Chapter 13

Microhabitat Shifts By Snails In Response To Fish Predators

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Contents

Introduction

A wide variety of terrestrial and aquatic organisms have adaptations that help them avoid predators (Chivers et al. 1997, Lürling and Van Donk 1997, Downes and Shine 1998, Lefcort 1998, Rochette et al. 1998). By increasing the likelihood that an individual prey survives long enough to reproduce, these predator-avoidance mechanisms facilitate long-term coexistence of predator and prey populations, and promote stability of biological communities by preventing localized or widespread extinctions of species (Diehl 1992, Gosselin and Chia 1995, Eklöv 1997, Brönmark and Vermaat 1998).

The primary objective of this laboratory exercise is to illustrate how one type of vulnerable prey, freshwater snails in the genus *Physella* (*Physa*), detect and avoid fish predators. Physids are ideal subjects for investigations of predator-prey interactions for several reasons. First, strong predation pressure on these thin-shelled snails by fish and other large shell-crushing predators has promoted evolution of predator-detecting chemosensory systems that are used in combination with behavioral strategies to avoid these predators (Alexander and Covich 1991a,b, Covich et al. 1994, Turner 1996, 1997, Brönmark and Vermaat 1998, McCollum et al. 1998, Stewart et al. 1999). *Physella* and other snails commonly evade predators by altering their microhabitat use. Behavioral responses include moving under objects, crawling into interstices between pebbles or other coarse substrate particles, or crawling out of the water (Turner 1997, McCollum et al. 1998, Stewart et al. 1999). Secondly, the relatively large size and slow, deliberate movements of snails make changes in behavior and microhabitat-use patterns very tractable. Finally, physid snails are relatively easy to obtain from ponds, streams, and lakes (Brown 1991, Brown 1997), or may be purchased from biological supply companies (see Appendix A for potential sources). Physids also survive and reproduce well in captivity. We have maintained a permanent laboratory population on a diet of commercial fish food.

In this exercise, an experiment is used to test hypotheses that physid snails 1) can detect predators through chemical cues originating from predators and/or conspecific snails that are injured or killed, and 2) subsequently the snails increase use of microhabitats that provide refuge from predators. This experiment is useful in supporting classroom discussions of evolutionary adaptations, predator-prey interactions, hypothesis testing, and experimental design. We also hope

to help instructors use statistics in undergraduate courses and stimulate increased student confidence and interest in data analysis. We feel this experiment and extensions of it are appropriate for use in high school biology classes, introductory college biology courses, and college-level courses in Animal Behavior, Evolution, General Ecology, and Aquatic Ecology. This experiment can be completed in 1 to 3 hours, depending upon the strength of prey responses to predators. It should take no longer than 1 hour to set up the experiment. However, we recommend the setup be completed between 1 and 24 hours before the experiment is run, so that snails in experimental microcosms have time to acclimate to initial experimental conditions. In addition, instructors should conduct preliminary trials to determine 1) what sources of chemical cues induce a behavioral response in their snails (e.g., predators or crushed snails), 2) the strength of chemical signal required to elicit this response, and 3) the amount of time required for the snails to exhibit a response that can be detected statistically. (See Notes for the Instructor: Predators.)

Materials

Collecting and Maintaining Animals in the Laboratory*

Aquatic dipnets
Enamel or plastic sorting pans**
Plastic buckets for collecting and transporting animals**
Commercial fish food
Aquaria
Aeration system (air pumps, tygon tubing, airstones)
Siphon***
Fishing pole, seine, or electroshocking unit***
Holding tank with source of oxygen***

The Experiment

Ten 19-L (5-gallon) aquaria (experimental microcosms)****
Two larger aquaria, at least 38-L (10-gallon) capacity
Enough dechlorinated water to fill all aquaria
Landscaping stones or similar coarse substrata
Ceramic tiles (optional)
Approximately 250 snails (preferably *Physella*) between 3 and 6 mm in shell length****
Molluscivorous fish (possibly optional, see Notes for the Instructor: Predators)
Ten 2-L containers

*Follow your institutional guidelines for use and care of captive animals.
**These items are only necessary if snails must be collected and transported.
***These items are only necessary if fish will be collected and transported.
****We have had success using the sizes and numbers of microcosms and snails suggested here. Smaller or larger sizes and numbers of microcosms and snails may also be used, but preliminary experiments should be conducted to determine their feasibility.

Notes for the Instructor

Snails

Although several species of snails might be used successfully in this study, we suggest using small or thin-shelled pulmonate (i.e., lunged) snails or small prosobranch (i.e., gilled) snails because these snails are most vulnerable to shell-crushing predators and are most likely to exhibit strong predator-avoidance behaviors (Alexander and Covich 1991a, b, Covich et al. 1994, Brown 1998). Pulmonates and prosobranchs constitute both subclasses of freshwater gastropods, and members of each group are easily distinguished from one another. Prosobranchs possess an operculum, a plate that covers the shell aperture (i.e., opening) when the snail withdraws into its shell (Strayer 1990, Figure 13.1). This operculum is absent in pulmonates (Strayer 1990, Figure 13.1). We feel that pulmonate snails of the genus *Physella* (also known as *Physa*) are the best snails to use in this experiment. Physid shells can be distinguished from shells of other North American freshwater pulmonates by 1) the presence of a raised spire (not a planospiral or disklike shell, as in the Planorbidae), and 2) sinistral rather than dextral orientation (Strayer 1990, Figure 13.1). A snail shell held with the aperture facing you and the spire pointing away from you is sinistral if the aperture is on your left, and dextral if it is on the right (Strayer 1990, Figure 13.1).

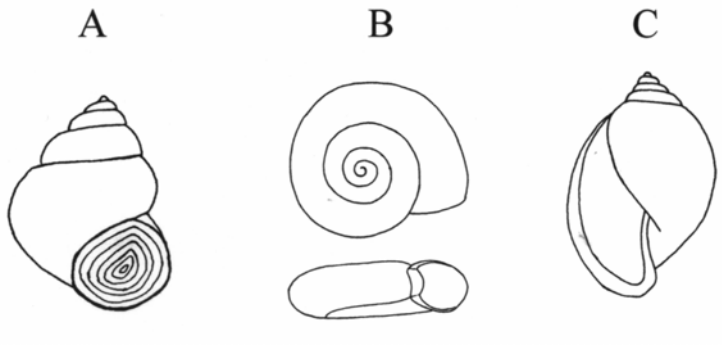

Figure 13.1. Shells of a prosobranch (i.e., gilled) snail with an operculum and dextral orientation (A), a planorbid snail (Family Planorbidae) with a planospiral shell (B), and a physid snail (Family Physidae, genus *Physella* or *Physa*) with sinistral orientation (C; drawings from Pennak 1989).

Snails can be found in almost every freshwater environment, ranging from roadside ditches to large lakes. Among the best habitats for physids are the margins of freshwater ponds, small lakes, and other slow-moving waters where aquatic vegetation is abundant (Brown 1991, 1997). Physids are easily collected using a large dipnet to collect aquatic plants, then picking or shaking snails from the plants. A white pan or tub is useful for separating snails from vegetation and physids from other snails (Brown 1998). Physids or other "pond" snails may also be obtained from biological supply companies (see Appendix A).

Regardless of what snail taxon or taxa are obtained, you should conduct preliminary experimental trials to determine if the collected snails exhibit the antipredator behavioral responses necessary to meet the objectives of this study. This is important because strengths of predator-avoidance responses differ across snail taxa, and may even differ across populations of the same species (Covich et al. 1994, Turner 1996, Brown 1998). Methods for detecting antipredator behavioral responses in snails are described in the Notes for Instructor: Predators section of this chapter.

Freshwater snails will generally survive well in the laboratory if they are held in aerated tanks and are not fed so much that ammonia levels become high in the aquaria. Physids are especially hardy, and permanent laboratory populations of these snails can be established. Several hundred physids can be maintained in an aerated, 100-L aquarium on a diet of commercial fish food. A few food pellets a day should be sufficient to promote growth and reproduction of these

snails. Mature snails will deposit horseshoe-shaped clumps of jellylike eggs on aquarium walls and other solid substrates. These eggs should hatch within a few weeks, and the young should develop into reproductively viable adults. Care must be taken to maintain snail cultures free of flatworms, leeches, water scavenger beetles, and other small predators of eggs and snails (Brown 1991). Several separate snail cultures are maintained in our laboratory in case one must be terminated due to infestation by these predators.

Predators

Snails may respond behaviorally to signals originating from either 1) injured or killed conspecifics, 2) predators themselves, or 3) by-products produced from predators eating snails (Covich et al. 1994, Turner 1996). Therefore, preliminary trials will be needed to determine what signal(s) generates a behavioral response in each snail population. Some physid snail populations respond to signals produced by tissues of injured or dead conspecifics (Turner 1996). In this case, actual predators are not required for the experiment. To determine if your snails respond to crushed conspecifics, crush a few snails, place them in an aquarium containing living snails of the same species, dechlorinated water, and some physical structure that can provide hiding places for snails. Record numbers of snails in "vulnerable" habitats every 5 minutes for a total of 30 minutes (See Notes for the Instructor: The Experiment and Student Outline for detailed methods of experimental setup and criteria for designating snails as "vulnerable"). If large numbers of snails hide in physically complex habitat or crawl above the water line after the addition of crushed snails, then predators are not needed to elicit behavioral responses in the snails. If living snails do not respond in this way within 30 minutes, replenish the chemical signal (which may be degrading by now) by adding more crushed snails to the aquarium, then record microhabitat shifts as described above. This procedure should be repeated every 30 minutes for up to 2 hours before concluding that the living snails will not respond to crushed snails alone. To verify the reliability of the experiment for a class exercise, conduct a complete preliminary experiment with replication and data analysis before using the experiment in class. (See later sections of this chapter for guidelines in designing this experiment and analyzing data). Finally, if the actual experiment is conducted without live predators, inform students that this act of crushing snails simulates actual predation and that the living snails are in effect responding to a predator.

If the use of live predators is necessary or desired, we suggest using molluscivorous fish that can consume large numbers of snails in a relatively short time period and consequently generate a strong chemical signal that living snails can detect. Fish are preferred predators because they are generally larger and consume more snails per unit time than crayfish and other potential predators. Molluscivorous fish can be obtained from ponds, lakes, and streams. They may be collected by several methods, including angling, trapping, seining, and electroshocking. Fish may also be obtained by contacting state hatcheries or privately-owned fish farms (see Appendix A). A variety of fish species may consume snails and elicit behavioral responses in these organisms. However, redear and pumpkinseed sunfish (*Lepomis microlophus* and *L. gibbosus*, respectively) are two species that readily consume snails and have elicited strong behavioral responses in snails in our studies and in other studies (Turner 1996, 1997, McCollum et al. 1998, Stewart et al. 1999; see Page and Burr 1991 or another fish identification guide to identify these species). These sunfish generally survive well in captivity, and can be maintained in aerated aquaria on diets of snails or commercial fish food. Allow at least 14 days for fish to begin feeding after they are brought into the laboratory. Fish may be maintained on a diet of prepared fish food, but they should be provided with snails for at least 1 week before the experiment begins to acclimate them to these prey. Be sure to monitor and regulate water-quality conditions within fish tanks to minimize fish mortality (e.g., oxygen, ammonia, temperature; refer to APHA (1989), Wetzel and Likens (1991), and Boyd

(1990) for water quality analysis techniques and acceptable water quality conditions). Avoid overfeeding fish. Remove feces from floors of fish tanks every 1 to 2 days to prevent accumulation of toxic chemicals and pathogens. A simple siphon consisting of a rubber tubing can be used to clean floors of tanks. Activate the siphon by submerging the rubber tube in water, then quickly pulling one end of the tube out of the water while maintaining the other end below the water. Fish that become visibly ill despite these preventative measures should be isolated from others so that disease transmission and mortality rates can be reduced. Because sick fish may not feed, only healthy fish should be used in this experiment.

The Experiment

Using previously described methods (See Notes for the Instructor: Predators), the experiment might be conducted by manually crushing snails to simulate predation. However, here we provide a protocol that involves using actual predators to evaluate antipredator responses through changes in snail microhabitat use. Before either type of experimental design is used, refer to methods described in Notes for the Instructor: Predators to determine what cues induce behavioral responses in your snail population, and what concentrations of crushed snails and/or predators are needed to generate these responses.

Complete the following steps 24 hours before the class meeting time (when the experiment will begin).

1. Construct an interstitial habitat for snails by placing a pile of landscape (i.e., patio) stones or similar coarse substrate particles in the center of each experimental microcosm (all ten 19-L aquaria; Figure 13.2). The same volume of stones should be placed in all microcosms to eliminate habitat variability as a potential explanation for differences in snail microhabitat use between experimental treatments. We typically add 1 liter of stones to each microcosm. This is equivalent to the amount of submerged stones required to displace the water line in a 2-liter volumetric container from the 1-liter to 2-liter mark. Spaces between stones in microcosms should be large enough to allow snails to crawl into these interstices, but small enough so this habitat will provide a refuge from fish and other large predators. Stones should also be stacked in multiple layers so that snails inhabiting interstices are completely hidden from view (Figure 13.2). Other types of habitat (e.g., cover) can also be provided in the form of ceramic tiles with one end resting just above the microcosm floor on a small stone. Snails seeking refuge from predators should inhabit undersides of these tiles.

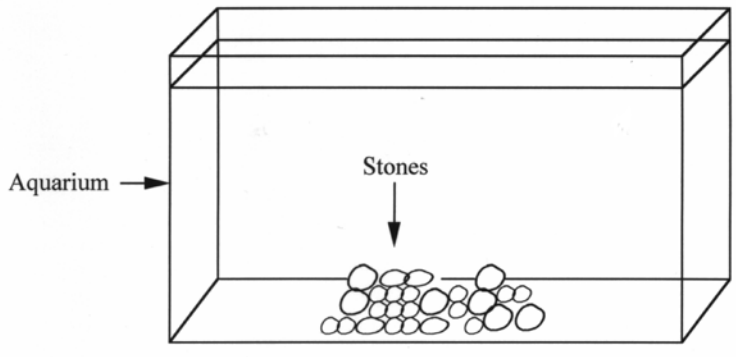

Figure 13.2. Example of an experimental microcosm used in this experiment.

2. Fill experimental microcosms to within 5 cm of their tops with dechlorinated water. Use of dechlorinated water is necessary because chlorine kills aquatic organisms. If your water supply is chlorinated, remove this chemical with a chlorine-detoxifier solution (available from Fisher Scientific, see Appendix A for address). Alternatively, chlorine is volatile and is naturally lost from standing water over time. If you allow chlorine to be reduced by volatization, you will need to fill microcosms several days before adding organisms. Chlorine volatization can be facilitated by aerating water.
3. Add snails to experimental microcosms. Release 20 snails with shell lengths between 3 and 6 mm at the water surface and in the center of each microcosm. Ideally, you should allow snails to disperse and become acclimated to initial environmental conditions for the next 24 hours. If this is not possible, try to give the snails at least 1 hour to acclimate and disperse before beginning the experiment.

Note: We have found that *Physella* from 3 to 6 mm in shell length (measured from the tip of the spire to the extreme tip of the aperture) respond well to cues from both molluscivorous fish and crushed snails. Other snail sizes and taxa may be used, but smaller snails are difficult to see in aquaria, and larger snails with a possible size refuge from predators may not respond well to predator cues. We have found that 20 snails per microcosm allows for enough statistical power to detect effects of chemical cues on snail habitat use. However, densities of 10 to 15 snails per microcosm may be sufficient if behavioral responses to predators are strong.

4. Fill two tanks of at least 38-liter capacity with dechlorinated water. One of these tanks will be used to generate chemical cues for microcosms constituting the "predator-cue" treatment, and the second will provide microcosms in the "predator-free" treatment with water lacking these chemical signals. Aerate water in both tanks using a pump, airline tubing, and airstones, but do not equip either tank with an activated charcoal filter because this will eliminate fish odors from the water. Place fish or other predators in the large predator-cue tank several days before the experiment is scheduled to begin so that predators can acclimate to tank conditions and begin feeding. Use only predators that have been observed to feed on snails in preliminary trials. The actual numbers and sizes of predators used will need to be pre-determined from numbers and sizes of predators required to elicit behavioral responses in your snails. We have observed strong microhabitat shifts in snails inhabiting 19-liter experimental microcosms when 2 liters of water in microcosms were exchanged with 2 liters of water from a 100-liter tank containing four redear sunfish (86 to 105 mm total length) that were fed 10 large snails 10 minutes before the experiment began. If necessary, chemical signal concentrations can be increased by using smaller (e.g., 38-liter) tanks to generate the chemical cues, by using smaller experimental microcosms, or by feeding fish in predator-cue generating tanks and repeating water exchange procedures every 30 minutes. We suggest starving predators for 24 hours prior to the pre-experimental feeding period (see below) so that hunger levels will be maximized and the likelihood of high pre-experimental predation rates and chemical-signal strengths are increased.

   Complete the following step 10 minutes before the experiment begins.

5. Provide fish in the predator-cue generating tank with between 10 and 20 physid snails. Add nothing to the predator-free tank. The actual number of snail prey needed to generate a sufficiently strong chemical signal in predator-cue microcosms will need to be pre-determined. (See Notes for the Instructor: Predators).

Student Outline

The following steps, constituting the actual experiment, are completed during the class meeting time.

1. The class is divided into 10 groups of students, and each group is provided with one of the 19-liter experimental microcosms (aquaria). Five student groups (e.g., students at odd-numbered tables) and their microcosms are assigned to the predator-free treatment, and remaining student groups and their microcosms are assigned to the predator-cue treatment.

2. Each group records initial numbers of snails in their microcosm that occupy microhabitats where they would be vulnerable to large predators. Snails are considered “vulnerable” if they are seen on microcosm walls, floors, or upper surfaces of stones or tiles where visually-oriented predators (i.e., redear and pumpkinseed sunfish) could easily find and eat them. In contrast, snails inhabiting undersides of tiles, interstitial spaces between stones, or that are found above the water line on microcosm walls are considered "invulnerable" or inaccessible to these predators. All non-visible snails are probably inhabiting interstices between stones or undersides of tiles and should be considered invulnerable. Only counts of vulnerable snails are needed for statistical analysis.

3. After these pre-experimental data are recorded, remove 2 liters of water from your microcosm and discard this water. Remove water carefully to avoid damaging or disturbing snails. Now transfer 2 liters of water from the appropriate large tank containing either fish (predator-cue generating tank) or dechlorinated water only (the predator-free tank). Microcosms in the predator-free treatment will receive water from the tank containing only dechlorinated water. Students working with microcosms constituting the predator-cue treatment need to replace water removed from their microcosm with water from the predator-cue tank containing fish recently fed snails.

4. After these water exchanges are made, observe behavioral responses and microhabitat shifts of snails in your own microcosm and those of students managing microcosms of the treatment differing from yours. Every 5 minutes, record the number of vulnerable snails in your own microcosm. As snails in the predator-cue treatment begin to detect and respond to predation risk, numbers of vulnerable snails in these microcosms should decline. It is important to monitor temporal changes in numbers of vulnerable snails so that 1) the instructor can identify a good time to end the experiment and 2) declines in snail refuge use, and thus chemical signal strength, can be detected in the predator-cue treatment. If snails in predator-cue treatments begin to emerge from hiding following an initial period of apparent increases in refuge use, the chemical signal in the microcosm is probably degrading. If this happens, or if strong microhabitat shifts are not observed in the predator-cue treatment within 30 minutes, repeat the water exchange procedures described above and continue the process of recording changes in numbers of vulnerable snails in microcosms. Water exchanges may need to be made three or four times before clear differences in snail microhabitat use occur between treatments. Therefore, the experiment could take up to 2 hours to complete. The experiment should end when 1) obvious declines in numbers of vulnerable snails have occurred in microcosms of the predator-cue treatment, and 2) few if any snails in the predator-cue treatment are still crawling about in microcosms. If chemical signals are sufficiently strong and snails have responded to these signals, the number of vulnerable snails should decline by at least 50% in microcosms of the predator-cue treatment.

5. At the end of the experiment, record post-experimental counts of vulnerable snails using the same methods for collecting pre-experimental data. Use paired-sample t tests to analyze class data and determine if numbers of vulnerable snails really differ in predator-free and predator-cue treatments. (See Appendices B and C).

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Appendix A: Suppliers of Organisms

Pond Snails (Call regarding availability of *Physella* or *Physa*.)

Carolina Biological Supply, P.O. Box 6010, Burlington, NC 27216-6010; 336-584-7686
Fisher Scientific, 485 South Frontage Road, Burr Ridge, IL 60521; 1-800-955-1177
Ward's Biology, P.O. Box 92912, Rochester, NY 14692-9012; 1-800-962-2660

Redear or Pumpkinseed Sunfish

Mr. Steve Muich, Missouri Department of Conservation, Route 1, Box 18, Hunnewell, MO 63443; 573-983-2201
Jack's Fish Farm, 14671 120th Avenue, Grand Haven, MI 49417; 616-846-5844
Northeastern Aquatics, 1 Kerr Road, Suite 2, P.O. Box 575, Rhinebeck, NY 12572; 914-876-3983
Also see Sources of Fish for Stocking Recreational Ponds on North Carolina's aquaculture web page (http://www.agr.state.nc.us/aquacult/recreational.html)

Appendix B: Data Analysis - The Paired-sample t Test

Paired-sample t tests can be used to test the hypothesis that perceived presence of predators causes declines in numbers of vulnerable snails. (See Zar 1999 for complete descriptions of this statistical test, assumptions that must be met for its proper use, and additional examples of its applications). Two paired-sample t-tests are needed to analyze our data. In one of these we test for pre- and post-experimental differences in numbers of vulnerable snails in the predator-free treatment. This is necessary to rule out physical disturbance associated with water exchange procedures as an important cause for shifts in microhabitat use, and to separate this disturbance from predator-cue effects in the predator-cue treatment. We should not find statistically significant changes in numbers of vulnerable snails in the predator-free treatment. However, in a second paired-sample t test that compares pre- versus post-experimental numbers of vulnerable snails in the predator-cue treatment, we should see statistically significant declines in numbers of vulnerable snails by the end of the experiment.

The paired-sample t test: An example

Here we provide a complete example of a paired-sample t test using simulated pre- and post-experimental data from the predator-cue treatment. A paired-sample t test is used to determine the significance of the difference between two sets of paired data (Zar 1999). Pre- and post-experimental counts of vulnerable snails in each microcosm are paired for this analysis. These pairings are based on our expectation that post-experimental counts of vulnerable snails in each microcosm should be affected by pre-experimental counts of vulnerable snails in addition to introduction of predator cues (Zar 1999). Procedures for conducting a paired-sample t test are provided below.

1. Using class data and the data analysis tables provided below (Table 13.1) and in Appendix C, fill in the student group or microcosm number (column number one) and pre- (initial) and post-experimental (final) numbers of vulnerable snails in all microcosms constituting a specific treatment (column numbers two and three). In this example we assigned even-numbered student groups to the predator-cue treatment.

2. Calculate "difference values" (d) for each pair of observations (pre- and post-experimental numbers of vulnerable snails). For each group or microcosm, subtract the number of post-experimental vulnerable snails (column number three) from the number of pre-experimental vulnerable snails (same row in column number two). Enter difference values in the fourth column of the table.

3. Calculate the mean, or average, difference value $\bar{d}$ from the difference values (d) present in column number four.

   $\bar{d} = (\sum d) \div n = 31 \div 5 = 6.2$

   Where $\sum$ = "sum"

4. Subtract the mean difference value from each individual difference value ($\bar{d}$ - d). Enter these values in the fifth column of the table. The sum of these values should be 0.

5. Square each of the values present in the fifth column of the table $(\bar{d} - d)^2$, and enter these values in the sixth column of the table. Then sum these values and enter the value in the last row of the sixth column.

Table 13.1. Completed data analysis worksheet for evaluating differences in pre- and post-experimental numbers of vulnerable snails in the predator-cue treatment.

Group number	Initial number vulnerable (X1)	Final number vulnerable (X2)	Difference values (d = X1 - X2)	$d - \bar{d}$	$(d - \bar{d})^2$
2	13	7	6	-0.2	0.04
4	11	4	7	0.8	0.64
6	16	6	10	3.8	14.44
8	8	5	3	-3.2	10.24
10	10	5	5	-1.2	1.44
n = 5			$\Sigma = 31$	$\Sigma = 0$	$\Sigma = 26.80$

6. Determine the number of degrees of freedom (DF) in the sample. This value is equal to the number of observations, or paired observations in our case, minus one.

$$DF = n - 1 = 5 - 1 = 4$$

7. Calculate the sample variance, or the variance of difference values (s^2d).

$$s^2d = \Sigma (d - \bar{d})^2 \div DF = 26.80 \div 4 = 6.70$$

8. Calculate sd, the standard deviation of difference values.

$$sd = (s^2d)^{1/2} = (6.70)^{1/2} = 2.59$$

9. Calculate $\overline{sd}$, the standard error of the mean difference value.

$$\overline{sd} = sd \div (n)^{1/2} = 2.59 \div (5)^{1/2} = 1.16$$

10. Calculate the t-statistic

$$t = \bar{d} \div \overline{sd} = 6.2 \div 1.16 = 5.34$$

11. Table 13.2 provides some critical values of the t distribution. Critical values for the paired-sample t test are based on: 1) the significance level chosen, or accepted probability of erroneously concluding that two paired samples differ when they actually do not (usually $p = 0.05$), 2) whether the investigator wishes to conduct a one- or two-tailed test (the two-tailed test provides the most conservative result), and 3) the number of degrees of freedom in the sample (Zar 1999). Table 13.2 provides critical t-statistic values for two-tailed tests and a 0.05 significance level. To identify the appropriate critical value in the table, first refer to the left column of the table to find a number corresponding to the number of degrees of freedom in your sample. Then look at the value in the adjacent column of the same row to find the appropriate critical value. Our hypothetical sample in the example above has 4 degrees of freedom. Therefore, the critical value is 2.776 and our t-statistic must equal or exceed this value if we are to conclude that there is a statistically significant difference in pre- and post-experimental numbers of vulnerable snails.

    Since our t-statistic of 5.34 has a greater value than the critical value of 2.776, we conclude that numbers of pre- and post-experimental vulnerable snails differ at the 0.05 significance level ($p = 0.05$). A visual examination of vulnerable snail counts in combination with our known treatment reveal the most likely reason for differences between pairs of observations: the number of vulnerable snails declined in response to the perceived presence of predators. This can be verified by conducting a second paired-sample t-test comparing pre- and post-experimental numbers of vulnerable snails in the predator-free treatment. Recall that in this treatment, water exchanges were made as in the predator-cue treatment, but no predator-cues were added to experimental microcosms. If snails in predator-cue treatments actually responded to predator-cues and not to physical disturbances caused by water exchanges, we should see no statistically significant difference in pre- and post-experimental numbers of vulnerable snails in the predator-free treatment (i.e., $p > 0.05$).

Table 2. Some critical values for the paired-sample t test (from Zar 1999).

DF	Critical value $p = 0.05$ (two-tailed)
2	4.303
3	3.182
4	2.776
5	2.571
6	2.447
7	2.365
8	2.306
9	2.262
10	2.228

Appendix C: Data Analysis Worksheets

1. Predator-free treatment

Group number	Initial number vulnerable (X1)	Final number vulnerable (X2)	Difference values (d = X1 - X2)	$d - \bar{d}$	$(d - \bar{d})^2$
n =			Σ =	Σ =	Σ =

What is your mean, or average, difference value ($\bar{d}$)? _____

$\bar{d} = (\sum d) \div n =$

How many degrees of freedom (DF) are in your sample? _____

$DF = n - 1 =$

What is the variance, or the variance of difference values (s^2d) in your sample?_____

$s^2d = \sum (d - \bar{d})^2 \div DF =$

What is the standard deviation of difference values (sd) in your sample?_____

$sd = (s^2d)^{1/2} =$

What is $\bar{sd}$, the standard error of the mean difference value?_____

$\bar{sd} = sd \div (n)^{1/2} =$

What is your t-statistic?_____

$t = \bar{d} \div \bar{sd} =$

What is your critical value? _____

A statistically significant difference exists if the value of your test statistic equals or exceeds that of the critical value ($p \leq 0.05$; Table 2). Is there a statistically significant difference in pre- and post-experimental numbers of vulnerable snails?

What do you conclude from looking at your *p*-value and the class data? _____

_____

2. Predator-cue treatment

Group number	Initial number vulnerable (X1)	Final number vulnerable (X2)	Difference values (d = X1 - X2)	$d - \bar{d}$	$(d - \bar{d})^2$
n =			Σ =	Σ =	Σ =

What is your mean, or average, difference value (d)? $\bar{d}$ _____

$\bar{d} = (\sum d) \div n =$

How many degrees of freedom (DF) are in your sample? _____

$DF = n - 1 =$

What is the variance, or the variance of difference values (s^2d) in your sample?_____

$s^2d = \sum (d - \bar{d})^2 \div DF =$

What is the standard deviation of difference values (sd) in your sample?_____

$sd = (s^2d)^{1/2} =$

What is $\bar{sd}$, the standard error of the mean difference value?_____

$\bar{sd} = sd \div (n)^{1/2} =$

What is your t-statistic?_____

$t = \bar{d} \div \bar{sd} =$

What is your critical value? _____

A statistically significant difference exists if the value of your test statistic equals or exceeds that of the critical value ($p \leq 0.05$; Table 13.2). Is there a statistically significant difference in pre- and post-experimental numbers of vulnerable snails?

What do you conclude from looking at your *p*-value and the class data?

_____

Appendix D: Additional Experiment

The following experiment is an extension of the experiment described previously.

Effectiveness of physical structural complexity in reducing fish effects on snail mortality

In a previous experiment, Stewart et al. (1999) evaluated combined effects of physical structural complexity and antipredator behavior of snails (i.e., increased use of refuges) in reducing fish effects on snail mortality. In the experiment described here, levels of structural complexity in the form of landscape stones and artificial plants are manipulated, and efficacy of this structure in reducing short-term fish effects on snail mortality is quantified. Because this experiment involves an assessment of the value of physical structure (i.e., refuges) in reducing predator effects on prey, this exercise is a logical extension to the experiment described in the main body of this chapter.

Like the setup of the predator-free and predator-cue microcosms described earlier, good results from this experiment are most likely obtained when structures and snails are introduced to experimental microcosms the day before the experiment is scheduled to begin. We have used this lab to illustrate how linear regression statistical techniques can be used in ecological experiments. In this case, we use several experimental microcosms that contain variable amounts of physical structure, ranging from aquaria lacking structure to those with plastic plants anchored to the floor by several layers of stones (See the Notes for the Instructor: The Experiment for methods used to measure volumes of these objects). Twenty four hours after structures and snails are placed in microcosms, we introduce one fish to each microcosm, and allow fish to feed until all or nearly all snails in microcosms without structure have been consumed. Some snails in microcosms without physical structure may avoid fish by crawling above the water line. If this happens, we usually end the experiment when no snails remain below the water line in these microcosms. Because we allow fish to feed until they can find no more snails, it is important that initial numbers and sizes of snails in microcosms do not exceed those which fish can eat in a short time period. In a previous experiment (Figure 13.3), we found that individual redear sunfish approximately 75 mm long in microcosms lacking structure ate 15 snails in 5 to 10 minutes.

We end this experiment by removing fish and then counting numbers of snails remaining in each microcosm. Stones and plants, if present, are carefully removed and examined to quantify numbers of remaining snails. Actual locations of surviving snails (e.g., above the water line, in interstices between stones, etc.) can also be recorded. Students will likely observe snails attempting to find refuges soon after fish begin feeding, suggesting that living snails are detecting and responding to the predator. Feeding rates of fish can also be determined by dividing number of snails consumed by the amount of time fish were allowed to forage. Lengths or weights of fish may also be measured so that any effects of variable fish sizes on snail survivorship can be quantified. This can be done by incorporating the independent variable “fish size” into the regression model along with the independent variable “volume of structure.” (See McClendon 1994 for descriptions and examples of simple and multiple linear regression techniques).

Results of analysis of actual class data from this experiment are provided in Figure 13.3. Note that 46% of the variation in snail survivorship is explained by the volume of the physical structures, and that there is a statistically significant positive relationship between the volume of physical structure and snail survivorship (Figure 13.3). Data were analyzed using the computer program SYSTAT 5.2.1 (SYSTAT Inc., Evanston, Illinois).

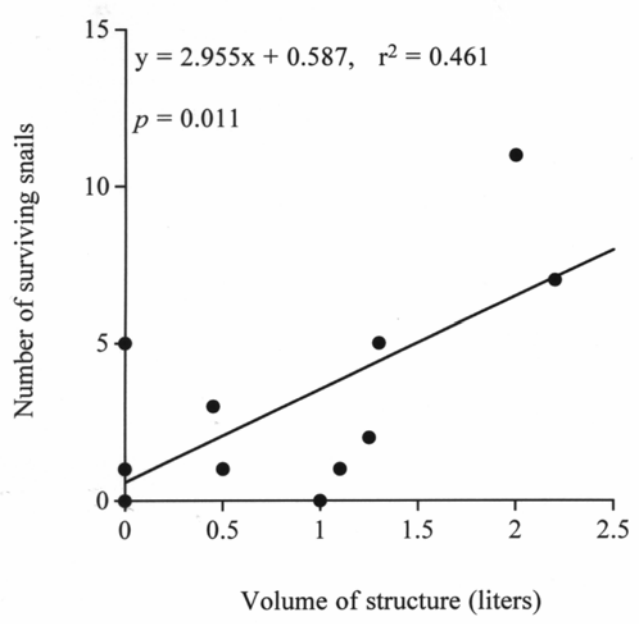

Figure 13.3. Number of surviving snails as a function of physical structure volume after 10 minutes of fish predation (n = 12 observations, 15 snails were initially present in each microcosm).