

**The Impact of Non-Morphological Criteria
on the Feeding Preferences of *Lymnaea
stagnalis* and *Physa* sp.**

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Abstract

It has recently been shown that herbivory plays an important role in aquatic macrophyte communities, but the role of snail herbivory specifically in shaping macrophyte communities is controversial. A recent study has shown, however, that the freshwater snails *Physa sp* and *Lymnaea stagnalis* consume macrophytes only when presented with no other food choice. Another study found that plant morphology plays a role in snail feeding choices, as *Physa* and *Lymnaea* preferentially consumed macrophytes reconstituted in gel form over those in natural form. In this study, feeding preference tests were run comparing- 1) macrophyte (*Potamogeton richarsonii*) gel and periphyton gel and 2) macrophyte gel and periphyton in its natural form. In the first test, there was a preference for periphyton gel, demonstrating that although morphology plays a role in food selection, periphyton is preferred for other reasons as well. The ratio of food types consumed was similar in the first and second tests, indicating that the gel itself does not affect feeding preferences.

Introduction

Macrophytes play an important role in lake ecosystems. Variation in macrophyte levels can affect the physical and chemical properties of lakes as well as other biota, which they provide with refuge, growth substrate and food (Carpenter and Lodge 1986). Until recently, herbivory has been considered an unimportant factor in macrophyte dynamics. However, recent studies have found that a variety of herbivores, including fish, crayfish, turtles, and aquatic birds, can affect macrophyte communities (Lodge 1998).

The role of freshwater snails in macrophyte herbivory has been a subject of some controversy. Snails are scrape-feeders whose primary food source is periphyton (Bronmark 1989). Analysis of crop contents has consistently indicated that live macrophytes make up only a very small part of freshwater snails' diets (Bronmark 1989). However, exclusion experiments have found that macrophyte growth is greater in areas where snails are excluded (Sheldon 1987). Bronmark (1990) suggests, however, that variation in the populations of other invertebrates can account for these results. Despite

the questionable role of snail herbivory on macrophytes, recent papers have used *Lymnaea stagnalis* to study comparative herbivory of different macrophyte species (Elger et al. 2002, Elger and Barrat-Segretain 2002).

Chambers and Lodge (Unpublished data) carried out microcosm experiments comparing the consumption of periphyton and *Potamogeton richardsonii* by the freshwater snails *Physa* sp. and *Lymnaea stagnalis*. They found that the snails consume periphyton preferentially to macrophytes. In fact, *Lymnaea* only consumed significant amounts of macrophyte if given no other option and *Physa* avoided macrophytes even if no other food was available.

Bronmark (1989) suggests that snails' avoidance of macrophytes as a food source could be due to a high C/N ratio, plant structure, or secondary plant compounds. Crone (Unpublished data) tested the influence of morphological structure on snail feeding preferences. The experiment compared the consumption of *P. richardsonii* leaves to that of leaves which were ground to a powder, then reconstituted as a gel. The snails' preference for macrophyte gel indicates that morphological structure does play a role in snail feeding choices. However, it is also possible that the snails' preference was influenced by the alginic gel used, rather than the change in morphology.

To control for the influence of the gel, this experiment compares the relative preferences of snails when offered macrophyte gel and periphyton gel and when offered macrophyte gel and periphyton in its natural state. If the relative preferences are similar in these two tests, it can be inferred that the gel itself is not altering feeding choices. By comparing *Lymnaea* and *Physa* preferences for macrophyte gel and periphyton gel, this experiment examines whether differences in structural morphology are sufficient to

explain snails' preference for periphyton or if other criteria also affect feeding preferences.

Methods

Physa sp. snails were collected from Tenderfoot Lake during June and July 2002. *Lymnaea stagnalis* were collected during the same time period from both Tenderfoot Lake and Forest Lake due to the low numbers of *Lymnaea* in Tenderfoot. Both Tenderfoot and Forest Lakes are located in Vilas County, Wisconsin, near the Michigan border. After collection, the snails were maintained in aquariums in the wet laboratory at the University of Notre Dame Environmental Research Center (UNDERC), also located in Wisconsin/Michigan. The aquariums were filled with ground water and contained rocks covered in periphyton collected from Tenderfoot Lake as a food source for the snails.

Potamogeton richardsonii was also collected from Tenderfoot Lake. The entire shoot portion of the plant was used in the experiments. After collection, the macrophytes were scrubbed to remove epiphytes, snails, and any other organisms from the plants. Periphyton was collected from stones in Tenderfoot Lake. Any organisms were also removed from the periphyton. Both the macrophytes and periphyton were freeze-dried. *P. richardsonii*, periphyton, and snails were all collected from the same 300 yard section along the shore of Tenderfoot Lake

The macrophytes and periphyton were ground into a powder with mortar and pestle. The powder was sifted through a 1000 micron mesh sieve to ensure that both foods consisted of similar particle sizes. Each powder was then mixed with 2% solution

of sodium alginate to form a gel solution. The amount of solution added was calculated to restore the original water content of the macrophytes and periphyton. Periphyton contained 66% water and macrophytes contained 90% water. The solutions were then spread onto small tiles to which a piece of window screen consisting of 720 squares was glued. The screens held the gel in place and allowed the amount of food consumed to be quantified. After the gel solution was placed on the tiles, it was scraped level with the screen using a razorblade. To harden the gel, each tile was then dipped in a 0.25 M solution of calcium chloride.

Six prepared tiles of each type were dried in a drying oven for twenty-four hours and then weighed. The gels were then scrubbed off, the tiles dried for another 24 hours, and weighed again. This allowed the average biomass on each type of tile to be estimated.

To prepare tiles of periphyton in natural form, hereafter called natural periphyton, twenty-two tiles were placed on a tray and submerged in Tenderfoot Lake. The tray was covered with a screen to exclude macro-organisms. Any organisms observed on the tray were removed. The tiles remained in the lake for two weeks. The amount of periphyton which grew varied from tile to tile, and on no tile were all screen squares covered. The number of empty squares on each tile was recorded. In order to present the snails with similar amounts of both food types, the eight tiles with the least periphyton were discarded. Four of the remaining tiles were randomly selected from those remaining to estimate the average biomass of the periphyton on the tiles.

The food preference experiments were carried out in a UNDERC laboratory. For the experiment, glass dishes 18 cm in diameter and 7 cm deep were 3/4 filled with water

from Tenderfoot Lake. Two tiles were placed adjacent to each other in the center of each dish. Two snails were placed on opposite sides of the bowl so that they were an equal distance from each type of food. This arrangement ensured that snails had an equal likelihood of finding either food first. Placing the tiles next to each other decreased the likelihood that snails would continue to eat one food type due to the inaccessibility of the other type. Two snails were placed in each bowl.

While the experiments were running, the replicates were checked each hour throughout the daytime (8 AM to 1 AM). The number of snails located on each tile was recorded. The position of the snail was determined based on the location of its mouth. Any snail that appeared to have died, was removed and replaced by another snail of the same species. Each replicate was ended when at least a third of one food type had been consumed. This was to prevent increased consumption of one type of food due to a scarcity of the other.

The number of empty squares was recorded for each tile. A square was considered empty if any of the tile was visible beneath the food. This was found to be the most consistent measurement criteria. The number of squares on each tile were counted twice, and then averaged if the counts differed. For macrophyte gel and periphyton gel tiles, the number of squares consumed equaled the number of empty squares at the end of the experiment. For natural periphyton tiles, the number of squares consumed equaled the number of empty squares after the experiment minus the initial number of empty squares. No control for autogenic change was necessary, since the measure of consumption, number of empty squares, would not undergo autogenic change during the time period studied (Peterson and Renaud 1989).

Experiment 1. One tile of periphyton gel and one tile of macrophyte gel were placed in the center of each bowl. Eleven replicates each of *Physa* and *Lymnaea* were carried out. The *Physa* replicates began at 12:00 noon on July 10th, 2002 and ran until 1 PM on July 11th. The *Lymnaea* replicates began at 1:00 PM on July 15th and ran until 2:00 AM on July 16th.

Experiment 2. One tile of natural periphyton and one tile of macrophyte gel were placed in the center of each bowl. Ten replicates of *Lymnaea* were used in this experiment. The experiment began at 11:00 AM on July 17th and ran until 5:00 PM that evening.

A T-test was used to compare the consumption of the two food types for each experiment and species (Peterson and Renaud 1989). The ratio of macrophyte consumed to periphyton consumed was calculated. The consumption ratios of *Lymnaea* and *Physa* in experiment 1 and of *Lymnaea* in experiments 1 and 2 were compared using a T-test. Finally, the location observations were summed separately for *Physa* in experiment 1, *Lymnaea* in experiment 1, and *Lymnaea* in experiment 2 to form time budgets. This observed data was compared using a chi-squared test to expected values representing equal time spent on each type of food tile.

Results

When given the feeding choice of either macrophyte gel or periphyton gel, both *Lymnaea* and *Physa* snails preferentially consumed periphyton gel. *Lymnaea* in the control experiment also preferred real periphyton to macrophyte gel. The replacement of periphyton gel with real periphyton did not significantly affect feeding behavior.

Tiles containing macrophyte gel had a mean biomass of 55.7 ± 8.2 mg. Tiles containing periphyton gel had a mean biomass of 176.8 ± 24.7 mg. Tiles containing natural periphyton had a mean biomass of 241.3 ± 46.0 mg.

In experiment 1, *Physa* consumed 14.32 ± 8.14 (mean \pm standard deviation) squares per tile of macrophyte gel and 201.82 ± 125.17 squares per tile of periphyton gel. In the same experiment, *Lymnaea* consumed 159.86 ± 119.91 squares per tile of macrophyte gel and 262.68 ± 82.51 squares per tile of periphyton gel. In experiment 2, *Lymnaea* consumed 170.70 ± 126.07 squares per tile of macrophyte gel and 209.35 ± 57.76 squares per tile of real periphyton. These results are shown in figure 1.

A paired, two-tailed Student's T-test was performed. *Physa* consumed significantly more periphyton gel than macrophyte gel ($P < 0.001$). There was not a significant difference between the periphyton gel and macrophyte gel consumed by *Lymnaea* ($P = 0.10$). However, the data did show a trend that *Lymnaea* preferentially consumed periphyton gel. There was also not a significant difference between the consumption by *Lymnaea* of real periphyton and macrophyte gel ($P = 0.47$).

In experiment 1, the ratio of macrophyte gel to periphyton gel eaten by *Physa* was 0.093 ± 0.078 . For *Lymnaea* the ratio was 0.750 ± 0.605 . When offered real periphyton and macrophyte gel, the ratio of macrophyte gel to real periphyton was 0.948 ± 0.801 . Using an unequal variance, two-tailed Student's T-test, *Lymnaea* consumed a significantly higher ratio of macrophyte gel to periphyton gel than *Physa* ($P < 0.01$). The ratios of consumption by *Lymnaea* in experiments 1 and 2 were not significantly different ($P > 0.5$).

In experiment 1, there were 90 observations of *Physa* on the periphyton gel tiles and 27 observations of *Physa* on the macrophyte gel tiles. In the same experiment, there were 67 observations of *Lymnaea* on the periphyton gel tiles and 25 observations of *Lymnaea* on the macrophyte gel tiles. In the second experiment, *Lymnaea* were observed on macrophyte gel tiles 22 times and on the natural periphyton tiles 25 times (Figure 2). A chi-squared test was used to compare the actual time budget with the null hypothesis of equal time spent on both tiles. Both *Lymnaea* ($P < 0.001$) and *Physa* ($P < 0.001$) spent significantly more time than expected on the periphyton tile in the gel experiment. In the control, there was not a significant difference between the observed and expected values ($P > 0.6$).

Discussion

These results indicate that both *Physa* and *Lymnaea* prefer periphyton to macrophytes, even when differences in morphology are removed. Thus, some other factor or combination of factors must also play a role in food choices. The possibilities suggested by Bronmark (1989) were carbon to nitrogen ratio, plant structure, and secondary chemicals. Plant morphology at the macroscopic level has been eliminated as a factor, but other smaller structural differences such as differences in cell walls, lignified tissue, remain. These may affect the digestion of periphyton or macrophytes and therefore influence feeding choice. The carbon to nitrogen ratio is unlikely to have been a factor in the preference for periphyton since both periphyton and macrophytes in Tenderfoot Lake have similar carbon to nitrogen ratios (Kreps, unpublished data). Secondary chemicals may play a role in the feeding preference since it is quite likely that the macrophytes

would produce more chemicals for herbivory deterrence. Recent studies have shown that many organisms are not deterred by macrophyte secondary chemicals (Lodge et al. 1998), but it is possible that these chemicals do affect snails.

One potential cause of the preference for periphyton gel is that the macrophyte gel contained more water and less plant matter per volume than the periphyton gel did. The snails may have chosen periphyton gel due to the higher concentration of plant matter. However, the proportion of water in the two plant gels is the same as that of the plants in their natural state. Therefore, although the concentration may have influenced the result of this experiment, it is also likely to influence feeding preferences in the natural environment.

The results show that *Lymnaea* have a somewhat, but not significantly, greater preference for periphyton gel relative to macrophyte gel than for natural periphyton relative to macrophyte gel. The role of alginic gel in snail's feeding preferences thus remains unclear. The potential magnitude of influence of the gel however is fairly small. It is unlikely to account for the highly significant differences in consumption of macrophyte gel and macrophyte leaf. Additionally, the slight preference for periphyton gel over natural periphyton may be a result of morphological changes, rather than a result of the gel. Though periphyton is affected to a lesser degree than macrophytes, the process of forming it into a gel likely affects its morphology as well. Thus periphyton gel may be slightly easier to digest than natural periphyton and therefore preferred.

The fact that *Lymnaea* consumed a higher ratio of macrophyte gel to periphyton gel than *Physa* is consistent with the findings of Chambers and Lodge. *Lymnaea* consumed macrophytes when no other food was available, but *Physa* presented with only

macrophytes had empty crops. Crone's findings that *Physa* ate more macrophyte gel and less macrophyte leaf than *Lymnaea* indicate that morphological structure affects *Physa* more than *Lymnaea*. This is not surprising since *Lymnaea* are much larger. However, the difference between *Physa* and *Lymnaea* feeding preferences was not eliminated by the removal of macrophyte morphological structure. Therefore, not just morphological structures, but also other characteristics, have differing effects on the two species' preferences.

The time budget data for experiment 1 is consistent with the conclusion that snails prefer periphyton gel to macrophyte gel. Both species spent significantly more time on periphyton gel tiles. However, in experiment 2, *Lymnaea* did not spend significantly more time on one tile than another. Time budget is a less direct and likely less accurate measure of preferences however, since snails located on the tiles were not always actively eating (personal observation).

The first study of this series, demonstrated that *Physa* and *Lymnaea* exhibit a strong preference for periphyton rather than macrophytes as a food source (Chambers and Lodge). Together with the control for the effects of alginic gel from this study, Crone's results indicate that plant morphology plays a role in snail feeding choices. However, this study demonstrates that other criteria play a role as well. Macrophyte morphology and other characteristics are more inhibitory of herbivory by *Physa* than the larger *Lymnaea*. Overall, freshwater snails are unlikely to consume macrophytes for both morphological and non-morphological reasons.

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Feeding Preference of Freshwater Snails

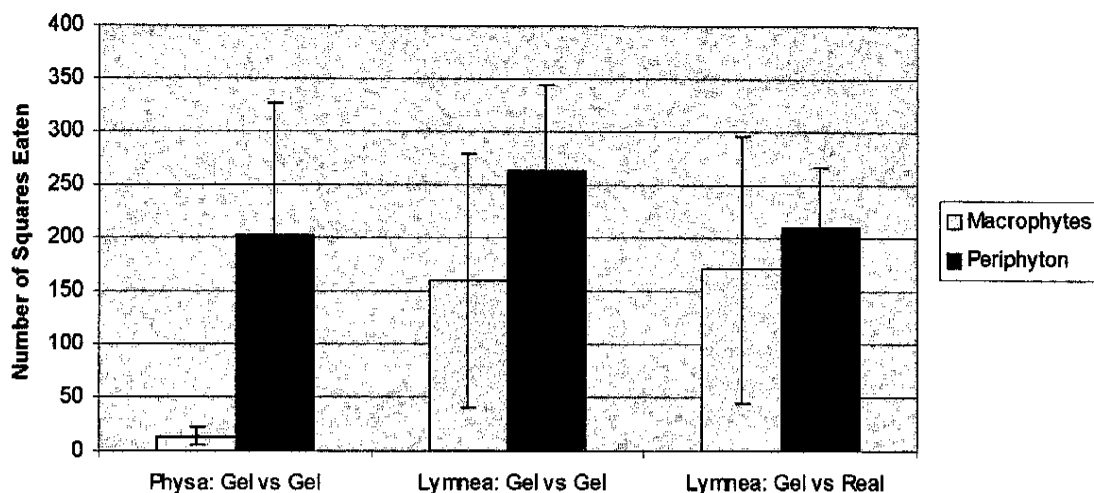


Figure 1- The mean number of squares eaten by *Physa* and *Lymnaea* in each experiment. Error bars represent standard deviation.

Time Budget of Freshwater Snails

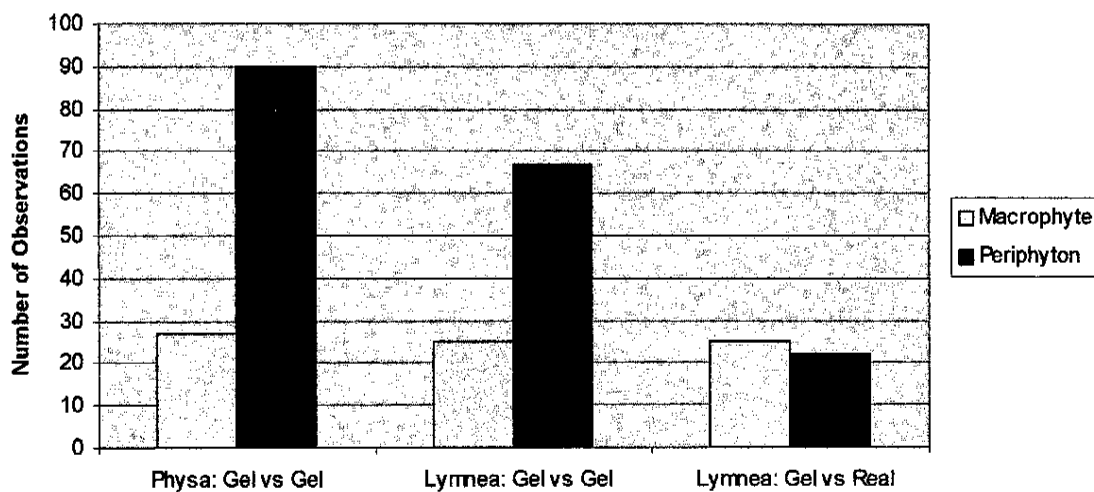


Figure 2- The number of observations of *Physa* and *Lymnaea* on food tiles for each experiment.