiment 3—testing the effect of food removal on behavior. In each case uninfected, nonbrooding females moved to the bottom of the rocks when food on tops of the rocks were not available. In two out of the three experiments (a and b),

correction set the 5% risk level at 0.008 within each experiment.

The data for experiment 4 are given in Figure 3. Significantly fewer snails were found on top of the rocks in the nonfood treatment, except for *Microphallus*-infected snails in the morning when compared to the afternoon. There was a significant class by time-of-day interaction (morning versus afternoon) on the proportion of snails found on top of the rocks from the food versus nonfood portions of the tank (Table 3). A significant class by time-of-day interaction was also found in the comparison between the morning and afternoon + evening samples (pooled) (Table 3). A significant class by time-of-day interaction was not found when comparing the morning and evening samples.

DISCUSSION

The results from this study address two alternative hypotheses to the manipulation hypothesis in the *Potamopyrgus–Microphallus* system. First, the behavioral effects of *Microphallus* on *Potamopyrgus* are unlikely to be exclusively a simple byproduct of parasitism because other castrating parasites do not induce the same foraging behavior. Second, the foraging behavior of *Microphallus*-infected individuals does not seem to be driven simply by hunger.

The effect of non-Microphallid parasites

Microphallus-infected snails behaved significantly different from snails infected by two other trematode groups (Table 2). Moreover, behavior of snails infected by the two other parasite groups were not significantly different from each other. These results suggest that effects on foraging behavior induced by *Microphallus* are specific to *Microphallus*. Effects on foraging behavior that are simply byproducts of parasitism are likely to be demonstrated by the unencysted-cercariae–infected and externally encysting-metacercariae–infected snails in this study because the two groups foraged similarly despite being infected with parasites with different life cycles. In this case, the unencysted-cercariae–infected and externally encysting-metacercariae–infected snails foraged on top of the rocks, similar to nonbrooding females (Figure 1), although *Microphallus*-infected snails were more likely to be on the bottom of the rocks, compared to the other infected groups, especially in the middle of the day.

Several studies have demonstrated different effects on behavior of multiple species of parasites on the same host (Bethel and Holmes, 1973; Curtis, 1985; Moore and Lasswell, 1986). Moore and Lasswell (1986) found that the nematode *Dispharnax nasuta* induced different behaviors in the isopod *Armadillidium vulgare* than in the acanthocephalan *Plagiorhyncus cylindraceus*. They suggested that this difference indicated that the change in behavior due to at least one of the parasites could not simply be a generalized response to infection.

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Microphallus-infected snails did not move to the bottom of the rocks when food was not available. If the letters above two bars are the same, then a significant difference between the two groups was not found in a chi-square analysis. If the letters are different within the same graph, a significant difference was found. In all cases where a difference was found, the level of significance was <.001, except in graph a between *Microphallus*-infected snails on food rocks and nonbrooding females on nonfood rocks (p = .005), and in graph b between nonbrooding females on nonfood rocks and *Microphallus*-infected snails on nonfood rocks (p = .018; marginally significant using a Bonferroni correction). The numbers at the bases of the bars represent the number of snails in each sample.
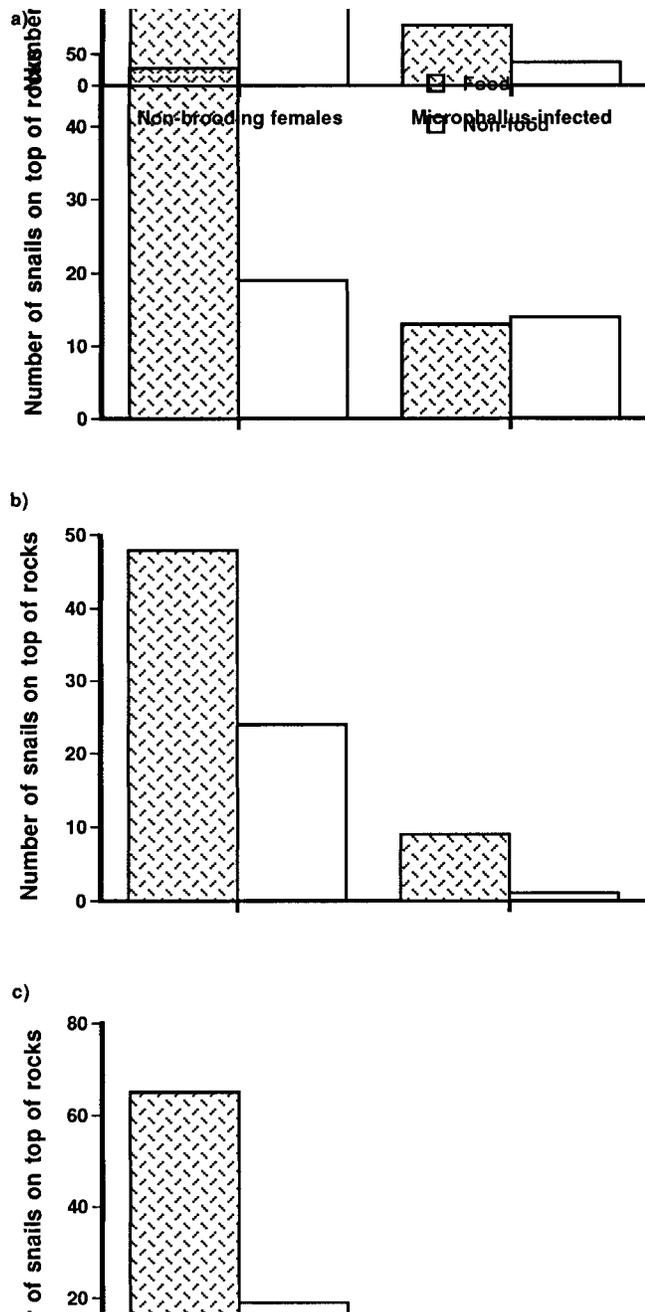
Figure 3
Results from experiment testing the effect of food removal on behavior over time. In the (a) morning, (b) afternoon, and (c) evening nonbrooding females were more likely to be found on top of the rocks in the food treatment than in the nonfood treatment. However, *Microphallus*-infected snails were just as likely to be found on top of the rocks in the food treatment as the nonfood treatment in the early morning (a), but behaved similarly to uninfected snails in the afternoon (b) and evening (c) (see Table 3). This suggests that the effect of *Microphallus* on behavior is time specific.

Table 3

Output from logistic regression analysis on the probability of snails being found on top of rocks from the food treatment or from the nonfood treatment

Variable	Likelihood ratio	df	*p*
Morning vs. afternoon			
Class	0.17	1	.67
Time	2.97	1	.08
Class*time	4.49	1	.03
Constant	10.04	1	<.01
Morning vs. evening			
Class	2.87	1	.09
Time	3.13	1	.08
Class*time	0.93	1	.33
Constant	16.29	1	<.01
Morning vs. afternoon + evening			
Class	0.45	1	.50
Time	5.23	1	.02
Class*time	3.82	1	.05
Constant	7.96	1	<.01

Unencysted-cercariae–infected and externally encysted-metacercariae–infected snails did not forage significantly different from nonbrooding females, although in both comparisons the results are close to being significant (Table 2). In any case, the foraging patterns of nonbrooding females, unencysted-cercariae–infected snails, and externally encysted-metacercariae–infected snails seem to parallel each other (Figure 1). This similarity may indicate that the general behavioral effect of parasitism is to cause individuals to forage similarly to nonbrooding females. This is perhaps not surprising because nonbrooding females feed more than any other class of snail (Levri and Lively, 1996), and infection is likely to be energetically costly to the host (Holmes and Zohar, 1990). Thus by feeding more, the snail (and the parasite) may survive longer.

It is possible that the behavior patterns of hosts infected with the two other parasite groups are simply a compilation of specific behavioral responses to the different parasite species. If this were true, the grouped results would mean little. When the results are examined for each species independently, they are similar to the patterns generated by the groups of species. The analysis could not be run on individual species because the sample sizes for each species were very small. In the externally encysting metacercariae, though, there are few species (two to three), and they are likely to be closely related based on morphological and life-cycle similarities.

In a previous study, the behavior induced by *Microphallus* was dependent on the transmissibility of the infection (Levri and Lively, 1996). Only snails infected by transmissible *Microphallus* (those parasites that were mature enough for transmission to occur) foraged on top of the rocks in the early morning. Snails with nontransmissible infections were more likely to be found on the bottom of the rocks throughout the daylight hours. If the general effect of parasite infection is to cause the snails to forage more on tops of the rocks throughout the day, as suggested in this study, then it would seem that nontransmissible *Microphallus*-infected snails also have a unique behavior pattern. The behavior pattern induced by nontransmissible *Microphallus*-infected snails may decrease the probability that the snail would be eaten by any large predator, most likely waterfowl or fish. There are few examples of

advantageous behavioral changes induced by nontransmissible parasites. Tierney et al. (1993) present data consistent with the idea that nontransmissible parasites may induce predator-avoidance behaviors in three-spined sticklebacks. Although the aim of their study was not specifically to address this idea, their data suggest that fish infected with nontransmissible cestode plerocercoids have a faster recovery time to a predator attack, a decreased duration of pectoral sculling, and an increased amount of time spent motionless compared to uninfected controls. Tierney et al. (1993) suggest that these behaviors seem to decrease the probability of predation on the fish.

The effect of food availability on behavior

The absence of food had a noticeable effect on nonbrooding females in each of the first three experiments. In each case nonbrooding females moved to the bottom of the rocks when food was not present on top of the rocks. This behavior makes sense in that the snails should not risk predation if the benefits of being on top of the rocks are removed. Previous studies have demonstrated that *P. antipodarum* responds to fish predators by moving to the bottom of the rocks (Levri, in press).

In the early morning, *Microphallus*-infected snails were on top of the rocks as frequently as nonbrooding females (Levri and Lively, 1996). If manipulation is occurring, snails, in the absence of food, should be on top of the rocks as frequently as nonbrooding females in the food treatment. In each experiment infected snails did not respond to the lack of food and remained on the top of the rocks as frequently as did nonbrooding females in the food treatment. This result suggests that *Microphallus* is inducing the snails to remain on the top of the rocks despite the absence of food, which is consistent with the manipulation hypothesis. These experiments were performed in the early morning, when *Microphallus*-infected snails are most likely to be found on top of the rocks and when the final host of *Microphallus* feeds most (Levri and Lively, 1996). Thus if *Microphallus* is manipulating the behavior of the snail, the parasite should induce the snail to be on top of the rocks in the early morning regardless of food availability. In the third experiment, *Microphallus*-infected snails did show a significant increase in the percentage on top of the rocks in the food treatment compared to *Microphallus*-infected snails in the nonfood treatment. The reason for the difference in behavior of the *Microphallus*-infected snails in the food treatment of the third experiment is not known.

Another interpretation of these data is that *Microphallus*-infected snails are nutrient limited due to parasitism, thus they are on top of the rocks more often in an effort to find food. This interpretation is not likely to be correct because in a previous field study, nonbrooding females were on top of the rocks more than any other group over the course of the day (Levri and Lively, 1996). Because the major benefit of being on top of the rocks is nutrient acquisition, it is assumed that nonbrooding females need to forage more than any other group, including infected snails. Given that nonbrooding females need to forage more, *Microphallus*-infected snails should have been on top of the rocks less than nonbrooding females in this experiment (i.e., the lack of food should have affected the nonbrooding females to the greatest degree). This was not the case. The behavior of *Microphallus*-infected snails was distinctly different from any of the uninfected classes (Levri and Lively, 1996).

Hence, it seems that infection by *Microphallus* results in the snails remaining on top of the rocks in the early morning regardless of food availability. In the field it was found that *Microphallus*-infected individuals moved to the bottom of the rocks in the late morning and afternoon. This period is when fish fed most intensely, and they cannot serve as the definitive host (Levri, 1998; Levri and Lively, 1996). If the lack of a response to the absence of food in parasitized snails observed in the present study is adaptive, then the response is expected to appear in the late morning and afternoon when waterfowl feed less and fish feed more. Snail samples in the fourth experiment were taken over time. In the fourth experiment, significantly fewer nonbrooding female snails were found on the top of the rocks in the nonfood treatment in the morning and the afternoon (Figure 3), suggesting that it is better to avoid predation when food benefits are eliminated. Similarly to the first three experiments, in the early morning *Microphallus*-infected snails were just as likely to be found on top of the rocks in the food and nonfood treatments, suggesting that the parasite is manipulating the snails to enhance transmission to the final host. However, in the comparison between morning and afternoon samples, a significant time-by-class interaction was found (Table 3), indicating that in the afternoon, there were fewer infected snails found on top of the rocks in the nonfood treatment than in the food treatment. The same time-by-class interaction was found when data from the afternoon and evening were pooled. This would seem to indicate that the parasite-induced effect is ''turned off'' when other predators are feeding more. The morning versus evening comparison did not yield a significant time-by-class interaction, but the trend was in the expected direction. These time-specific effects are exactly what was predicted by the manipulation hypothesis.

In summary, the results suggest that the foraging behavior induced in *P. antipodarum* by *Microphallus* is specific to *Microphallus*. *Microphallus*-infected snails behaved differently from snails infected with two other independent groups of parasites. Also, *Microphallus*-infected snails responded differently from uninfected snails when food availability changed. *Microphallus*-infected snails remained exposed to predators when the final host feeds most, regardless of food availability. Uninfected snails moved to a safer habitat when food was removed. Taken together with previous findings (Levri, 1998, unpublished data; Levri and Lively, 1996), these results provide support for the hypothesis that *Microphallus* is manipulating the behavior of *P. antipodarum* to enhance its probability of transmission.

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