ASSESSING ALGAE SURVIVORSHIP FOLLOWING SNAIL GRAZING

Diego Garcia, Elizabeth Post, Ahmad Allan

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Prof. Robert Pillsbury

Abstract:

A study by Porter (1976) showcases planktonic chlorophyta within the family of *Sphaerocystis* to be the best at surviving digestion by grazers due to presence of a complicated polysaccharide sheath found on the cells. This sparked interest in studying the survivorship of different algal groups after undergoing digestion by a two species of grazing snails (*Physa sp.* and *Lymnaea sp.*). Fecal samples were carefully examined to identify the cells and their abundance in order to compare a digested sample of algae to one uneaten. We hypothesized that cyanobacteria will have the highest survivorship amongst the algae groups due to the presence of a mucous membrane. Our results show a shift in the total cell distribution following digestion. *Physa sp.* had ~79% of the overall bacillariophyceae cells from the uneaten algae sample in the fecal sample, with an increase of ~580% for cyanobacterial cells and an increase of ~88% for chlorophyta. Bacillariophyta for *Lymnaea sp.* decreased to ~68% compared to the original uneaten algae sample along with an increase of ~492% for cyanobacteria and an increase of ~150% for chlorophyta. We learned that cyanobacteria had the highest survivorship.

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Diego Garcia

garciadi@umich.edu

University of Michigan

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A study by Porter (1976) showcases planktonic chlorophyta within the family of *Sphaerocystis* to be the best at surviving digestion by grazers due to presence of a complicated polysaccharide sheath found on the cells. This sparked interest in studying the survivorship of different algal groups after undergoing digestion by a two species of grazing snails (*Physa sp.* and *Lymnaea sp.*). Fecal samples were carefully examined to identify the cells and their abundance in order to compare a digested sample of algae to one uneaten. We hypothesized that cyanobacteria will have the highest survivorship amongst the algae groups due to the presence of a mucous membrane. Our results show a shift in the total cell distribution following digestion. *Physa sp.* had ~79% of the overall bacillariophyceae cells from the uneaten algae sample in the fecal sample, with an increase of ~580% for cyanobacterial cells and an increase of ~88% for chlorophyta. Bacillariophyta for *Lymnaea sp.* decreased to ~68% compared to the original uneaten algae sample along with an increase of ~492% for cyanobacteria and an increase of ~150% for chlorophyta. We learned that cyanobacteria had the highest survivorship.

Introduction:

Herbivory can cause change a community composition and alter the succession in aquatic systems. Grazers in aquatic systems will feed on algae and as they digest the proportion of algal groups can change with some algae surviving better than others, which can affect the overall algae distribution (Tuchman and Stevenson, 1991). Previous studies have found that herbivory done by grazing can prevent some algae groups from becoming dominant and therefore can increase species diversity (Peterson, 1987). The community of algae within aquatic systems changes over time and some algal groups can become more common over others, which can have different effects on the entire system. If some taxa of algae are being digested more than others this will cause the community composition to shift and can cause effects on the diets of herbivores and can affect their chances for survival. This can also decrease the chances for other groups of algae to attain light or nutrients if the dominant group is consuming most of the necessary components needed by others. In a study by Arnold (1971), *Daphnia pulex* that fed on cyanobacteria had a lower survivorship compared to others who fed on chlorophyta. The digestive system of some grazing organisms cannot successfully break up some types of algae with a mucous sheath, and therefore it is likely that species that feed on cyanobacteria do not get nutritional benefits from it. A study by Porter (1976) showcases planktonic chlorophyta within the family of *Sphaerocystis* to be the best at surviving digestion by grazers because these colonies have a complicated polysaccharide sheath.

Eutrophication can result in displacement of algal species by an increase in cyanobacteria (Arnold, 1971). Grazer's digestive properties are important as they have an impact in the types of algae that are being randomly selected for in their guts. A decrease in species richness within ecological systems and a loss of diversity can lower the productivity of an ecosystem (Tilman et al., 1996). Because grazers are very common in aquatic systems it is a possibility that they contribute to giving cyanobacteria an advantage in increasing their numbers and outcompeting other species of algae. During accelerated growth of cyanobacteria, we can often see that their respiration demands are greater than that of their oxygen production. Therefore, cyanobacteria are depriving the system of oxygen. This eventually results in death of a large portion of the population of cyanobacteria, which will progress to more oxygen deprivation as bacteria will begin to breakdown the dead cells and use up the oxygen in the system. The accelerated succession through the advantage of increased survivorship for algae groups can therefore have a variety of effects on the system (Cole and Weihe, 2016). Algal blooms caused by some species of cyanobacteria that produce toxins can have negative impacts on the aesthetics of the water and also make water unavailable for drinking. We will be able to gain insight into the groups of algae that survive digestion and that can therefore contribute to the process of eutrophication.

The purpose of the study is to evaluate which groups and species of algae are surviving being digested by two different snails (*Physa sp. and Lymnaea sp.*). Through this, we will investigate the digestive behavior of the two snail species. We can learn about which groups are being digested for and if those surviving groups are related to those found in algal blooms involved in eutrophication. We hypothesize that if an algal cell has a mucous membrane, it will not be as digested as other cells. We predict that algae in the cyanobacteria group will have the highest survivorship compared to bacillariophyta and chlorophyta because they have the largest mucous membrane of these algal groups.

Methods and materials

Two snail species, *Physa sp*. and *Lymnaea stagnalis* (great pond snail) were collected from Douglas Lake, MI located near the University of Michigan Biological Station (UMBS). The snails were starved for a 24-hour period and moved to individual containers (2.54cm x 5.08 cm). An algal sample was collected at the UMBS stream-lab facility’s experimental streams and was examined using a microscope under 400x to count and identify algal cells. The identification of algae species was recorded along with a cell count of at least 100 cells. The starved snails were fed a 3 ml stream lab algal sample for a period of 24 hours. Snail fecal samples were retrieved the following day after removing the snails from the container and returning them back to the lake. The fecal pellets were smeared underneath the microscope slide to release all of the contents. The fecal samples were carefully examined under the microscope at 400x. The algae were identified and counted until 100 cells were reached. The uneaten algae composition was compared to the fecal algal composition. Chi-square tests were done in order to asses if the snails processed the living and dead cells differently.

Results

Bacillariophyta: The percent composition of algal communities shows a decrease in the percentages of bacillariophyta for both species of snails (Figure 1). In the uneaten group, bacillariophyta made up ~76% of the overall algal communities. In the *Physa sp.*

feces, bacillariophyta only made up ~61% and for *Lymnaea sp.* they were only ~52% (Appendix 1).

Cyanobacteria: The uneaten groups starting percentage was ~3.71%. The uneaten group showed an increase of ~22% for *Physa sp.* and an increase to ~18% for *Lymnaea sp.*.

Chlorophyta: Chlorophyta made up ~20% of the initial uneaten algae. We saw a decrease in chlorophyta cells only for *Physa sp.* at ~18%, but there was an increase for *Lymnaea sp.* at ~30%.

Based on our percent starting material, we were able to determine how much change in the composition of algal communities happened after digestion (Figure 2). *Physa sp.* had ~79% of the uneaten bacillariophyta group in the fecal sample, an increase of ~580% for cyanobacteria and an increase of ~88% for chlorophyta. Bacillariophyta for *Lymnaea sp.* decreased to ~68% along with an increase of ~492% for cyanobacteria and an increase of ~150% for chlorophyta (Appendix 2). *Fragilaria sp.*, at ~25% is the algal species that is the most common in the dead cell count (Table 1). *Aphenocapsa*, *Fragilaria* and *Coccoid green algae* are the species with the most surviving cells in their respective groups (Table 2). *Fragilaria* cells were found to have the largest shift from live to dead cells after digestion (Table 3). The chi-square test based on living cells for the two snail species gave us an alpha value of 31.4 (Chi-square=116.83, p=31.4, df=20). Our comparison for the dead cells composition between the two snails gave us an alpha value of 25 (Chi-square=54.08, p=25, df=15). Both of these tests showed significance.

Discussion

We were able to find that the distribution of the algal groups changed after they were digested. Bacillariophyta cells that had originally made up ~76% of the overall algal communities in the uneaten group decreased for both of the snails. *Lymnaea sp.,* having ~52% after digestion, showed that they broke down more bacillariophyta cells than *Physa sp.* by a ~9% difference. *Lymnaea sp.* was therefore consuming more bacillariophyta cells compared to *Physa sp.*. Cyanobacteria had a starting percentage of ~4%. This increased for both of the snails with *Physa sp*. at ~22% and ~18% for *Lymnaea sp.*. This suggests that cyanobacteria actually were not digested much by the two snail species. *Lymnaea sp.* was slightly better at digesting cyanobacteria by a ~3% difference as compared to *Physa sp.*'s digestive capabilities. This difference is however is very small suggesting that the two snails actually similarly process cyanobacteria, though more statistical tests to research this difference were not performed. An increase in the distribution of cyanobacteria cannot be accounted for because this increase would suggest that there was a proliferation in the amount of algal groups within the digestive track of the snails (Figure 1). Cyanobacteria were found in the highest percentages after digestion because they were able to survive the most (Figure 2). *Daphnia pulex* in a separate study also were not able to digest cyanobacteria similar to the snails used in our study (Arnold, 1971).

The two snails digested Chlorophyta differently. The uneaten group had a starting distribution of ~20% for chlorophyta cells. *Physa sp.* had a decrease in the amount of chlorophyta cells found and therefore digested these cells more compared to *Lymnaea sp.* which showed an increase in the chlorophyta distribution at ~30%, almost a 10%

difference. This increase in percentage for *Lymnaea sp.,* similar to the increase in cyanobacteria cell distribution for *Physa sp.*, suggests that chlorophyta were able to survive digestion. Therefore, *Lymnaea sp*. is not digesting chlorophyta cells as efficiently as *Physa sp.*. The grazer *Hypophthalmichthys molitrix* (silver carp) in Russia was found to have similar digestion habits compared to those of both of our snails. Silver carp digest bacillariophyta cells the most followed by chlorophyta and lastly cyanobacteria (Moriarty and Pullin, 1987). Our results on the survivorship of algal cells are not restricted to grazers in Douglas Lake, and can be traced to different locations around the world.

In our dead cell count of the overall communities, we found that *Fragilaria* is the species of algae that is the most digested by both of the snails, followed by *Cymbella*, *Natricula* and *Synedra* (Table 1). These cells all represent bacillariophyta, and this group makes up most of the dead cells within our counts. The only bacillariophyceae specie that was not digested as much was *Gomphonema* (Table 1). We learned that bacillariophyceae cells have very low survival and that the snail guts can break these down easily. *Fragilaria* is the species with the most digested cells because the post-digestion proportion of dead-to-live cells changed the most out of all the individual cells (Table 1, Table 3). A study done by Knobloch (1991) found similar results to ours with a species of *Fragilaria, Fragilaria vaucheriae*, being the most digested as it goes during gut-passage and dying off in greater numbers. Although we did not determine the exact algal species within our fecal samples, it appears as though the genus of bacillariophyceae, *Fragilaria,* can be broken down rather easily during digestion. Another study by Lowe and Hunter (1988) was interested in the effects of *Physa integra* on the periphyton community and found the bacillariophyceae *Synedra* and chlorophyta *Mougeotia* to have a decrease in their distribution after grazing. Their results along with ours similarly showcase both of these algal species having a lower live-cell count after digestion. The results that they obtained also show that *Synedra* had more dead cells when compared to *Mougeotia*, similar to our findings (Table 1). Because we assessed the entire community, the species that are highlighted (*Aphenocapsa,* F*ragilaria, Coccoid green algae*) show the highest survival within their respective groups (Table 2). This suggests that the algal species mentioned are those that are being selected for and could have an advantage over other cells in their own groups.

Our Chi-square result allows us to reject our null hypothesis and say that the proportion of live algal cell species is different between the two snails. Our alpha value of 31.4 for our living cell comparison is high and statistically significant. This means that the difference in live algal species between the two snails is high. For our dead cells comparison, we had an alpha value of 25 that allows us to also reject our null hypothesis as it is statistically significant. We can therefore say that there was a difference in the composition of dead and live cells within the fecal matter of the two snail species. Based on our assessment of the data gathered, we have found the species and the groups that are the most digested and least digested. We were also able to find the overall change from living to dead cells for the two snails. Our findings show that cyanobacteria are the best at surviving the digestive tract of our grazers, which supports our hypothesis and predictions.

We learned that the high survivorship of cyanobacteria gives them an advantage over other algae. A higher survival rate for cells could mean that they have a chance to increase their numbers in a colony of dead cells that now can also provide them with nutrients. Also mentioned by Porter (1976) was that some species of algae can gain a nutrient boost when undergoing gut passage which can therefore compensate for the loss of some of their colonies. Therefore the high survivorship of cyanobacteria could also increase their chances at receiving a nutrient boost. It is important to mention that some species of cyanobacteria are able to produce a toxin called microcystin, which can be harmful to humans. Lake Erie in August of 2014 suffered an algal bloom caused by cyanobacteria (Smith et al., 2015). Microcystin toxins released by cyanobacteria upon cell death have rendered water sources undrinkable to people during algal blooms. Our results help to support possible relationships between cell survivorship and how under the right conditions can cause cyanobacteria to proliferate leading to algal blooms.

Community succession for algal species can depend on the survivorship of the group. Bacillariophyta groups may have an accelerated succession cycle compared to the other two groups. Our results showcase bacillariophyceae as the algal group that is the most digested. Digestion by grazers can randomly select out species of bacillariophyceae and slightly speed up of the rate of succession of bacillariophyta in the aquatic community (Tuchman and Stevenson, 1991). This is also similar to the random selection for cyanobacteria which could also allow harmful species to proliferate and cause an algal bloom. Conway and Trainor (1972) reflect on algal species of *Scenedesmus* having small bristles on their morphology, which can increase their motility in the water column. They explain that if algal species are able to change their morphology during a change in nutrient availability it will increase its chances of survival, as it will allow them to change their depths. Because of accelerated succession due to herbivory, it would be interesting to investigate if a change in morphology could be done by cyanobacteria which would increase their advantage even more. A focus should be brought to maintaining a diverse set of species of algae groups within an aquatic ecosystem as the interspecific competition between algal species can prevent the proliferation of harmful algal species which can harm both humans and animals (Cardinale et al., 2011, Titman, 1976).

Possible errors during the study may have occurred. The snails sometimes tried to crawl out of their individual compartments seeking food sources or to escape that could have affected their stomach contents. The sample examined under the microscope could have been mistaken for uneaten algae as opposed to a fecal pellet. As different students were involved in the microscope analysis, there could have been a possibility of inexperience in identifying and counting cells. Future studies should focus on having 2 experienced individuals doing cell counts and identification together with a third individual as a “tie-breaker.” The identification of specific snail and algal species could also improve the study by allowing us to compare individuals within the study. Also, an increase in the total number of cells counted can allow for a more intensive comparison of the algal distributions. Investigating the characteristics of an organism’s digestive system, such as pH, would also provide more information about the challenge that algal cells must undergo during digestion and how this affects their survivorships.

Literature cited

Arnold D. 1971. Ingestion, assimilation, survival, and reproduction by Daphnia Pulex fed seven species of cyanobacteria. Limnology and Oceanography 6: 907-920.

Cardinale BJ, Matulich KL, Hooper DU, Byrnes JA, Duffy E, Gamfeldt L, Balvanera P, O'Connor MA, and Gonzalez A. 2011. The functional role of producer diversity in ecosystems. American Journal of Botany 3: 572-592.

Cole GA, and Weihe PE. 2016. Textbook of limnology: fifth edition. Long Grove, IL : Waveland Press, 13-21

Conway K., and Trainor FR. 1972. Scenedesmus morphology and flotation. Journal of Phycology.8: 138-143.

Kinzig AP., Pacala S., and Tilman GD. 2001. The functional consequences of biodiversity: empirical progress and theoretical extensions. Princeton: Princeton University Press, 120-150.

Knobloch, T.J. 1991. Viability of diatom species following ingestion by the algivorous minnows, Campostoma anomalum and Pimephales notatus. Unpublished M.S. Thesis. John Carroll University, University Hgts., Ohio. 118 pp

Lowe, RL., and Hunter RD. 1988. Effect of grazing by Physa Integra on periphyton community structure. Journal of the North American Benthological Society 1: 29-36.

Moriarty DJW, and Pullin RSV. 1987. Detritus and microbial ecology in aquaculture. Manila, Philippines. International Center for Living Aquatic Resources Management, 253-255.

Peterson, C.G. 1987. Gut passage and insect grazer selectivity of lotic diatoms. Freshwater Biology 18: 455-460.

Porter, KG. 1976. Enhancement of algal growth and productivity by grazing zooplankton. Science 192: 1332-1334.

Smith DR., King KW., and Williams MR. 2015. What is causing the harmful algal bloomsin Lake Erie? Journal of Soil and Water Conservation 2: 27A-29A.

Titman D. 1976. Ecological competition between algae: experimental confirmation of resource-based competition theory. Science 4238: 463-465.

Tuchman NC., and Stevenson RJ. 1991. Effects of selective grazing by snails on benthic algal succession. Journal of The North American Benthological Society, 10:430-443.

Tables and Figures

Table 1 – Distribution of dead cells: Comparison of algal species and their individual proportions relative to the total dead-cell counts.

Total Dead Cells		
Algae Group	**Algal Genus**	**Percent Total**
Bacillariophyta	*Fragilaria*	24.78
Bacillariophyta	*Cymbella*	22.28
Bacillariophyta	*Natricula*	19.62
Bacillariophyta	*Synedra*	16.73
Cyanobacteria	*Aphenocapsa*	2.97
Chlorophyta	*Dictyos phaerium*	2.5
Chlorophyta	*Mougeotia*	2.19
Chlorophyta	*Coccoid Green Algae*	1.80
Chlorophyta	*Scenedesmus*	1.49
Chlorophyta	*Closterium*	1.33
Chlorophyta	*Cosmarium*	1.25
Cyanobacteria	*Microcystis*	1.02
Cyanobacteria	*Merismopedia*	0.94
Bacillariophyta	*Gomphonema*	0.63
Chlorophyta	*Green Cells*	0.47
Chlorophyta	*Pediastrum*	0
Cyanobacteria	*Blue Green*	0
Cyanobacteria	*Chrococcus*	0
Chlorophyta	*Green Filament*	0

Table 2 – Total live cells: Comparisons in distributions of live cells for species of algae relative to their respective groups.

Total Live Cells		
Algae Group	**Algal Genus**	**Percent Total**
Cyanobacteria	*Aphenocapsa*	7.95
Cyanobacteria	*Merismopedia*	4.60
Cyanobacteria	*Microcystis*	0.97
Cyanobacteria	*Blue Green*	2.99
Cyanobacteria	*Chrococcus*	3.08
Bacillariophyta	*Cymbella*	17.52
Bacillariophyta	*Natricula*	10.44
Bacillariophyta	*Fragilaria*	23.63
Bacillariophyta	*Synedra*	2.99
Bacillariophyta	*Gomphonema*	0.87
Chlorophyta	*Coelastrum*	0.55
Chlorophyta	*Coccoid Green Algae*	10.21
Chlorophyta	*Mougeotia*	2.574
Chlorophyta	*Pediastrum*	0.41
Chlorophyta	*Cosmarium*	3.72
Chlorophyta	*Scenedesmus*	2.71
Chlorophyta	*Dictyos phaerium*	2.25
Chlorophyta	*Closterium*	1.93
Chlorophyta	*Green Filament*	0.51
Chlorophyta	*Green cells*	0.09

Table 3 – Individual species distribution of *Fragilaria*: Individual species comparisons of Fragilaria cells show the proportion of dead *Fragilaria* cells in the fecal samples. A higher percentage shows a higher abundance of dead cells.

Sample	**Dead/Living Proportion**
***Fragilaria* - uneaten**	**0.013**
Fragilaria - Physa sp.	**0.45**
Fragilaria – Lymnaea sp.	**0.75**

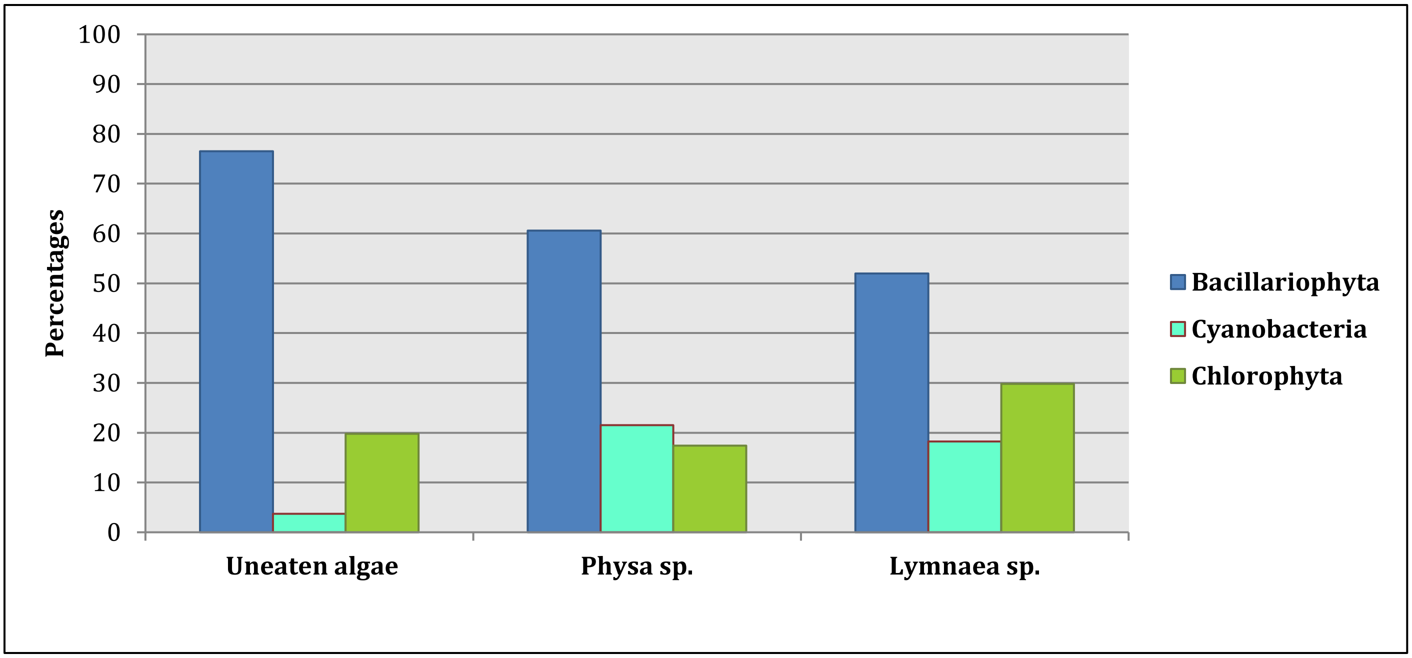

Figure 1 - Distribution of living algal communities: The distribution of algal communities shows the shifts in distributions for each group in comparison to the uneaten sample.

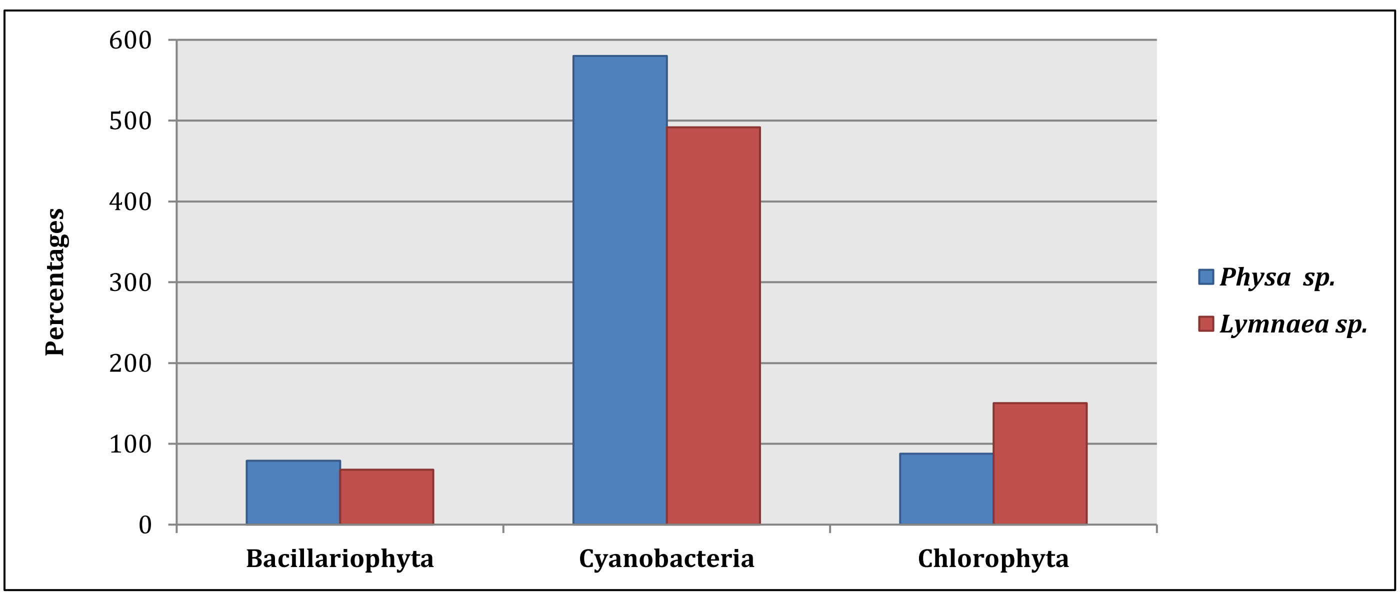

Figure 2 - Amount of surviving material: The surviving material count shows the change in percentages of the algal groups for each snail in comparison to the uneaten algae's percentage.

Appendix:

Appendix 1 - Distribution of living algal communities: The distribution of algal communities in each of the samples shows the shifts in distributions for each group in comparison to the uneaten sample.

Percent Distribution of Algal Communities				
Algae group	**Uneaten Algae**	***Physa sp.* - feces**	***Lymnaea sp.* – feces**	***Physa sp.* – *Lymnaea sp.* Difference**
Bacillariophyta	76.49	60.60	51.96	8.65
Cyanobacteria	3.71	21.54	18.26	3.29
Green	19.80	17.40	29.79	-12.40

Appendix 2 - Amount of surviving material: The surviving material count shows the change in percentages for the algal groups in the fecal samples in comparison to the uneaten algae's percentage of 100%.

Percent of Surviving Material		
Algae group	***Physa sp.***	***Lymnaea sp.***
Bacillariophyta	79.23	67.92
Cyanobacteria	580.25	491.75
Chlorophyta	87.85	150.46