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Chemical signaling and avoidance responses between *Orconectes propinquus* crayfish and *Phsya integra* and Planorbidae snails

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Abstract

Snail avoidance of predators due to chemical signaling was studied in a freshwater stream between *Physa integra* and planorbid snails and *Orconectes propinquus* crayfish. The behavior of snails downstream from no crayfish, crayfish only, and crayfish plus snails treatments was observed over a two-week period. Algal growth in each of these treatments was also analyzed to determine if algal consumption was altered as a result of avoidance behavior. Avoidance behavior was exhibited by both species of snails only in the crayfish plus snails treatment, suggesting that chemical signals from both foraging crayfish and hunted or injured snails are necessary to elicit a response. The algal analysis did not reveal a change in algae consumption among the different treatments.

Introduction

Predator-prey interactions elicit behavioral responses from organisms. Often, changes in behavior can be elicited by non-visual stimuli. Dodson *et al.*, (1994), found that prey species respond to chemical signals by altering many features including their life history strategy, feeding habits, morphological development, and predator avoidance behavior (1994). These cues are especially important to organisms whose eyes do not form images, including molluscs. Kairomones are chemicals transmitted between species that provide an advantage to the receiver, including predator-prey signals. Alarm substances are those signals given by damaged conspecifics. Both chemicals have also been shown to initiate avoidance behavior in many species, including snails. *Physa virgata* snails have been previously shown to exhibit avoidance behavior when in the presence of both predators and injured conspecifics, but do not respond to inactive crayfish (Dodson *et al.*, 1994).

Tenderfoot Creek, a lake-outflow located on the University of Notre Dame Environmental Research Center property in the upper peninsula of Michigan, is home to many types of vertebrates, invertebrates and algae. The crayfish *Orconectes propinquus* is abundant in this stream. Crayfish are generalist feeders, they consume both algae and invertebrates, as well as other food resources (Charlebois and Lamberti, 1996). Snails present in the stream, including *Physa integra* and snails in the family Planorbidae, are grazers, and their main food source is algae.

In this experiment, the behavior of *P. integra* and snails in the family Planorbidae was observed when they were placed in divided artificial channels downstream from no crayfish, crayfish only, and crayfish plus snails. Direct contact between the organisms was prevented by a permeable barrier to allow for behavioral changes in response to chemical signals. It was expected that, consistent with previous research (Dodson *et al.*, 1994), the snails would exhibit the most avoidance behavior in the crayfish plus snails treatment. A study comparing the algae in the different environments also was conducted. If the snails' behavior is affected by chemical signals from upstream predators, their foraging habits may be altered. Covich *et al.* (1991) considered this effect to be a cost to the snails if they crawl out of the water to avoid a predator. It was hypothesized that if the snails exhibited avoidance behavior (defined as hiding under substrate or crawling out of the water), they would not be as effective at grazing and, therefore, the abundance of algae would increase in these treatments. In the downstream halves, the amount of algae in the no crayfish treatment should be the lowest, intermediate in the crayfish only cages, and highest in the crayfish plus snails treatment.

A second experiment also was completed, which attempted to eliminate vision as a source of behavioral change. Fake crayfish were placed upstream from the snails and avoidance behavior and algae consumption were assayed in the same manner. If there was no avoidance response in this experiment, then visual perception of the crayfish could be eliminated as the cause of the avoidance behavior in snails. It was expected that the snails in this treatment would behave similarly to those in the no crayfish treatment of the first experiment.

Materials and Methods

These experiments were conducted in Tenderfoot Creek, located on the UNDERC property in Gogebic Co, Michigan. Cages were composed of plastic gutters 1m in length, with chicken wire (1/8") covering each end and dividing the gutter in half. The entire gutter was covered with a Plexiglas lid. Gutters (n = 9) were placed in the creek, parallel to the current. Four tiles (7.5 cm^2) were placed in each half of each cage to serve as substrate. These were slightly elevated using small rocks from the stream. The tiles were placed in the stream at least two weeks prior to the initiation of the experiment to allow them to gather algae to serve as a food source for the snails and crayfish.

P. integra and planorbid snails and *O. propinquus* were collected from the stream. Cage A was designated the control with no crayfish or snails in the upstream half. Cage B was the crayfish only treatment with two *O. propinquus* upstream, and Cage C was the crayfish plus snails treatment with two crayfish and two of each kind of snail upstream. Five of each species of snail were placed in the downstream half of each cage. The treatments were replicated 3 times, for a total of nine cages in the stream in a stratified random design.

The day that these organisms were placed in the cages was day 0. On this day, multiple observations of the snails were taken. After a four hour adjustment period, counts were made every hour for 3 – 5 hours. The number of visible snails on the tiles or floor of the gutter was counted, and it was inferred that the other snails were exhibiting avoidance behavior. Observations were made on days 1, 3, 6, 7, 13, and 14 in the morning, afternoon, evening and night as much as possible. If weather conditions prevented these observations, attempts were made to observe the snails on other days. On days 5 and 11, the snails and crayfish alive in the cages were counted, and any organisms that had died or escaped were replaced. The number of each kind of snail exhibiting avoidance behavior was averaged for all observations taken over the two weeks and expressed as a percentage. A one-way Analysis of Variance (ANOVA) was conducted to detect significant differences in snail behavior between the treatments.

In the second experiment, Cage D referred to the fake crayfish treatment. One plastic crayfish was placed in the upstream half near the wire mesh that split the cage in half. One of each of cages A, B, and C were run simultaneously with 3 replicates of cage D. Observations of snail behavior and assays of algae were made and analyzed in a similar manner.

Algal abundance was assayed twice during each two-week experiment, on days 7 and 14. One tile from each half of each gutter was removed and replaced with another tile from the stream. Algae were scraped off the tile and diluted into 100 mL of distilled water. This mixture was homogenized by shaking to ensure even distribution of algae in the water. Two samples of 20 mL were filtered onto Fisherbrand Glass Fiber Filters (G6). One of these was assayed for biomass and one for chlorophyll a. Biomass was determined by drying the algae on the pre-weighed filters for 24 hours to constant weight at 105°C. This dried algae was weighed, oxidized at 500°C for one hour, and then reweighed. AFDM was calculated as the weight of the DM minus the ash, divided by the area of the substrate (Steinman and Lamberti, 1996). Chlorophyll a was determined by steeping the second filter in 25 mL of 90% acetone (90 parts acetone, 10 parts saturated magnesium carbonate solution) for 24 hours at 4°C in the dark. Three mL of the acetone were placed in a 1 cm cuvette. The absorbance was read at 750 and 665 nm. The acetone was then acidified with 0.1 mL 0.1 *N* HCl, incubated 90 seconds, and absorbance at each of these wavelengths was again read. The chlorophyll a content was determined with the equation Chlorophyll a ($\mu g/cm^2$) = $26.7\ (E_{665b} - E_{665a})\ X\ (V_{ext}$ / area of tile (cm^2)) X L (Steinman and Lamberti, 1996). Statistical comparisons were made among the different treatments using one-way ANOVA.

Results

In the first experiment, there was a significant difference in the behavior of both kinds of snails between the no crayfish and crayfish and snails treatments (Figure 1). *P. integra* in the no crayfish treatment had a mean of 31.8% snails exhibiting avoidance behavior, the crayfish treatment had a mean of 43.2%, and the crayfish plus snails treatment a mean of 45.3% (Table 1). Tukey's Test showed a significant difference between no crayfish and crayfish and snails ($P < 0.05$). The planorbid snails also changed their behavior in the presence of foraging crayfish. For this snail, the mean percentage of snails exhibiting avoidance behavior was 20.4% for no crayfish, 29.2% for crayfish, and 33.0% for crayfish plus snails (Table 1). Tukey's Test revealed a significant difference between the no crayfish and crayfish plus snails treatments ($P < 0.05$).

A significant difference was also found in AFDM on day 14. The no crayfish treatment had a mean of 1.8×10^{-3} mg/cm^2 while the crayfish treatment mean was 3.0×10^{-4} mg/cm^2 and the crayfish plus snails treatment mean was 1.7×10^{-4} mg/cm^2 (Table 1). Tukey's Test showed significant difference between no crayfish and crayfish and between the no crayfish and crayfish plus snails but not between the crayfish and crayfish and snails ($P < 0.05$).

A one-way ANOVA was also run comparing AFDM on day 7 for crayfish, AFDM on days 7 and 14 for snails, and chlorophyll a on both days 7 and 14 for both crayfish and snails. No significant differences were found between treatments for any of these contrasts.

The avoidance behavior between the two species of snails was compared (Figure 1). The percent of Planorbid snails exhibiting avoidance behavior was always less than that of the *P. integra*. The avoidance behavior of the snails showed no clear pattern over time in the no crayfish treatment (Figure 2A), the crayfish only treatment (Figure 2B), and the crayfish plus snails treatment (Figure 2C). Finally, the behavior of the snails in all three treatments was compared at different times of the day—in the morning, afternoon, evening, and night. The avoidance behavior did not follow any pattern related to the time of day (Figure 3).

When the data from the second experiment (with the fake crayfish) is combined with that from the first experiment, different results are found. There is no significant difference in snail behavior. The only differences among the treatments are in the amount of algae in the crayfish halves of the gutters. The AFDM is different on Day 7 between the no crayfish and crayfish treatments and on day 14 between no crayfish and crayfish plus snails. The chlorophyll a differs on day 7 between no crayfish and crayfish plus snails.

Discussion

The first experiment revealed a change in snail behavior in the cages where the snails were downstream from crayfish plus snails. This, along with previous research, suggests that snails respond to chemical signals from crayfish predators, injured conspecifics, or both. The fact that the behavior was only significantly different in the crayfish plus snails treatment suggests that the alarm substances transmitted by conspecifics are essential factors in eliciting a behavioral response. Some previous experiments ensured that the upstream snails gave off these alarm signals by crushing their shells prior to putting them in the cages (Dodson *et al.*, 1994). This step was not taken in this experiment. Sometimes the upstream snails would live for days, and thus, it cannot be determined when alarm signals were transmitted.

Some difference was also observed between the behavioral responses of the two species of snails. Most of the planorbid snails typically remained at the end mesh of the downstream half while the *P. integra* were dispersed throughout the cage. Also, the *P. integra* exhibited more avoidance overall in all of the treatments (Figure 1). This could be due to the feeding habits of the crayfish. If they prefer to prey upon *P. integra*, those snails will probably respond the most to their signals. Alexander and Covich (1991) found that *Physa virgata* was more vulnerable than *Planorbella trivolis,* when preyed upon by *Procambarus simulans*, was significant. *P. virgata* was more vulnerable. This is consistent with the increased tendency of the physid in this experiment to exhibit avoidance behavior. Covich *et al.* (1994) also found that physid species exhibited more avoidance behavior than planorbid species of snails.

Also in this experiment, in the crayfish half of the gutter, it was expected that algae would be more abundant in the crayfish plus snails treatment than in the crayfish only treatment. However, the crayfish did not seem to preferentially feed on the snails. No difference was observed between the algae in these two treatments. This could also be due to the small number of snails in the upstream half being insufficient to feed the two crayfish there. It was visually obvious that the crayfish were consuming the algae. Also, the crayfish at some points preyed on each other, indicating that food was very limited. Much more algae appeared to be present in the no crayfish treatment than the others. However, the data only showed this in the AFDM analysis on day 14, where a difference was seen between the no crayfish treatment and the crayfish or crayfish plus snails treatments. This disagreement between the visual observations and the experimental results could be due to difficulties in measuring the algae. After the tiles were scraped, it was attempted to make an even mixture of the algae in 100 mL of water. Perhaps this technique did not accurately distribute the algae in the water and therefore influenced the results. Also, if this experiment was replicated more times, random error, and thus the deviations, could be reduced and more precise data could be obtained.

Another goal of this experiment was to examine differences in algae consumption between the downstream halves of different treatments, but none were revealed. If there was a difference, the technique used was probably not sensitive enough to detect it. Also, this comparison was complicated due to the differences in the algae upstream. The snail behavior was hypothesized to increase algae in the crayfish only and crayfish plus snails treatments, leaving less algae in the no crayfish treatment. However, the accumulation of algae in the upstream half of the no crayfish treatment probably affected the amount of algae downstream.

Many problems arose during the course of this experiment. One of the most detrimental was the mortality rate of the snails and crayfish. Often over 50% of the snails had to be replaced on days 5 and 11. This suggests that the method used to determine avoidance behavior may not have been ideal. If the snails had escaped, then they were not seen and counted as exhibiting avoidance behavior, and if they had died, then they were either counted or not, depending on where their shell lay. In future experiments, it may be beneficial to thoroughly survey each cage each time an observation is made so that only live snails are included in the results.

Also because of this problem, this experiment was not successful in determining if the snail's behavior would change over two weeks. There is no pattern showing a change in avoidance over the two-week experimental period in any of the treatments (Figure 2). It would be interesting to see if the snails would become accustomed to the constant signals from the crayfish and snails and when they don't encounter immediate danger, would stop exhibiting avoidance behavior. Because many of the snails died, an observation like this cannot be made.

Another problem during this experiment resulted from the changing water levels. In the beginning of the experiment, the water level was very high—almost to the Plexiglas lids. Toward the end, it was much lower, about an inch or two over the tiles. This made it difficult to standardize the results. These water levels could have had an effect on the behavior of the snails and the accumulation of algae. This change in water levels also made it difficult to determine when snails were crawled out. This behavior has previously been defined as crawling above the water line (Alexander and Covich, 1991). However, because the water reached the Plexiglas lids in the beginning of the experiment, any crawling on the sides of the gutters towards the water line was judged as avoidance.

The second experiment, which examined the possibility of visual cues, was unsuccessful. In this experiment, fake crayfish were used to determine if they would initiate the same behavioral responses as living crayfish. In this experiment, the snails downstream from the fake crayfish exhibited avoidance behavior at the same levels as those downstream from the crayfish plus snails in the first experiment. Treatments A, B and C also showed this same behavior. These data are unreliable because of the many problems and changing conditions during the experiment. Fluctuating water levels again caused many problems. Early in the experiment, the gutters dried out as water levels lowered, but after being adjusted to the new levels, they flooded in a rainstorm. Most of the snails were lost. The cages were then flooded a second time toward the end of the experiment, and many of the snails and crayfish again escaped or died. It is unknown exactly how the data were affected by these conditions. In addition, two of these observations were taken in the rain, and it was observed that most of the snails were crawled out under these conditions. These circumstances probably played a role in the elevated percentages of snails exhibiting avoidance behavior. Other researchers have noted that snails do not have image forming eyes, and thus visual signaling probably does not play a role in this predator-prey relationship (Dodson, *et al.,* 1994). This suggests that the avoidance is probably due to chemical signaling; however, this experiment could be repeated to effectively eliminate this as a possibility.

Future experiments could look at these predator-prey interactions in a laboratory stream in order to control many of the changing variables. It would probably be most effective to use *P. integra* in this study due to its increased behavioral responses. In more controlled conditions, it will be easier to assay the algae in each treatment in order to determine if the foraging behaviors are affected by this increased avoidance behavior.

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Works Cited

Alexander, James E., and Alan P. Covich. 1991. Predation risk and avoidance behavior in two freshwater snails. Biological Bulletin 180: 387-393.

Alexander, James E., and Alan P. Covich. 1991. Predator avoidance by the freshwater snail *Physella virgata* in response to the crayfish *Procambarus simulans*. Oecologia 87: 435-442.

Covich, Alan P., Todd A. Crowl, James E. Alexander, Jr., and Caryn C. Vaughn. 1994. Predator-avoidance responses in freshwater decapod gastropod interactions mediated by chemical stimuli. Journal of the North American Benthological Society 13: 266-7.

Charlebois, Patrice M., and Gary A. Lamberti. 1996. Invading crayfish in a Michigan stream: direct and indirect effects on periphyton and macroinvertebrates. Journal of the North American Benthological Society 15(4): 551-563.

Dodson, Stanley I., Todd A. Crowl, Barbara L Peckarsky, Lee B. Kats, Alan P. Covich, and Joseph M Culp. 1994. *Non-visual communication in freshwater benthos: an overview.* Journal of North American Benthological Society, 13(2): 268-282.

Steinman, Alan D., and Gary A. Lamberti. 1996. Biomass and Pigments of Benthic Algae. *Methods in Stream Ecology.* Academic Press.

Figure 1 Avoidance Behavior Exhibited by *Physa integra* and Planorbid Snails

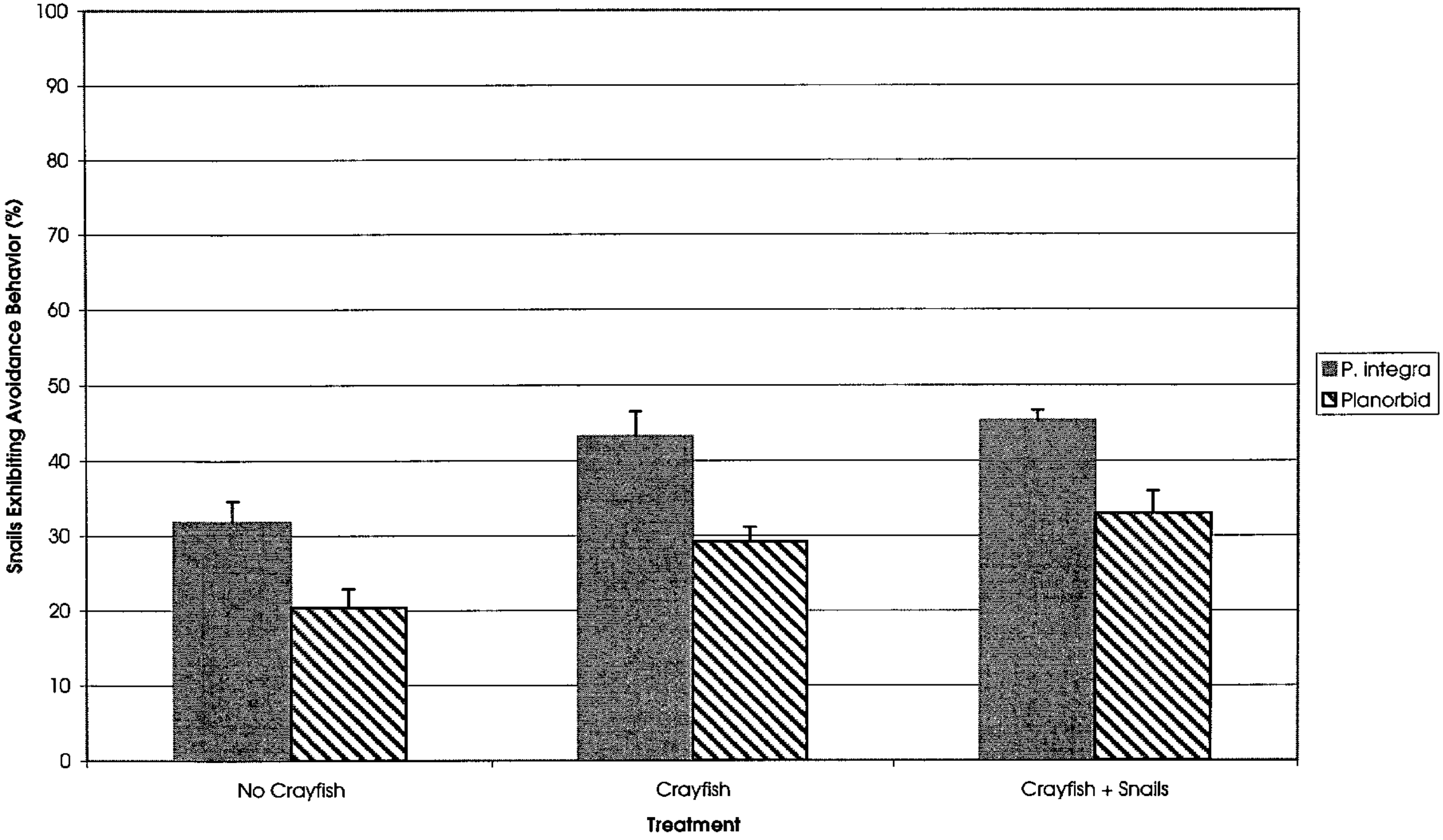


Table 1. Mean values of percent avoidance, ash-free dry mass (AFDM), and chlorophyll a (Chla) for the three replicates of each experiment. The first two columns show percent avoidance for the two snails. AFDM and Chla are shown for the crayfish and snail halves on both days 7 and 14.

Treatment	Avoidance of P. integra (%)	Avoidance of Planorbids (%)	AFDM Crayfish Day 7 (mg/cm^2)	AFDM Crayfish Day 14 (mg/cm^2)	AFDM Snails Day 7 (mg/cm^2)	AFDM Snails Day 14 (mg/cm^2)	Chla Crayfish Day 7 (mg/cm^2)	Chla Crayfish Day 14 (mg/cm^2)	Chla Snails Day 7 (mg/cm^2)	Chla Snails Day 14 (mg/cm^2)
No Crayfish	31.8	20.4	1.2 X 10-3	1.8 X 10 -3	1.0 X 10-3	1.0 X 10-3	1.66	2.92	1.37	2.17
Crayfish	43.2	29.2	2.0 X 10-4	3.0 X 10-4	1.1 X 10-3	1.4 X 10-3	0.758	-0.178	1.24	2.35
Crayfish Plus Snails	45.3	33	2.0 X 10-4	1.7 X 10-4	1.1 X 10-3	1.1 X 10-3	0.854	0.0593	1.2	2.37

Figure 2A

Snail Avoidance Behavior Over Time in No Crayfish Treatment

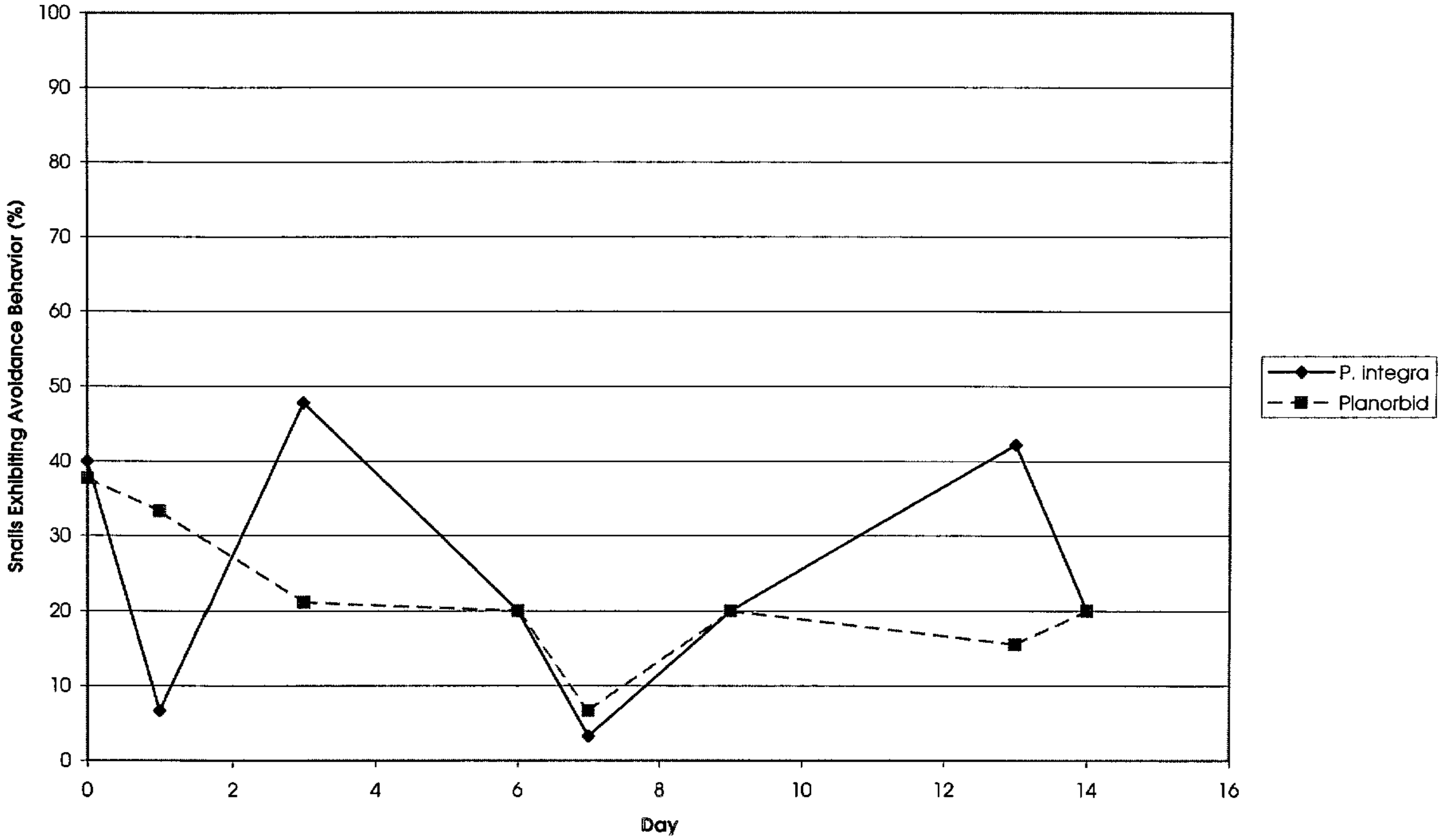


Figure 2B

Snail Avoidance Behavior Over Time in Crayfish Treatment

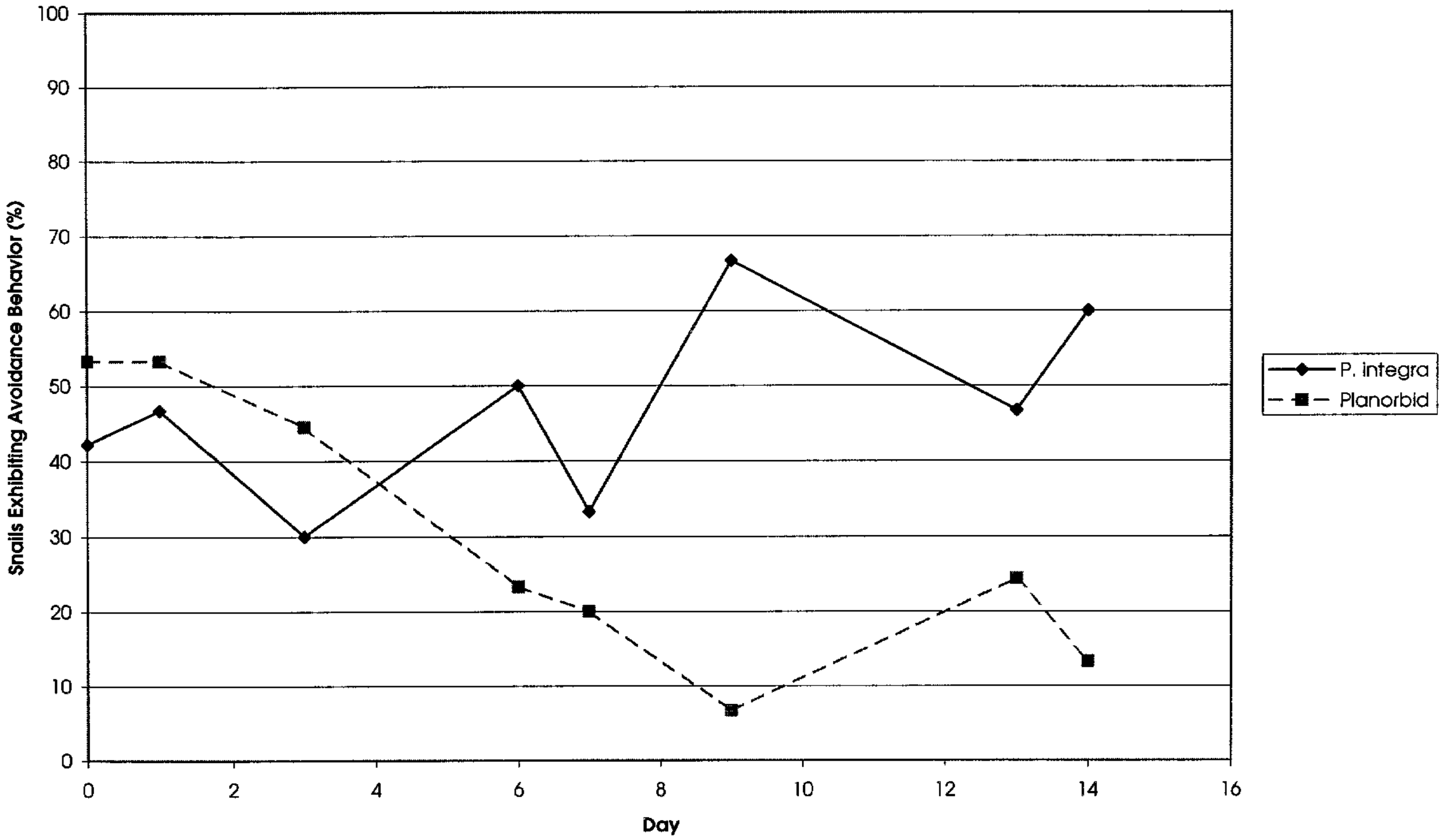
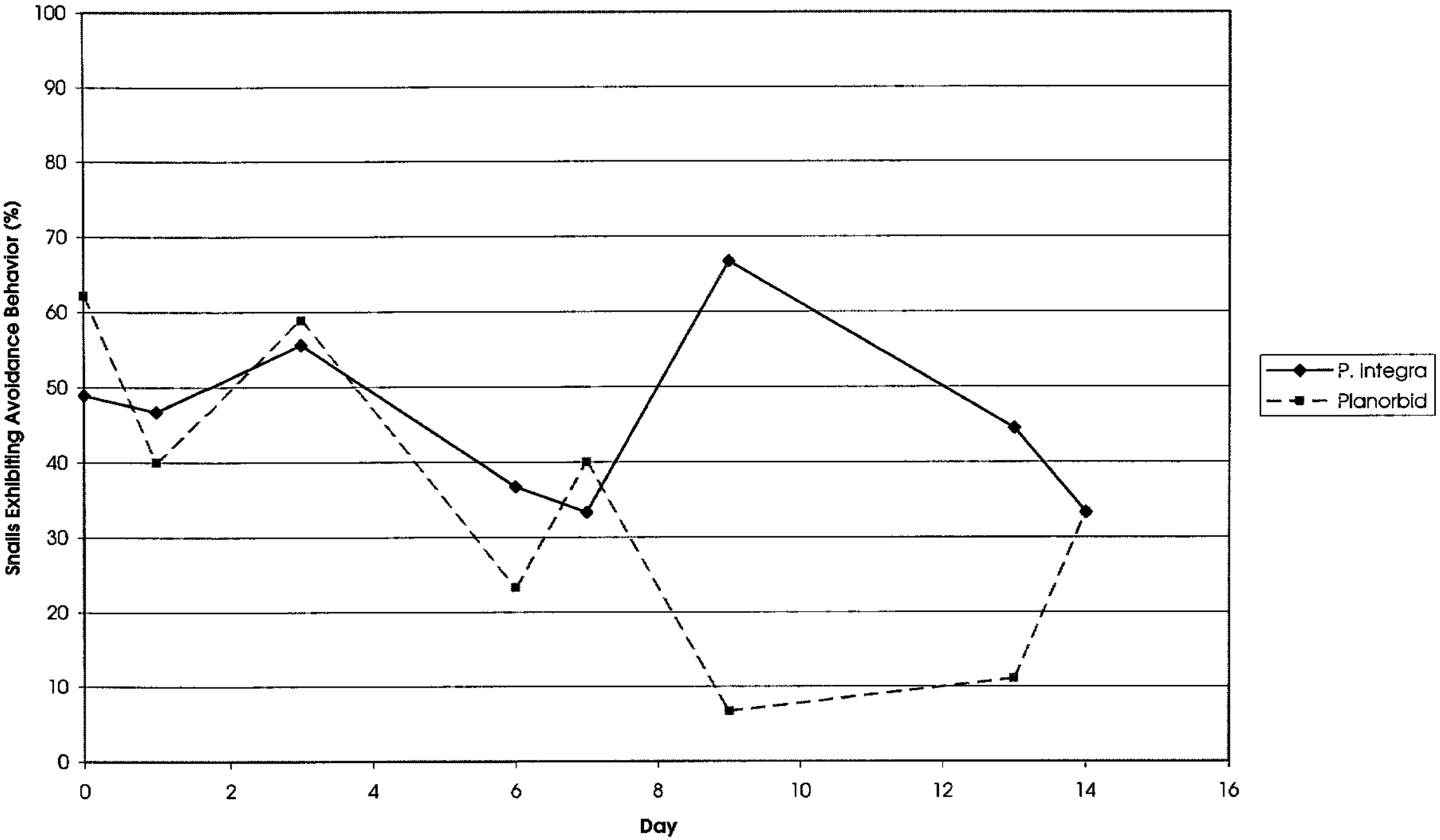
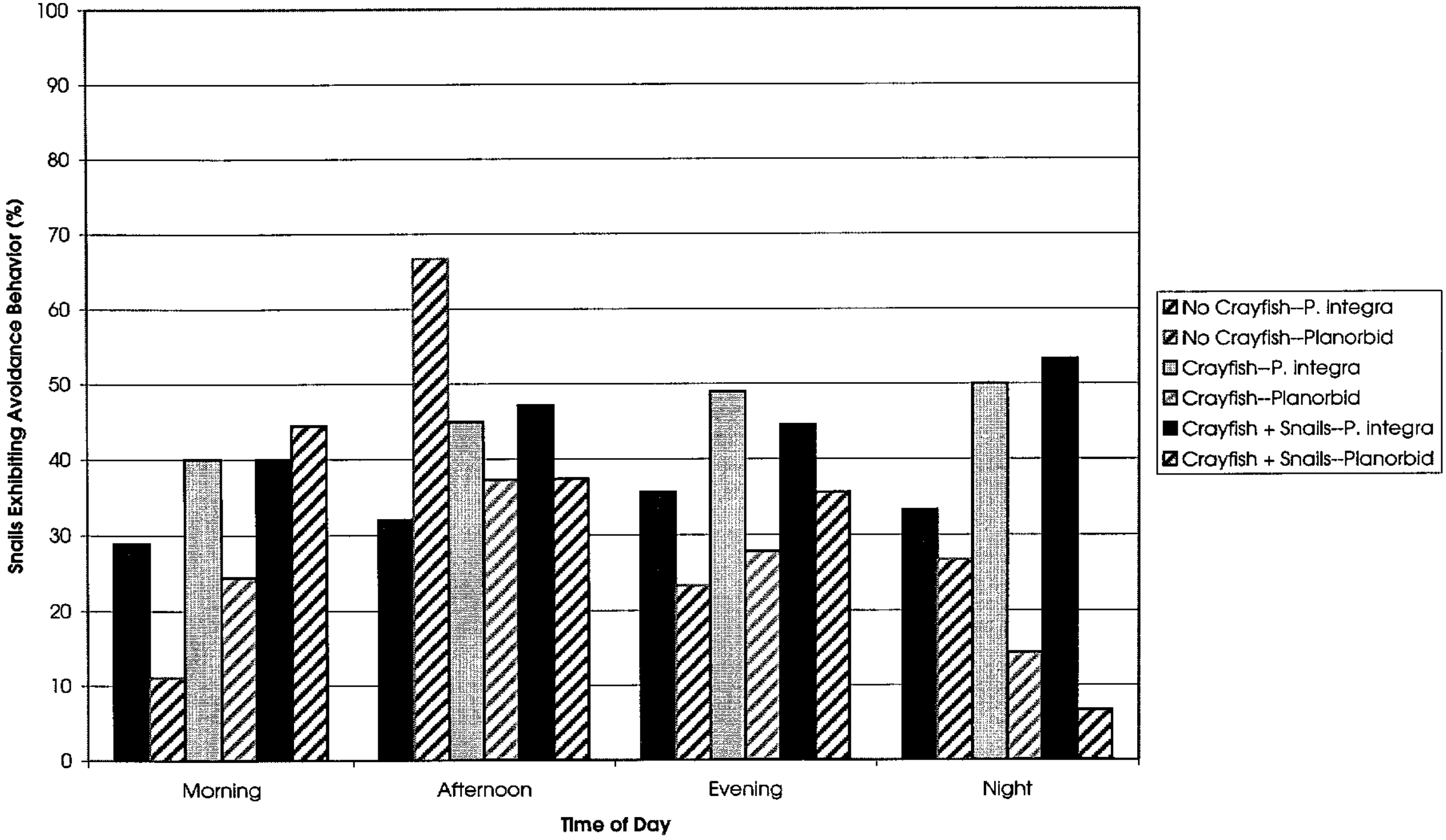


Figure 2C



Snail Avoidance Behavior Over Time in Crayfish + Snails Treatment

Figure 3



Snail Avoidance Behavior at Times of Day

Figure 1. Percent of *P. integra* and Planorbid snails exhibiting avoidance behavior in the no crayfish, crayfish, and crayfish plus snails treatments. The solid bars represent the behavior of the *P. integra* and the stripes represent that of the planorbids. Error bars are represent standard error. There is a significant difference in the behavior of both snails between the no crayfish and crayfish and snails treatments ($P < 0.05$).

Figure 2. Percent avoidance of both snails on each day that they were observed over a two-week period. On days that more than one observation was made, the percentages for each observation were averaged. Figure 2A shows the avoidance behavior of snails in the no crayfish treatment, figure 2B shows that in the crayfish only treatment, and figure 2C shows that in the crayfish plus snails treatment.

Figure 3. Avoidance behavior of the *P. integra* and planorbid snails in the morning, evening, afternoon, and night. Percentages of avoidance at these times observed over the two-week period were averaged. Values are shown for avoidance behavior in all three treatments.