Forming foci of transmission: the effects of resource utilization, species interaction, and parasitism on molluscan movement

T.T. Gray, J.T. Detwiler, and D.J. Minchella

Abstract: Animal aggregation to environmental cues provides opportunities for parasite transmission between individual hosts of the same or different species. Better characterization of host behavioral responses to environmental stimuli in the absence and presence of parasites will improve our understanding of how foci of transmission form. The behavioral response patterns of two co-occurring freshwater snail species (*Lymnaea elodes* (Say, 1821) and *Helisoma trivolvis* (Say, 1817) (= *Planorbella trivolvis* (Say, 1817))) were assessed in response to three environmental stimuli (crayfish (genus *Orconectes* Cope, 1872) carrion, vegetation, or temperature gradient). Experiments were conducted with single species and species interactions. In addition, parasitized *L. elodes* were included in a single-species experiment and a species-interaction experiment. Snail species differed in the direction and magnitude of their responses to the environmental stimuli. Species interactions did not affect the responses to two of the stimuli for either species; however, interspecific interactions affected the response to high temperature in both species. Behavioral responses were altered in the presence of parasites for both the infected and uninfected hosts, suggesting parasitism is an important biotic factor in animal movement. This experimental study indicates co-occurring species respond to environmental factors in different ways. Furthermore, species interactions and parasitism within a guild can have strong effects on animal movement and potentially on parasite transmission.

Résumé : Les rassemblements animaux en réaction aux signaux du milieu offrent des occasions pour la transmission des parasites entre les hôtes individuels de même espèce ou d'espèces différentes. Une meilleure caractérisation des réactions comportementales des hôtes aux stimulus de l'environnement en présence et en l'absence de parasites devrait améliorer notre compréhension de la formation des noyaux de transmission. Nous avons évalué les patrons de réactions comportementales de deux espèces syntopiques de gastéropodes d'eau douce (*Lymnaea elodes* (Say, 1821) et *Helisoma trivolvis* (Say, 1817) (= *Planorbella trivolvis* (Say, 1817))) en réaction à trois signaux environnementaux (charogne d'écrevisses (le genre *Orconectes* Cope, 1872), végétation et gradient thermique). Les expériences portaient sur des espèces isolées et sur des interactions d'espèces. De plus, des *L. elodes* parasités ont été introduits dans une expérience monospécifique et une expérience d'interaction d'espèces. Les espèces de gastéropodes diffèrent par le sens et l'amplitude de leurs réactions au stimulus environnementaux. Chez les deux espèces, les interactions interspécifiques n'affectent pas les réactions à deux des stimulus; cependant, les interactions interspécifiques affectent les réactions aux hautes températures chez les deux espèces. La présence de parasites modifie les réactions comportementales à la fois des hôtes infectés et sains, ce qui indique que le parasitisme est un important facteur biotique dans le déplacement des animaux. Notre étude expérimentale montre que deux espèces cooccurrentes peuvent réagir aux facteurs de l'environnement de manière différente. De plus, les interactions interspécifiques et le parasitisme au sein d'une guilde peuvent avoir des effets importants sur le déplacement des animaux et potentiellement sur la transmission des parasites.

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Introduction

Animal aggregations are common in heterogenous environments (Parrish and Edelstein-Keshet 1999). Resource availability and quality are two factors that influence animal movement and stimulate the formation of groups (Bovbjerg 1975; Hylander et al. 2005). Negative consequences of aggregation include increased competition, as well as an increased opportunity for disease transmission. Infected hosts may serve as disease vectors to members of their own species, but also to individuals within interspecific aggregations. Many parasites can colonize a wide array of host species at particular points in their life cycle. Parasites can affect fitness traits of their hosts (Minchella et al. 1985), and alter host behavior (Holmes and Bethel 1972).

Host movement patterns can strongly influence parasite dispersal and the probability of parasite transmission (Kuris 2005; Miura et al. 2006). Previous studies have focused on how vertebrate host movements affect the genetic structure of parasite populations (Mulvey et al. 1991; Blouin et al. 1995; Thiele et al. 2008). Many parasite life cycles, however, utilize invertebrate hosts at some point in their life cycle. Understanding the factors underlying host movements could help predict foci of infection. By examining host be-

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Table 1. Overview of experiments with two snail species (*Lymnaea elodes* and *Helisoma trivolvis* (= *Planorbella trivolvis*)) and the environmental stimuli.

Experiment	Number of replicates per stimulus		
	Crayfish (*Orconectes* sp.) carrion	High temperature	Vegetation
Single snail (*n* = 12)			
Lymnaea elodes	15	15	15
Helisoma trivolvis	15	15	15
Infected *Lymnaea elodes*	8	—	—
Snail interaction (*n* = 6 per species)			
Lymnaea elodes + *Helisoma trivolvis*	15	15	15
Infected *Lymnaea elodes* + *H. trivolvis*	15	—	—

Note: *n* is the number of snails within a chamber of each species per replicate. A dash indicates that the experiments were not attempted.

havior with and without parasites, we can address whether parasites influence host movement patterns in ways that influence transmission opportunities within or between host species. Molluscan behavior is of particular importance because they are hosts for most trematode parasites (Platyhelminthes: Digenea). These parasites are common in marine, freshwater, and terrestrial habitats with over 18 000 described species (Olson et al. 2003).

Although snails are often conspicuous in freshwater communities, their movement patterns are relatively unstudied. Resource availability and quality can affect whether snails move randomly, or in nonrandom ways that lead to aggregation near particular resources. Bovbjerg (1975) found that snail movement became directed in the presence of vegetation, crayfish (genus *Orconectes* Cope, 1872) carrion, and high temperature. Snails aggregated to vegetation and crayfish carrion, but moved away from high temperatures. The purpose of our experiment is to ask how general Bovbjerg's (1975) results are across snail species, and to ask how they might be altered by biotic interactions between snails and parasites.

More specifically, our study has three objectives: (1) to determine how two snail species respond to the presence of resources or environmental cues (crayfish carrion, vegetation, high temperature); (2) to determine how those responses are influenced by the presence of a second snail species; and (3) to determine whether parasites have a significant effect on the behavioral responses of snails either alone or in the presence of a second snail species. Of particular interest is the possibility that snail species will respond in ways that lead to intra- and inter-specific aggregation, which in turn might influence the ability of the parasite to colonize additional hosts. Our experiments use two snail species (*Lymnaea elodes* (Say, 1821) and *Helisoma trivolvis* (Say, 1817) (= *Planorbella trivolvis* (Say, 1817))) that are commonly found together in freshwater communities throughout North America and therefore exposed to similar parasite communities. The parasite *Echinostoma revolutum* is an ubiquitous digenean trematode that utilizes both a first and a second intermediate host, as well as one definitive host, to complete its life cycle. Experimental infections indicate that *L. elodes* is the only first intermediate host (Sorensen et al. 1997). The parasite develops in *L. elodes* into a mobile larval form (cercariae) that leaves the snail and can infect either snail species (*L. elodes* or *H. trivolvis*) as their second intermediate host.

Materials and methods

Our experimental design examined snail movement patterns in response to multiple combinations of environmental stimuli, species interaction, and parasitism (see Table 1). We placed snails at the foot of a Y-shaped Plexiglas® aquarium with the following dimensions: the body was 37 cm × 14.5 cm, the arms were 23.5 cm × 14 cm, and the depth was 4 cm. The environmental stimulus (see below) was placed in either the right or the left arm of the aquarium. We recorded the location of each snail within the experimental chamber using a grid divided into 6.25 cm^2 squares every 5 min for a period of 1 h (adequate time for a response; Bovbjerg 1975). We designated a snail as "responding" to an environmental stimulus if it approached within 5 cm of the stimulus at any time during the experiment.

Snail-host dispersal was assessed in three ways. First, we compared the number of responders to nonresponders at a single time point (at 1 h). Second, we compared the tortuosity of the paths between responding and nonresponding snails and among responders from different experiments to determine whether interactions and parasitism alter snail movements. Fractal dimension (Fractal D) was calculated to determine the degree of tortuosity of the movement paths (Fractal version 5.18.0). Fractal D equal to one indicates a straight path, whereas values greater than one reflect a more tortuous path (Doerr and Doerr 2004). Third, we measured the time to first response (the time it took the first snail in an experiment to approach <5 cm from the stimulus) and compared it among the experiments to determine how species interaction and parasitism affected how quickly snails reach a resource.

Experimental protocol

Nonparasitized single-snail and snail-interaction experiments

Following Bovbjerg (1975), the response of snails to three environmental stimuli was investigated: crayfish carrion, common duckweed (*Lemna minor* L.), and high temperature. The crayfish and duckweed were collected from a freshwater pond in northeastern Indiana (41°18′N, 85°28′W), where they coexist with the snail species being evaluated. The crayfish, with a mean length of 25 mm, were finely sliced directly before the experiment. The duckweed was a 9 cm^3 sample that was large enough for multiple snails to investigate. A personal immersion heater was placed into a 250 mL beaker of water to create a zone of 30 °C at the center of the arm (9 cm × 14 cm). This temperature is commonly observed within the aforementioned freshwater pond (J.T. Detwiler, 2006 personal observation).

Before the experiment, only 2 cm of well water were placed in the aquarium to limit snail dispersion to two dimensions. A collection of nonparasitized, laboratory-raised snails (*L. elodes* and *H. trivolvis*) were size-matched within species (*L. elodes*: 19–30 mm; *H. trivolvis*: 12–17 mm) and individually numbered with permanent marker. After a 5 min acclimation period within the aquarium (well water, 22 °C), the snail slime trails were eliminated by hand and the snails were placed along the bottom of the aquarium, spread out, and positioned with the snail aperature forward. One of the environmental stimuli was placed into one arm of the chamber, which was alternated with each trial. The snails were released and their position recorded every 5 min for a 1 h period (Bovbjerg 1975; Curtis 1985). For single-species trials, 12 *L. elodes* or 12 *H. trivolvis* snails were treated as described above. The species-interaction trials were performed exactly as above, but with 6 *L. elodes* with 6 *H. trivolvis* alternating in location along the bottom of the aquarium. At the end of each trial, the aquarium was hand-cleaned to eliminate slime trails, which can cause directed snail dispersion (Bovbjerg 1975; Clifford et al. 2003). A total of 15 trials were conducted for each of the 9 experiments. A total of 1080 and 540 laboratory-raised snails were utilized in the single-species and species-interaction experiments, respectively.

Parasitized single-snail and snail-interaction experiments

Infections were established within the snail *L. elodes* using the trematode parasite *E. revolutum*. Larval parasites were collected from field-caught snail hosts (northeastern Indiana; 41°18′N, 85°28′W) and fed to chickens (*Gallus gallus* (L., 1758)). After 2 weeks, adult worms were removed from the rectum. Eggs were teased from the parasites and incubated in the dark in water-filled Petri dishes at 22 °C. After 19 days of incubation, the ciliated larval stage (miracidia) hatched from the eggs. Two miracidia were pipetted into 10 mL of well water with each *L. elodes* snail (<10 mm). Following 3 h of exposure, snails were individually placed into 125 mL screw top jars and maintained by feeding a 25 cm^2 square of lettuce twice a week, and changing well water once per week. Patent infections resulted in about 4 weeks. At this time, snails were placed under lights for 2 h to stimulate the emergence of larval parasites from the snails. Snails shedding parasites were immediately utilized in experimental trials. Infected snails were used in one single-species experiment (12 parasitized *L. elodes* with the crayfish carrion; replicates = 8) and in one snail-interaction experiment (6 parasitized *L. elodes* + 6 unparasitized *H. trivolvis* with crayfish carrion; replicates = 15). A total of 96 and 90 infected snails were used in the latter experiments.

Parasite biomass determination

Although each snail was exposed to two miracidia, the intensity of parasitism could differ among the snails. To determine the extent of parasitism and how it may affect behavioral responses, we decided to measure the parasite:snail biomass ratio. After the parasitized interaction experiment (15 replicates), the 90 infected *L. elodes* snails were placed into individual 1.5 mL microcentrifuge tubes filled with 100% ethanol. Specimens were stored at –20 °C until time of dissection. Each snail was briefly blotted to remove the excess ethanol and shell length was measured with digital calipers. Total mass (with and without shell) was measured to the nearest 0.001 g (Vicon Digital Scale model VIC 303; Acculab, Bohemia, New York, USA). Finally, the larval parasites (rediae and cercariae of *E. revolutum*) were separated from the snail tissue and placed into a 1.5 mL microcentrifuge tube (previously weighed) containing 100% ethanol. The sample was spun at 10 000 r/min (9300*g*) for 3 min. The supernatant was decanted and the parasite pellet was dried for 10 min (until pellet moisture approximated that of the snail body). To determine the mass of parasites, the mass of the empty tube was subtracted from the combined mass of the tube and pellet. The parasite:snail biomass ratio was calculated by taking the mass of the parasite divided by the mass of the snail without the shell.

Statistical analysis

Snail-host dispersal

We evaluated snail response to the stimuli (after 1 h) by fitting a negative binomial generalized linear model (GLM) to the data (Agresti 2002). A separate model was fitted to each experiment, and model selection was conducted by comparing the log-likelihood values of the most complex (including all possible interactions) to that of the most reduced (main effects only) model. Area was also included as a fixed factor to account for the three areas (two arms and body) where snails could be located at the end of the hour. Likelihood-ratio tests (LRT; Agresti 2002) were performed on the parameter estimates of each final model to determine whether snails responded to the stimulus and whether responses were different between the host species.

Fractal *D* was calculated for each individual snail with the same scale settings (minimum = 1; maximum = 22, 30 divisions) used for all paths (Fractal version 5.18.0). Using general linear models (GLM), the differences in tortuosity (Fractal *D*) between the responding and nonresponding snails were determined for both species when crayfish carrion was present. Tortuosity among snails responding to crayfish carrion was compared for both snail species. We compared the response of *L. elodes* from four experiments: single, interaction, infected single, and infected interaction. The responses of *H. trivolvis* were compared in three experiments: single, interaction, interaction + parasites. Mixed models with compound covariance structure were used to account for the random effect of replicates because snails within a chamber might be influencing each other's behavior. The response variable Fractal *D* was transformed by a $\log(D - 1)$ function to meet the assumptions of normality and homogeneity of variance.

Because we were counting the number of snails that re-

Table 2. (*a*) Responses and (*b*) mean responses of snails *Lymnaea elodes* and *Helisoma trivolvis* to environmental stimuli during single-species trials.

(*a*) Responses to stimuli by single species.

Host species	Stimulus	Factors	df	LRT	*P*
Lymnaea elodes	Crayfish (*Orconectes* sp.) carrion	Stimulus	1	24.26	<0.0001*
		Area	2	31.54	<0.0001*
	Temperature	Stimulus	1	5.62	0.0178*
		Area	2	43.31	<0.0001*
	Vegetation	Stimulus	1	7.67	0.0056*
		Area	2	50.92	<0.0001*
Helisoma trivolvis	Carrion	Stimulus	1	0.55	0.4594
		Area	2	8.36	0.0153*
	Temperature	Stimulus	1	0.47	0.4928
		Area	2	17.22	0.0002*
	Vegetation	Stimulus	1	21.90	<0.0001*
		Area	2	16.67	0.0002*

(*b*) Mean responses to stimuli by single species (df = 1 for all tests below).

Host species	Stimulus	Response	Mean	SE	LRT	*P*
Lymnaea elodes	Crayfish (*Orconectes* sp.) carrion	0	0.8638	0.1404	23.91	<0.0001*
		1	2.0027	0.1346		
	Temperature	0	1.3088	0.1013	5.37	0.0205*
		1	0.7102	0.2167		
	Vegetation	0	0.0713	0.1115	7.19	0.0073*
		1	1.5965	0.1284		
Helisoma trivolvis	Carrion	0	1.1102	0.1455	0.55	0.4587
		1	1.3160	0.2096		
	Temperature	0	1.1963	0.1437	0.47	0.4910
		1	1.3859	0.2065		
	Vegetation	0	0.8854	0.1430	23.56	<0.0001*
		1	2.0445	0.1479		

Note: LRT, likelihood-ratio test. *, $P < 0.05$.

sponded at a particular time, the time to first response among the experiments was analyzed with negative binomial generalized linear models (GLM). Snails that did not respond during the experiment (time intervals 1–13) were included in a 14th time interval. We consider this a conservative approach that underestimates potential differences in snail responses.

Parasite biomass determination

For infected snails, a logistic regression model (Agresti 2002) was fitted to assess the effects of parasite biomass (parasite:host tissue ratio) and snail length on the response to the stimulus. The relationship between parasite biomass and snail length was also examined with Pearson's correlation coefficients. All data were analyzed using SAS version 9.1.3 (SAS Institute Inc., Cary, North Carolina, USA) and results were considered significant at $P < 0.05$.

Results

There were species-specific responses to each of the environmental stimuli (Table 2*a*, Table 4). *Lymnaea elodes* responded positively to crayfish carrion and vegetation, and negatively to high temperature (Table 2*b*). *Helisoma trivolvis* did not respond to crayfish carrion or high temperature (Table 2*a*), but did respond positively to vegetation (Table 2*b*). Positive or negative responses occurred when a significant number of snails were either at the stimulus or not at the stimulus at 1 h, respectively. The outcome was considered a nonresponse when there was no difference between the mean number of responding and nonresponding snails. Responses to one of the environmental stimuli were altered during the interaction experiments (Table 3*a*). From the single species to the interaction experiment with high temperature, the response of *L. elodes* changed from a negative response to a nonresponse (Table 3*b*, Table 4). Similarly, the response to high temperature of *H. trivolvis* shifted from a nonresponse to a positive response between the single-species and species-interaction experiments (Table 3*b*, Table 4).

Parasite infection did not alter the response of snails in the single-species experiment with infected *L. elodes*. As in uninfected snails, there was a significant response to carrion ($LRT_1 = 9.98$, $P = 0.002$). During the interaction experiment with parasitized *L. elodes*, both species were attracted to the carrion ($LRT_1 = 3.96$, $P = 0.047$; Fig. 1). Therefore, *H. trivolvis* altered its behavior only in the presence of infected snails but not uninfected ones. Table 4 summarizes the species responses to the environmental stimuli in single-species, species-interaction, and parasitized-snail experiments.

Tortuosity between the responding and the nonresponding snails was different in both *L. elodes* ($F_{[1,163]} = 36.45$, $P < 0.0001$) and *H. trivolvis* ($F_{[1,156]} = 59.65$, $P < 0.0001$) single-species experiments. Responding snails were less tortuous than nonresponding for both species (least squares

Table 3. (*a*) Responses and (*b*) mean responses of snails *Lymnaea elodes* and *Helisoma trivolvis* to environmental stimuli during species-interaction experiments.

(*a*) Responses to stimuli with species interactions.

Stimulus	Factors	df	LRT	*P*
Crayfish (*Orconectes* sp.) carrion	Stimulus	1	1.22	0.2695
	Area	2	46.90	<0.0001*
	Host species	1	2.84	0.0922
	Stimulus × host species	1	7.38	0.0066*
Temperature	Stimulus	1	4.36	0.0369*
	Area	2	33.17	<0.0001*
	Host species	1	1.65	0.1987
	Stimulus × host species	1	11.62	0.0007*
Vegetation	Stimulus	1	13.39	0.0003*
	Area	2	34.11	<0.0001*
	Host species	1	0.00	0.9799

(*b*) Mean responses to stimuli with species interactions (df = 1 for all tests below).

Stimulus	Host species	Response	Mean	SE	LRT	*P*
Crayfish (*Orconectes* sp.) carrion	*Lymnaea elodes*	0	0.3978	0.1274	7.66	0.0057*
		1	1.0373	0.1653		
	Helisoma trivolvis	0	0.5476	0.1215	0.34	0.5588
		1	0.3774	0.2435		
Temperature	*Lymnaea elodes*	0	0.5958	0.1218	0.08	0.7835
		1	0.5211	0.2229		
	Helisoma trivolvis	0	0.2874	0.1392	16.67	<0.0001*
		1	1.2005	0.1459		
Vegetation	*Lymnaea elodes*	0	0.2632	0.1635	9.86	0.0017*
		1	1.1642	0.1973		
	Helisoma trivolvis	0	0.2857	0.1623	8.60	0.0034*
		1	1.1301	0.2001		

Note: LRT, likelihood-ratio test. *, $P < 0.05$.

means: *L. elodes* = 1.17 vs. 1.32; *H. trivolvis* = 1.16 vs. 1.36). Among the experiments, the responding snails had similar tortuosities (*L. elodes*: $F_{[3,161]} = 1.68$, $P = 0.17$; *H. trivolvis*: $F_{[2,123]} = 1.51$, $P = 0.23$). There were, however, differences in the time to first response among the experiments for both species (*L. elodes*: $\mathrm{LRT}_3 = 9.29$, $P = 0.026$; *H. trivolvis*: $\mathrm{LRT}_2 = 14.94$ $P = 0.0006$). Compared with their response to carrion in the single-species experiment, infected *L. elodes* responded later in the interaction experiment ($\mathrm{LRT}_1 = 8.95$, $P = 0.0028$) and infected single-species experiment ($\mathrm{LRT}_1 = 4.82$, $P = 0.028$). *Helisoma trivolvis* also responded later in the interaction experiment compared with its responses in both the single-species experiment ($\mathrm{LRT}_1 = 15.54$, $P < 0.0001$) and the interaction + infected *L. elodes* experiment ($\mathrm{LRT}_1 = 8.70$, $P = 0.0032$). It is notable that the response time for *H. trivolvis* was different between the two interaction experiments (with and without parasites), as *H. trivolvis* responded more quickly to the stimulus when paired with infected *L. elodes* (Fig. 2). The response time between the species was only different in the interaction experiment ($\mathrm{LRT}_1 = 11.13$, $P = 0.0009$).

Among the 90 snails examined, the parasite to snail biomass ratio varied from 0.082 to 0.714. The mean parasite to snail biomass ratio per replicate (15 total) ranged from 0.22 to 0.56. The Pearson's correlation coefficient (Neter et al. 1996) between parasite biomass and snail length was –0.31, indicating that multicollinearity may be present in a model fitted with both of these factors included. However, all three models (single variable only and both variables) showed no relationship to the response (parasite biomass: $\mathrm{LRT}_1 = 1.10$, $P = 0.29$; snail length: $\mathrm{LRT}_1 = 0.14$, $P = 0.70$; both variables — parasite biomass: $\mathrm{LRT}_1 = 0.96$, $P = 0.33$; snail length: $\mathrm{LRT}_1 = 0.00$, $P = 0.94$). Similarly, there was no relationship between time to first response and parasite biomass (all three models: $\mathrm{LRT}_1 = 0.0$, $P = 1.0$).

Discussion

Behavioral responses to environmental stimuli differed between snail species. Species differences were observed after 1 h and in the time to first response, but not in the tortuosity of their movement paths. The responses of species to the environmental stimuli were variable (Table 4). Interspecific aggregation to a stimulus only clearly occurred in the case of one environmental stimuli, i.e., vegetation (Tables 2*b*, 3*b*). Based on the movement patterns after 1 h, we concluded that when both species were together, each species response to the environmental stimuli usually remained similar to their single-species experimental response. One exception was in response to high temperature, which tended to be more attractive to both species when they were together (Table 4). Despite a significant presence of parasites (as determined by the parasite biomass measure), the effects of parasitism on infected hosts were only apparent by examining response time as opposed to the mean number of responding snails. In contrast, the other potential host

Table 4. Responses of snails *Lymnaea elodes* and *Helisoma trivolvis* to environmental stimuli, species interaction, and parasitism.

	Lymnaea elodes				*Helisoma trivolvis*		
Stimulus	Single	Interaction	Interaction + parasite	Parasite	Single	Interaction	Interaction + parasite
Crayfish (*Orconectes* sp.) carrion	+	+	+	+	0	0	+
Temperature	–	0			0	+	
Vegetation	+	+			+	+	

Note: + (positive response) and – (negative response) are statistically significant at $P < 0.05$ (see Tables 2*b* and 3*b*). 0, no response.

Fig. 1. Response of snails *Lymnaea elodes* and *Helisoma trivolvis* to crayfish (*Orconectes* sp.) carrion during species-interaction experiments. Solid bars represent results from the species-interaction experiment without parasites. Hatched bars represent the species-interaction experiment with infected *Lymnaea elodes* and uninfected *Helisoma trivolvis*. 0, no response; 1, response. Error bars are standard errors.

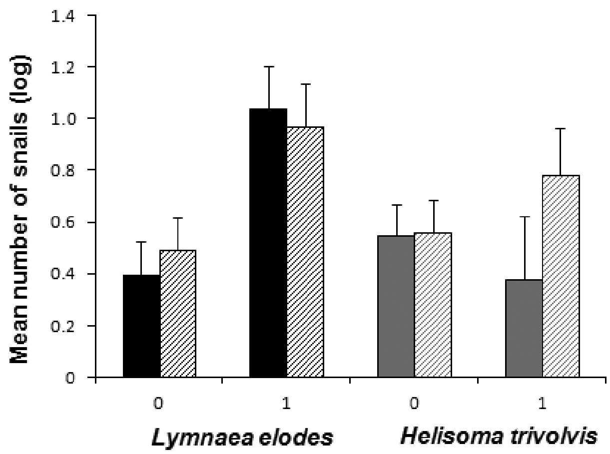
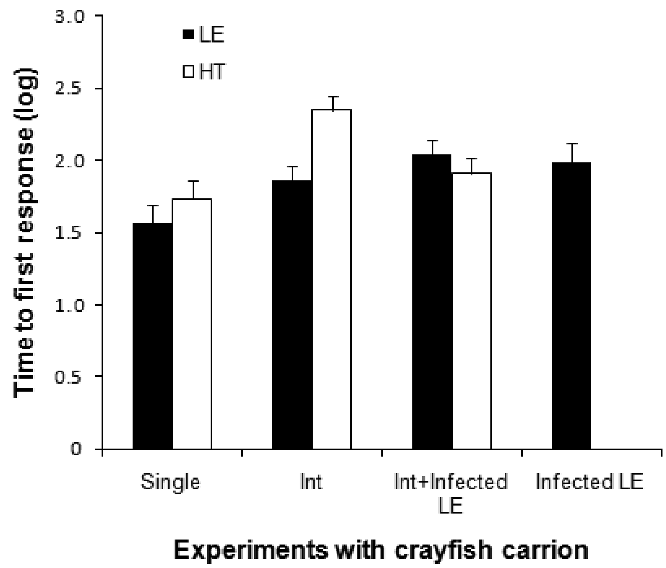


Fig. 2. Time to first response with crayfish (*Orconectes* sp.) carrion as the environmental stimulus for the two snail species (LE, *Lymnaea elodes*; HT, *Helisoma trivolvis*). Results from seven experiments are shown: single species (Single), species interaction (Int), species interaction with infected *L. elodes* (Int+Infected LE), and single species with infected *L. elodes* (Infected LE). Error bars are standard errors.



species response was altered in the presence of infected hosts according to both the single point (at 1 h) and the time to first response. More *H. trivolvis* responded to the stimulus (Fig. 1) and responded earlier (Fig. 2) in the presence of infected *L. elodes* than they do when in the presence of uninfected *L. elodes*.

Our study indicates that resource type affects snail movements and may act as stimuli for interspecific aggregations. After 1 h, we found that *L. elodes* aggregated around each of the environmental stimuli, whereas *H. trivolvis* only responded to vegetation. Our results are complementary to that of Chase et al. (2001), who characterized the resource utilization of both species after 1 month. Relative to *H. trivolvis*, *L. elodes* were considered grazing snails because they discovered resources more quickly but left more resource behind. *Helisoma trivolvis* were considered digging snails because once a resource was discovered, they greatly exploited it. Differences in foraging strategy may promote coexistence and lead to spatially distinct interspecific aggregations (Brown 1982; Chase et al. 2001; Lombardo and Cooke 2004). Given that co-occurring snail species utilize the same resources differently, it was expected that species interactions would not change their responses. The temperature gradient was the only stimulus to alter responses, and both species responses were changed from the single-species experiments. However, the direction of the change still caused them to be separate in the experimental chamber. This spatial separation may reduce competition, which can occur as the result of high resource overlap and lead to reduced fecundity (Brown 1982). We cannot distinguish whether the difference in the behavioral responses is due to resource competition or interference competition.

Benefits of aggregation include reduced susceptibility to predation, improved opportunities for mate choice, and increased food finding (Erlandsson and Kostylev 1995; Parrish and Edelstein-Keshet 1999). Groups may also experience negative consequences such as increased risk for parasite transmission owing to the presence of infected individuals within the group. Some parasites can alter snail behavior to increase the likelihood of transmission to the next host (Curtis 1985, 1987). The single-time-point assessment suggested that the behavioral responses of *L. elodes* were similar when infected or uninfected. In both the single-species and species-interaction experiments, snail aggregation around the stimulus increased the probability of transmission, though among already infected snails. The cost of parasitism became apparent for these snails only when the time to first response was examined. There was a significant increase in the time it took for infected snails to first reach the stimulus (Fig. 2). The probability of infection was also increased for uninfected snails (*H. trivolvis*), as more of these snails were in close proximity to the infected hosts (Fig. 1). The response time for *H. trivolvis* was particularly interesting because it showed a later response time in the species interaction without infected *L. elodes*, but an earlier response time with infected snails. Therefore, the mean response time became more similar to that of infected *L. elodes*, increasing the amount of time they spent in close proximity. This response gave the parasite two options for second intermediate hosts: colonize already infected snails or colonize uninfected heterospecific snails. These behavioral changes clearly benefit the echinostome parasites, as they seem to preferentially utilize snail hosts that cannot serve as first intermediate hosts (Detwiler and Minchella 2009). The free-swimming infective stage, the cercariae, has limited dispersal and encystment abilities after 24 h (Fried and Bennett 1979). Cercariae penetrate and encyst in snail tissue, ensuring the continuation of the parasite life cycle.

The host–parasite interaction may have altered the composition of secretions in *L. elodes*. This change in chemical components seems to be more attractive to *H. trivolvis*. It is tempting to hypothesize that this change is parasite-induced and thus an adaptation that favors parasite transmission, but it could also be a nonadaptive side effect of the interaction. Nevertheless, parasites may have affected the chemical components of the slime trails of *L. elodes*, leading to an increase in the number of *H. trivolvis* aggregating with *L. elodes*. Slime trails contain chemical cues that allow snails to follow conspecifics (Wells and Buckley 1972; Clifford et al. 2003). Snail species can follow heterospecifics, especially when they are prey species (Cook 1985; Clifford et al. 2003), but Townsend (1974) found that some snail species did not follow heterospecific trails. Given that the snail species in our study co-occur naturally, we suggest that they recognize the presence of other species via the slime trails, which may be useful when foraging. Unfortunately, we did not directly evaluate whether this occurred in our experiments.

Understanding the behavioral responses of snails to different resource types allows a better understanding of how aggregations form and the nature of interspecific or intraspecific groups. Their different responses to the environmental stimuli suggest that these two snail species may overlap while foraging. When interspecific interactions occur, they can but do not always alter behavioral responses. This study is novel because in addition to examining responses of single species and species interactions, we determined how parasites may affect snail behavior. Whereas parasites did affect the behavior of the infected host, the fundamental change in the behavior of uninfected yet susceptible heterospecific snails in the presence of infected hosts is a novel finding that warrants further investigation.

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