EXPERIMENTAL EVALUATION OF THE HERBIVORY INTERACTION BETWEEN *PHYSA SP.* AND PERiphyton*

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Abstract – The influence of the herbivorous snail (*Physa sp.* ) on the algal periphyton community from the Gamasiyab River, Kermanshah province in western Iran was examined by establishing a gradient of *Physa sp.* density in artificial streams. The impact of *Physa* grazing was evaluated by measuring dry mass, ash-free dry mass (AFDM) and chlorophyll *a* in the periphyton community. Snails significantly depressed periphyton biomass. Following twelve days of grazing of the grazer densities of 0, 0.015, 0.03 and 0.06 individuals/cm$^2$, the dry masses of the periphyton communities were 3.04, 2.9, 2.66 and 1.07 mg/cm$^2$ respectively. Snails also decreased ash-free dry mass by 2.04, 1.78, 1.63 and 0.71 mg/cm$^2$ and chlorophyll *a* by 8.6, 8.06, 5.25 and 4.6 mg/m$^2$. During this experiment the survival percentage for 0.015, 0.03 and 0.06 (individuals/cm$^2$) treatments was 77.7%, 58.3% and 30% respectively. During the 12 days of the grazing period in the artificial stream the snail growth also showed a density dependent pattern. Diatom biovolumes calculated in this study are $57.1 \times 10^6$, $36 \times 10^6 \times 31 \times 10^6$, and $5.8 \times 10^6$ μm$^3$/cm$^2$ for snail densities of 0, 0.015, 0.03 and 0.06 individuals/cm$^2$ respectively. A group of species which are found primarily in the grazed treatments include *Achnanthes lanceolata*, *Cocconeis pediculus*, and *Ulothrix sp.* In contrast, ungrazed assemblage had high relative abundance of non adnate diatoms such as *Nitzschia linearis*, *Gomphonema sp.* and non filamentous chlorophytes such as *Scenedesmus sp.*

Keywords – Periphyton, herbivorous snail, artificial streams, Gamasiayab River, *Physa sp.*, diatoms

1. INTRODUCTION

Historically, lotic research on interactions of benthic algae and their consumers have involved both field studies and *in situ* experiments [1]. Recently, the use of artificial streams to study the effects of herbivory in lotic ecosystems has increased [2]. Artificial stream research focusing on invertebrate grazing began with studies of *Physa gyrina* snails in indoor recirculating artificial streams [3] and the manipulation of grazing chironomid larvae in outdoor channels [4]. Paradoxically, Kehde and Wilhm [3] found that algal chlorophyll *a* increased by treatment with grazers, but algal biomass and algal species diversity were not affected. In contrast, Eihenberger and Schlatter [4], like many others, reported reduced algal biomass and increased algal export by macroinvertebrates (midges). Lamberti and Resh [5] have shown that during a three week period of grazing by *Helicopsyche borealis* algal biomass (as indicated by chlorophyll *a*) has been decreased significantly. Hill and co-workers [6] showed that periphyton build up in experimental channels can be strongly influenced by snail density.

The periphyton assemblage is a complex community with heterothrophic bacteria, fungi, protozoa, and small metazoa, as well as autotrophic components in close spatial proximity. Production in both freshwater and marine littoral is strongly dependent on the assemblage of algae and heterothrophic

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organisms that exist in the periphyton community [7]. Several studies have documented that macroconsumers (gastropods, crustaceans and insect larva) can have a strong effect on the community production [5, 8]. Freshwater grazers such as snail, caddisflies, isopods, minnows, daphnia, and other grazers can restrict primary production by feeding on algae standing crop [1, 5, 9, 10]. Herbivory (grazing or consumption of living plants by animals) is often a major factor influencing the trophic structure and food web dynamics [11]. Macrograzers may exert strong effects on vertical strata or horizontal patches of the assemblages [12, 13].

The periphyton community provides food for many invertebrates and vertebrate animals in aquatic ecosystems [14, 15]. Almost all streams consist of herbivorous snails for grazing periphyton. The effects of insect grazers have been assessed by several investigators [15, 16, 17]. They revealed that herbivory activities of insect larva decreased algal abundance and species composition. Considerably more literature exists about the influence of freshwater snails on algae [16]. The general interactions between periphytic algae and grazer snails have been well documented in various ecosystems [15, 18]. In a number of studies the grazing activity of snails has been shown to decrease the periphyton biomass [15]. Other studies have indicated a positive relationship between nutrient addition and algal biomass, as well as a negative relationship between herbivory and algal biomass [4, 19]. Also, Bönmark [20] has suggested that low grazing pressure is coupled with high periphyton biomass and intermediate grazing pressure with a biomass decline. Higgins and Hann [21] have indicated that overgrazing of the periphyton community causes considerable reduction in both biomass and productivity of periphyton algae.

The previous record of research on artificial streams in Iran is restricted to the present authors [22]. In the present study, our basic aim is to establish causal relationships between an important component of the grazer community in inland freshwaters of Iran and the periphyton community by looking at the influence of the herbivory of this snail on various indicators of the standing crop, survival of the snail, and also on the species composition of the periphyton community.

2. METHODS

a) Experiment design

This study was conducted in three fiberglass streams, each 2 meters in length, 0.5 meters in width and 0.4 meter in depth. Each fibreglass stream has been divided into two equal sections by placing a fibreglass sheet in the middle of the streams. Water from upstream of the Gamasiayab River (34 40E, 48 20N) was used in the artificial streams at a depth of approximately 0.08 m. Using in water pumps water velocity in the channels averaged 17cm/s. Light was supplied by 18 metal halide lamps (6 for each channel), which provided a broad spectrum of photosynthetically available irradiant. Quantum flux density levels were 90 \( \mu \text{mol.m}^{-2}.\text{s}^{-1} \) when measured at the water surface in the artificial streams.

To inoculate the streams with algae, pebble were collected from the Gamasiab River and scraped with a brush into a water container. Inoculums were prepared by scraping periphyton from the Gamasiayab River. The water containing scraped algae was filtered through a mesh and an equal volume of the filtrate containing algae was added to each channel. Water temperature ranged between 20-23°C during the experiments. In order to enhance the colonization processes commercial P and N fertilizer were added to achieve a nominal concentration of nitrate and a phosphate of 0.04 and 0.004 mg/L respectively.

We made mesh cages (20L×30W×15Dcm) and placed 16 tiles in each of them in order to be colonized by the periphyton community. Snails (\textit{Physa sp}) of similar size (5-7mm long) were collected from the Gamasiab River. The snails were added to the cages at 0, 0.015, 0.03 and 0.06 densities (individuals/cm²).
b) Analytical methods

Dry mass - After 12 days of the grazing period four tiles were randomly selected and dried for 24 hours at 60 °C and attached periphyton was scraped from the tiles with a razor blade and weighed. Ash-free dry mass (AFDM) was measured by combustion. Four tiles were randomly selected and dried for 24 h at 60 °C and periphyton organisms were scraped similar to dry mass, weighed, combusted in a 500°C furnace for four hours, and reweighed. Representing all organic matter in the periphyton (detritus, algae, bacteria, protozoan, fungi, etc.), AFDM was the difference in the mass before and after incineration. AFDM was calculated as grams per square centimetre of the original substrate.

Chlorophyll a - We randomly selected four tiles in each cage and scraped the attached periphyton into experiment tubes containing 10 ml of 95% ethanol. The samples were then stored overnight in a freezer. The light absorbency at 665nm of the supernatant was determined both before and after adding two drops of 0.1 N HCL using a spectrophotometer. The chlorophyll a concentration was determined from the absorbency reading using the equation proposed by Nusch [23].

Statistical analysis. Biomass, ash free dry mass and chlorophyll a contents of the samples were compared at different treatments using single factor analysis of variance (ANOVA).

3. RESULTS AND DISCUSSION

Dry mass. Information obtained on the herbivory effects of the introduced snails on the periphyton biomass is shown in Fig. 1. It is evident from the current experiment that dry mass of the periphyton community decreases with increasing in the snail density. Statistical comparison between dry mass of the periphyton community show a significant (P<0.05; F=5.6) difference using single factor analysis variance (ANOVA). The extent of changes due to the herbivory effects of Physa sp. at densities of 0, 0.015, 0.03 and 0.06 individuals/cm² on the periphyton algal community were 3.04, 2.9, 2.66 and 1.07 mg of dry mass per cm² respectively (Fig. 1).

![Fig. 1. The effects of snail on periphyton dry mass. Dry mass in the artificial streams decreases as density of the snail increases](image)

Periphyton biomass, as indicated by ash-free dry mass (AFDM), decreased with increasing grazing densities of Physa sp. when expressed as a function of the total biomass grazing. A significant difference in ash-free dry mass is shown among the artificial streams with differing snail densities (P < 0.05; F=13.5) using one-way analysis variance. Periphyton ash-free dry mass (AFDM) were 2.04, 1.76, 1.63 and 0.71 mg/cm² at densities of 0, 0.015, 0.03 and 0.06 individuals/cm² respectively. AFDM appeared to decline as a function of applied Physa sp. density with the greatest reduction occurring at 0.06 individual/cm² (Fig.2).
Figure 2 demonstrates changes in the Chlorophyll \(a\) as an indicator of the periphyton biomass studied in the artificial streams at different densities of \textit{Physa sp.} We demonstrated that chlorophyll \(a\) declines by increasing the snail density \((P < 0.05; F=84.2, \text{ANOVA})\). Periphyton chlorophyll \(a\) was 8.6, 8.06, 5.25 and 4.6 mg/m\(^2\) for 0, 0.015, 0.03 and 0.06 snail densities (individuals/cm\(^2\)) respectively. The ratio of chlorophyll \(a\) to periphyton AFDM increased with increasing \textit{Physa} densities (Fig. 4), even though chlorophyll decreased in absolute term. The ratio was 0.42, 0.045, 0.032 and 0.064 \(\mu\)m/mg for 0, 0.015, 0.03 and 0.06 snail densities (individuals/cm\(^2\)) \((P < 0.05; F=33.6 \text{ ANOVA})\) (Fig. 4).

Another parameter that was measured in the artificial streams was the survival of \textit{Physa sp.} We demonstrated that the survival of the snail increased with the decrease in the number of snails. Survival for 0.015, 0.03, and 0.06 individuals/cm\(^2\) was 77.7%, 58.3% and 30% respectively (Fig.5).
Algal composition- Algal assemblages at all densities were influenced by grazers. Assemblages at low snail densities are dominated by adnate diatom and non filamentous green algae. In contrast, assemblages at high snail densities had high relative amounts of erect (non adnate) diatoms and filamentous green algae (Fig. 6).
Fig. 6. Relative abundance of dominant periphyton algal taxa are grouped in various types of snail densities (ind/25cm²) (0, 0.015, 0.03, and 0.06) after 12 days of grazing. Adnate diatoms include *Achnanthes lacerata*, *Cocconies* sp. Non-adnate diatoms *Gomphonema* sp., *Nitzschia linearis*, and *Synedra ula*. Filamentous chlorophytes include *Ulothrix* sp. Non-filamentous include chlorophytes and *Senedesmus* sp. Ad-dia=Adnate diatoms, Non. Ad-dia=Non Adnate diatoms, Fil-chlo=Filamentous chlorophytes, and Non. Fil-chlo=Non Filamentous chlorophytes.

All measures of periphyton productivity including dry mass, ash-free dry mass (AFDM) and chlorophyll *a* declined with increasing grazer density. The pattern of decrease in all three component of the periphyton community when expressed as a function of the grazer density includes a sudden decrease in the highest density of *Physa* sp. The overall decreasing pattern in chlorophyll *a* appeared somewhat more linear in the dry mass and ash-free dry mass. Single factor analysis of variance (ANOVA) resulted in *F* values for all three variables (Table 1) indicating significant differences. The relationship between snail densities and various components of the periphyton community is also shown by linear regression analysis (Table 2).

Table 1. Results of ANOVA of various characteristics during 12 days experimental periods

<table>
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<th>Attributes</th>
<th>df/</th>
<th></th>
<th>F</th>
</tr>
</thead>
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<td>Dry mass</td>
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</tr>
<tr>
<td>AFDM</td>
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<td>0.04</td>
<td>13.5***</td>
</tr>
<tr>
<td>Chlorophyll <em>a</em></td>
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<td>0.04</td>
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</tr>
<tr>
<td>Chlorophyll <em>a</em> / AFDM</td>
<td>3</td>
<td></td>
<td>33.4*</td>
</tr>
</tbody>
</table>

*P < 0.05, ** p < 0.01, *** p < 0.001
Table 2. Regression derivatives for relationships between snail densities and various measures of the periphyton standing crop

<table>
<thead>
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<th>Y units</th>
<th>β</th>
<th>α</th>
<th>r²</th>
</tr>
</thead>
<tbody>
<tr>
<td>AFDM (mg/cm²)</td>
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<td>0.85</td>
</tr>
<tr>
<td>Chlorophyll a (mg/m²)</td>
<td>10.33</td>
<td>-0.48</td>
<td>0.91</td>
</tr>
<tr>
<td>Dry mass (mg/cm²)</td>
<td>3.95</td>
<td>-0.61</td>
<td>0.75</td>
</tr>
<tr>
<td>Survival</td>
<td>101</td>
<td>-22.35</td>
<td>0.99</td>
</tr>
</tbody>
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\[ Y = \alpha x + \beta \]

Y = Periphyton biomass parameter
\( \beta \) = Regression constant
\( \alpha \) = Regression coefficient

Many studies in the field of freshwater ecology involve manipulation of the natural environment including using in situ artificial streams [16]. However, it appears that the current experiment is successful in demonstrating a density dependent relationship between the grazer effects and various measures of the periphyton community. The results of this experiment indicated that the grazer (Physa sp.) is capable of reducing the periphyton standing crop in artificial streams. Reduction of biomass by grazing has been well established in lotic periphyton investigations [24]. The majority of the grazing experiments conducted with various herbivorous or grazers have shown a reduced rate of primary production under moderate to high grazing pressure [9]. Results obtained from this study indicated that grazers (Physa sp.) in the artificial streams played a significant role in limiting various measures of the periphyton productivity. The results are in general agreement with grazing experiments conducted to evaluate Physa impact upon periphyton standing crop.

The patterns of Physa sp. grazing on various components of the periphyton community are not similar (Table 2). The pattern of Physa sp. grazing as indicated by AFDM demonstrated decreasing periphyton AFDM with increasing Physa sp. densities. However, this relationship (Fig. 2) is not a perfect linear one (\( r^2 = 0.85 \)). Several explanations are possible for this pattern of standing crop reduction. At the highest density snails may have physically interfered with each other’s grazing activity, reducing the time for grazing per individual. Since no agonistic behaviour was observed among the grazing snails, this seems to be an unlikely explanation for the current trend.

Sudden decrease in AFDM at the highest density of snails may be partially due to structural changes in the periphyton community as a result of the high grazing pressure of Physa sp. The grazer may have harvested much of the loose layer of the periphyton community at low densities, leaving mostly adherent organic matter that was progressively more difficult to harvest as the snail density increased. Similar structural change has been reported for periphyton communities [25]. Effects of grazing on periphyton algae depend on the grazer species [26]. Snails have a fine toothed radula that is capable of removing filamentous algae and diatoms [9]. Therefore, Physa sp. can eat diatom and filamentous algae. Snails affect the structure of periphyton because algae species have different resistance to physical disturbance while feeding. Upper-layer diatoms that are loosely attached to the substrate are most susceptible to grazers, whereas small and adherent diatoms are resistant to grazer pressure [5, 27]. Grazers also affect the benthic algal assemblage by disturbing the substrate surface [28], which reduce the abundance of loose-layer diatoms [9].

Another explanation for the non-linear reaction of the periphyton community to the grazing pressure may be an increase in productivity of the algal community as a result of the increased efficiency of primary production. A Grazing-induced increase in primary production has been reported for a wide
variety of herbivores [29, 5 and 16]. An increase in chlorophyll $a$ per unit of algal biomass with increasing *Physa* sp. biomass suggested that the efficiency of primary production might have been increased under grazing pressure. This has been reported by Hill and Knight [16]. Other mechanisms for increasing chlorophyll $a$ per unit of biomass is the possible role of the grazer in fertilizing the periphyton algae. Moreover, the grazer may ingest and thereby remove, dead and senescent algal cell. By decreasing the absolute number of cells and the thickness of the algal layer, grazing may actually enhance primary production through facilitating the access of light and nutrients to the benthic community [5].

In this study no territorial or aggressive behaviour between individual snails was observed in the mesh cages. The apparent absence of such agnostic behaviour, combined with the evidence of resource depletion, indicated that the density-dependent growth of *Physa* sp. was due to food limitation. Similarly the pattern of mortality in the course of the experiment also indicated a strongly density-dependent trend. Mortality of *Physa* could have been caused by several density dependent processes affecting their survival amongst which the food availability may play a major role. However, it is apparent that further studies are necessary to identify, precisely, the mechanisms responsible for grazer-accelerated turnover rates. The present study was performed to determine the extent to which the snail community can affect the food resource (periphyton) and the extent to which food resource can reduce the snail survival.

In this study taxa associated with the loose layer periphyton reduced in relative abundance while adnate taxa increased. Sumner and McIntire [29] have suggested that grazing by snail *Juga plicfera* decreased the relative abundance of some erect taxa (*Nitzschia palea, N. linearis, Surirella*) that dominated the loose layer. These taxa were associated with an overstory matrix constructed by the filaments of *Melosia varians*, a large diatom especially susceptible to the snail scraping radula [16]. Grazed assemblages were distinguished from ungrazed assemblages by a greater relative abundance of adnate diatom such as *Cocconies placentula* and *Achnanthes lanceolata*, filamentous chlorophytes such as *Stigeocloium tenue* and prostrate *Phormidium tenue* [16]. In contrast, ungrazed assemblage has been shown to have high relative amounts of erect diatoms such as *Synerda ulna, Nitzschia Linearis*, and *Gomphonema parvulum*, and non filamentous Chlorophytes such as *Scenedesmus obliquus* and *Characium sp.* [26]. Our results showed that by grazing *Physa* communities were dominated by the adnate diatom and non filamentous algae, whereas ungrazed communities were dominated by filamentous and non adnate (erect) diatoms. Several authors [13, 30] have suggested that grazing affected mostly filamentous and erect chain-forming algal species, which is a persistent in grazing treatment on periphyton, presumably based on higher mechanical vulnerability of filaments. Upright and filamentous species from the periphyton are presumably inedible for many small herbivores [insect larva], but large consumers (snails) could remove large algae, thus shifting the species composition toward a dominance of small cells which are more easily ingestible by small grazers [7].

It has been documented that some functional aspects of aquatic ecosystems are under the influence of grazing. Hill and his co-workers [6] demonstrated that rates of carbon fixation were significantly reduced by *Elimia* grazing compared to the streams without snails. In another study conducted by Hill and Knight [16] it was shown that the pattern of the declining effect of consumers on primary productivity is not linear, while the decrease in chlorophyll $a$ appeared somewhat more linear. Similar results were obtained in another experiment in which following 23 days of grazing by *Ameletus validus* (a mayfly), at different densities the periphyton ash-free dry mass (AFDM) was depressed. Freshwater herbivory such as snails affect the physiognomy of periphyton. For example, Hill and Knight [16] suggested that larger algal cells are susceptible to grazing by freshwater snail, thereby the composition of periphyton was changed by freshwater grazers.

The importance of resource limitation was widely appraised by many researchers. Several ecologists believe that resource limitation is a major factor regulating population size [24, 31], whereas others
believe that population are seldom restricted by their resources [32]. Various authors have proposed that the importance of limitation depend on either the trophic level or the physical harshness of the environment [33]. However, the interaction effects of nutrients and grazers on periphyton have received less attention [27].

Eisenberg [34] studied the effects of food limitation by a freshwater snail and demonstrated that density-dependence in adult fecundities was largely responsible for adjustments in population density. We conclude that early survival of young snails may have been responsible for the large numerical response by *Physa*. The presence of the predators of snail in natural conditions may significantly reduce snail densities in which competition does not occur; in this experiment there were no snail predators.

REFERENCES


