An ASSESSMENT of TORCH LAKE SNAIL GRAZING on BENTHIC ALGAE DIATOMS

AUTHORS: Three Lakes Association's 2021 Summer Interns

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Executive Summary:

This assessment was based on microscopic examinations of fecal samples of two species of snails obtained from Torch Lake to determine if the nature of diatoms in the feces of these snails was similar or different from the diatoms in benthic algae. The basic question was to evaluate whether the process of snails grazing on benthic algae may be part of a natural ecological process for reducing the growth of golden brown algae.

Snail enclosures with 12 snails of each species were placed on mats of golden brown algae for about a week, and then the feces from individual snails were collected and mounted on slides. The five interns then tallied the number of each of five diatom genera in each of five fields per slide at 400X magnification. The data was used to compare the profile of diatom genera from two locations on Torch Lake: one sandy area and one cobblestone area.
There was no significant difference between the diatom genera living in benthic algae from the two different substrates, nor was there a significant difference between the diatom genera in the two snail species.

An unexpected finding was that about 80% of the diatoms consumed by both species of snails were still alive in their feces, which suggested that snails were not grazing on the benthic algae as a source of nutrition. Although we did not measure the snails consumption of the mucilage associated with mats of golden brown algae, the snails may have also been grazing and obtaining nutrition from the mucilage. The only other unexpected finding was the enriched abundance of *Centric* genera of diatom in the snail feces. We speculated that *Centric* diatoms may have been growing on the surface of golden brown algae mat, which may have made them more available for grazing snails and could help explain the color of golden brown algae. Although grazing snails do not appear to be part of the natural ecological management of golden brown algae, other organisms that graze on benthic algae may be part of the ecological control of the golden brown algae.

Introduction

The locals around the beautiful Torch Lake water have noticed an unappealing golden brown algae becoming more present, which inspired us to investigate the algae by taking a closer look at the organisms that consume it and how they influence the algae population. A study by R. Jan Stevenson provided us with further reason to question the relationship between the nuisance brown algae and the diatom population by his findings of the diatoms being responsible for the discoloration. With Dr. Stevenson's findings in mind we set out to specifically study the diatoms that make up the golden brown algae. To study the diatoms we decided it would be beneficial to track them through the snail species in Torch Lake.

We wanted to find out how the snails fit into the benthic algae population. We knew they were grazing on the bottom of Torch where the benthic algae is present, but are they eating the nuisance algae? Are they helping to reduce the population of the nuisance algae? To guide this project, we hoped to answer three main questions: what diatoms were available to the snails for consumption, what did the snails ingest, and what did the snails digest?

We set out to answer these questions by analyzing two different snail species that we found to be available in the locations of our project. The *Pleurocera* and *Physa* snails allowed us to conduct an experiment where we could track the diatoms from the lake floor, through the snails, and into their feces to give us answers on how the snails are affecting the golden brown algae population. Diatoms are enclosed in a glass shell that makes it easier to track them through the snail and into their feces because the shell protects the diatom and allows us to identify them through a microscope. By analyzing the diatoms in the benthos and the diatoms in the snail's feces we can get a better understanding of how the snails contribute to the consumption and population of the golden brown algae in Torch Lake.

Materials and Methods

Enclosure Building

The snail enclosures were made using small, clear storage boxes. We cut a section out of the top and attached a piece of cross stitch plastic mesh using pop rivets on one side so the other side would flip up, making snail insertion and removal smoother.

Intern, Vivien Felker, trimming a hole in the enclosure for the plastic mesh

Snail Collection

To collect the snails, we went to two different locations on Torch Lake. We collected *Pleurocera* snails from the Robson/Morse property just north of deep-water point, and *Physa* snails from the Lake Street access to Torch Lake off of Crystal Beach Road. We snorkeled and collected snails then separated them into individual cups filled with lakewater, and let them defecate overnight. We kept the feces as a baseline for what diatoms are already being consumed by the snails.

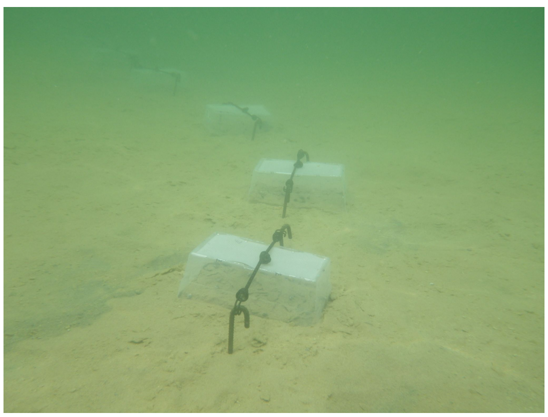
Snails were separated into cups, 1 snail per cup, directly after collection

Enclosure Placement

At the Gourley site, we placed ten enclosures over benthic algae and two control enclosures with no algae. At the Hayo-Went-Ha site, we placed 4 enclosures over benthic algae and two control enclosures.

We chose the Gourley site because it had a sandy lake floor, known phosphorus concentrations in the groundwater and surface water, and known seasonal growth patterns of benthic algae. To place the enclosures, we hammered two steel rebar rods about a foot apart into the lake floor. We then placed the enclosure horizontally between the rods and dropped twelve of a species through the top of the enclosure. To make the bungee cord tighter, we tied three knots and strapped it over the enclosure to secure it.

Six of the enclosures at the Gourley site were washed away from their original location due to wave action. Most of the snails were unable to be seen inside the surface of the enclosure, so we dug through the sand to find as many as possible. We believe the snails crawled under the flap of mesh or crawled underneath the enclosure by burrowing in the sand. The harvested snails went back into individual cups to defecate overnight. We saved their samples of feces for making slides.



Underwater photo of enclosures at Gourley(left) and Hayo-Went-Ha(right) directly after placement

The Hayo-Went-Ha site was chosen because of the cobble benthos and because Dr. Jan Stevenson suggested it might be an interesting comparison to the sandy benthos at the Gourley site. At the Hayo-Went-Ha site, we upgraded the way we secured the enclosures. Because so many enclosures drifted away at the other site, instead of just two steel rebar rods, we hammered four into the lake floor and made a cross with two bungee cords. We placed another bar over the mesh to keep the enclosures in place more effectively and to try to keep the snails in the enclosures.

Only one enclosure was washed away after a second storm, so the crossing of bungee cords did keep the enclosures in place more effectively. Although the enclosures were mostly in place, the majority of the snails had either escaped or were buried under the sediment buildup inside the enclosures.

We derived benthic samples in and around the enclosures after snail placement. At both sites, we collected one sample of benthic sediment inside every enclosure and one from outside of the enclosures in both the benthic algae area and control area to count diatoms in the algae before and after the snails were introduced.

Slides

______We decided to use wet mount slides because they were equally as efficient as syrup slides, but only took a fraction of the time. Also, the view of diatoms was the same so it didn't make sense to spend more time waiting for syrup slides to cure.

To make the wet mounts, we mixed the harvested material (benthic algae or snail feces) with a small amount of distilled water to make a slurry. A drop of that slurry was placed on a slide then covered with a cover glass. It was sealed with nail polish and allowed to dry before viewing. We used 400X microscopy and oil immersion to view the diatoms.

We viewed five fields for every sample, both benthic and fecal, and calculated the relative abundance, how many were alive, and the total quantity of each genus type, *Encyonema*, *Cymbella*, *Fragilaria*, *Diatoma*, *Epithemia*, *Centric*, and other, for every slide. These listed genus types are specific to diatoms found only in the Golden Brown Algae and are abundant in Torch Lake, making them significant in our findings on whether or not they are selectively eating and controlling the Golden Brown Algae.

Originally, the tally sheets we used had both *Encyonema* and *Cymbella* diatom genuses, which was later combined into the category *Cymbelloids*. At the magnification available in the study, we could not confidently distinguish between them, and, therefore, couldn't be accurately observed. Furthermore, our consultant, Professor Stevenson, told us that diatoms that resemble one another tend to behave similarly and agreed with our naming of the two as *Cymbelloid*. Data collected on the tally sheets was then put into an excel sheet and organized by quantity, abundance, and percentage alive, see Appendix, and was used for analysis.

Date: Sample ID: Slide ID: [ ] Syrup mount [ ] Wet mount

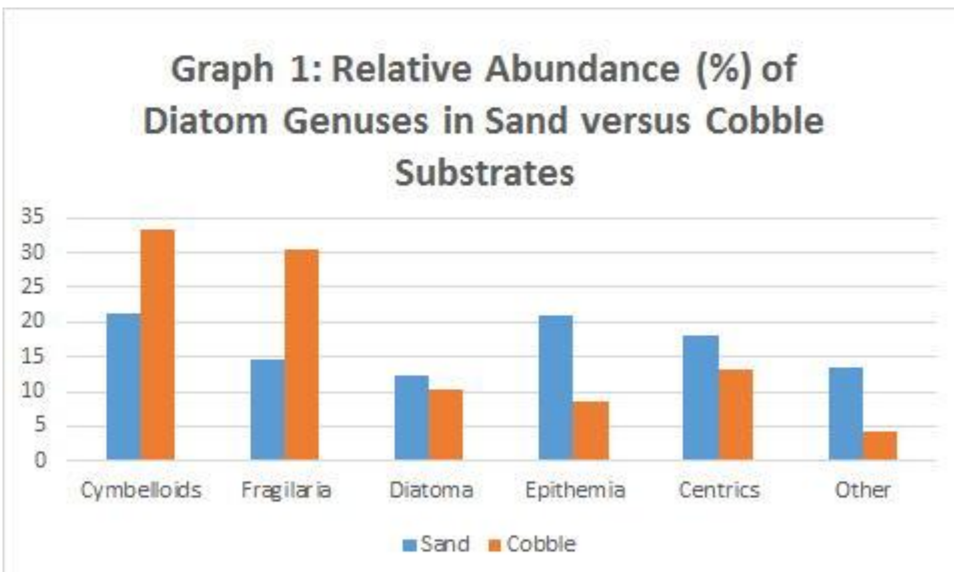
	Field One		Field Two		Field Three		Field Four		Field Five		Totals	Relative
	Total	Alive	Total	Alive	Total	Alive	Total	Alive	Total	Alive	Sum	Abundance
Encyonema Cymbelloids Cymbella												
Fragilaria												
Diatoma												
Epithemia												
Centrics												
Others												
TOTAL												

The original tally sheet used while observing slides for diatom counts

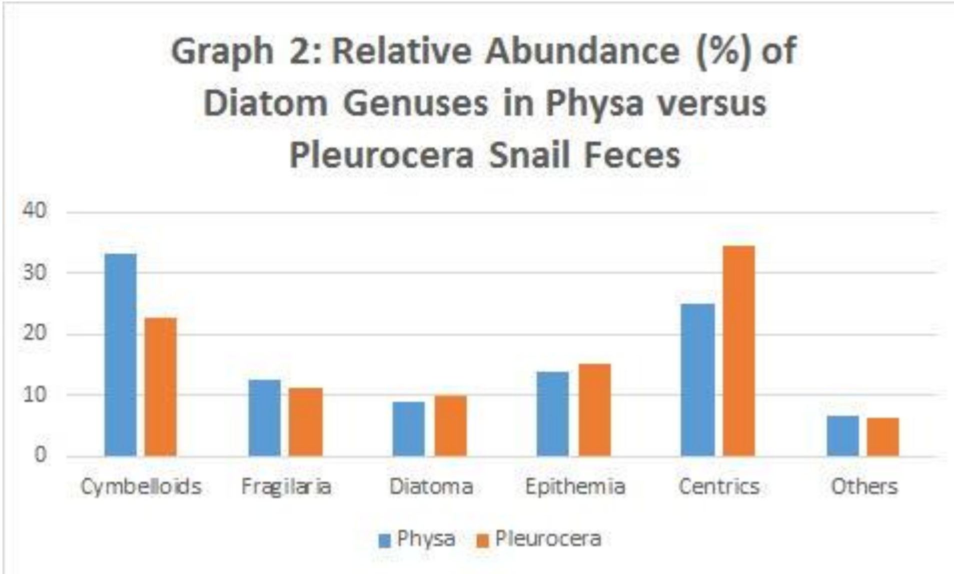
Results

The loss of some of our study enclosures and snails, made our sample size smaller than planned. This could have prevented us from finding what may have been true differences because our numbers were small and the ranges of our observations were large; however, after examining the remaining data, we had a handful of sure conclusions.

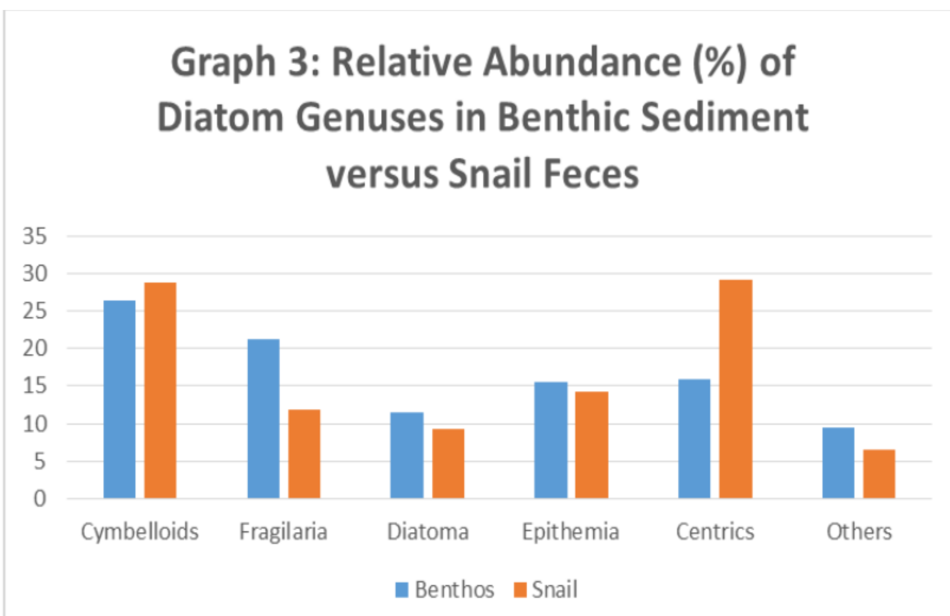
Graph 1 shows the relative abundance of the diatoms found in the two different substrates in our study (sand at Gourley and cobble at Hayo-Went-Ha). Despite the different bar heights, most notably for *Cymbelloids*, *Fragilaria*, and *Epithemia*, with the small number of samples, no statistically significant differences could be shown between these substrates. Therefore, the results were pooled for comparisons. Overall, the benthic algae showed the percent relative abundance of the diatoms to be *Cymbelloids* 26.3, *Fragilaria* 21.3, *Diatoma* 11.4, *Epithemia* 15.6, *Centrics* 15.9, and all others 9.4. This data provides the answer to our first question, what does the benthic diatom population consist of.



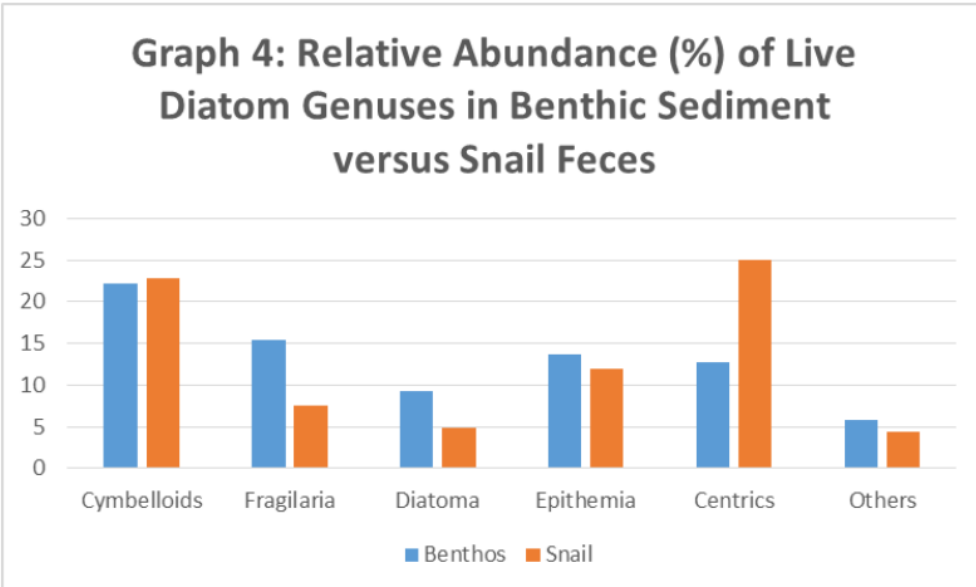
Graph 2 shows the relative abundance of diatoms in both *Physa* and *Pleurocera* fecal matter. As with the substrates, no statistically significant results prevailed from a Student T test, meaning that an average of both snail types from both locations would provide us with a large, more accurate representation of the collected data. This data was also pooled for comparison. Overall, the snail feces showed the percent relative abundance of the diatoms to be *Cymbelloids* 28.8, *Fragilaria* 11.9, *Diatoma* 9.4, *Epithemia* 14.3, *Centrics* 29.1, and all others 6.6. This data provides the answer to our second question, what diatoms are the snails consuming.



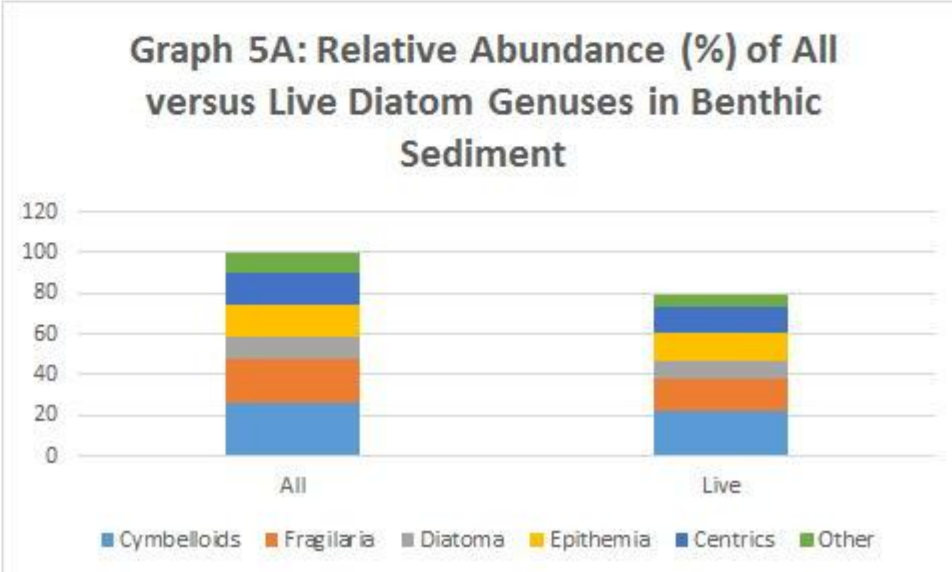
Graph 3 shows a comparison of the relative abundances of diatoms in the benthic sediment and in the snail feces. An interesting observation concerning the *Centrics* surfaced during this study. Graph 3 shows that there appears to be a substantial enrichment of the *Centric* diatoms in the snail feces relative to the benthic algae. An explanation for this has been suggested by Dr. Jan Stevenson, our consultant: The *Centric* diatoms tend to be on the surface of the benthic sediment, less mixed in than the other types, so as the snails graze, they may ingest the *Centrics* disproportionately in the benthos simply because these diatoms are presented to the snails first.

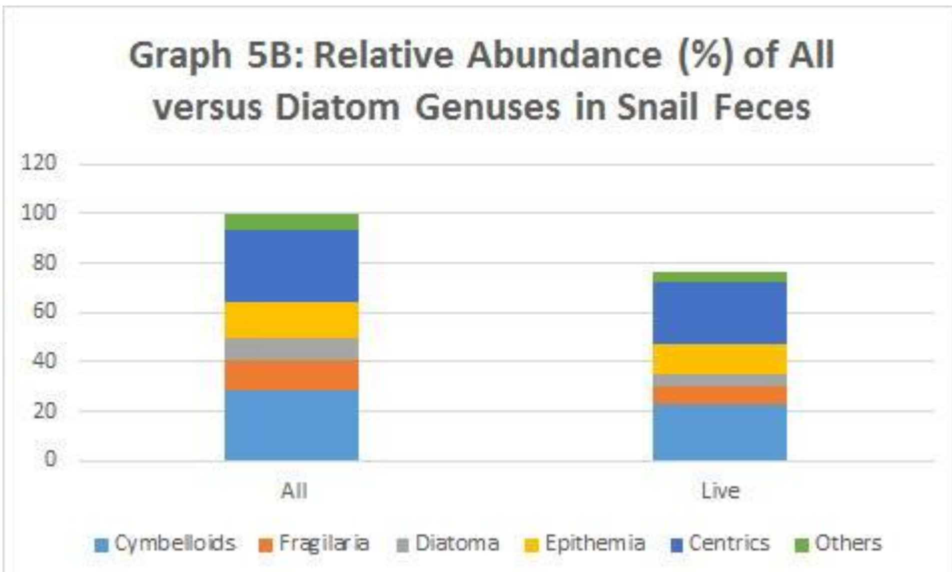


One of the more remarkable findings in our data is the high percentage of live diatoms excreted in the snail feces. Graph 4 shows that the enriched percentage of *Centric* diatoms was unexpected. We had assumed that what the snails ingested would be digested. Microscopic examination allows us to readily distinguish between living and dead diatoms, the dead being where there is only an empty, colorless glass shell and live where there is cellular material inside the shell.

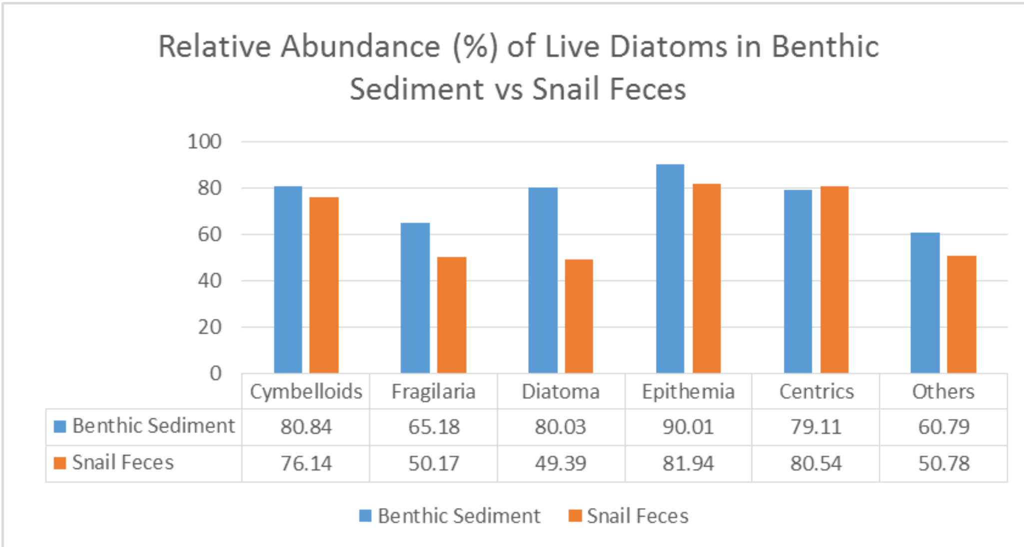


Looking at Graph 5A, we see that out of all of the diatoms in the benthic sediment available to the snails, most, 80% were alive. In Graph 5B, in the snail feces, after consumption, just under 80%, 79% still remained alive. This answers our third question, what did the snails digest, or lack thereof, given that the bulk of snail excretion consisted mainly of live diatoms. What was likely digested for nutritional value, bacteria and mucilages, was not observable in this study.





Graph 6 shows the data graphically. The one thing that stands out is the substantial drop in the percentage of live *Diatoma* diatoms from benthic sediment to feces, suggesting that the snails are actually deriving nutritional value from this type of diatom. To confirm this impression we performed a Student's T-test and found confirmation in the form of a highly significant p value of <0.003. It is important to note that graph 4 and graph 6 are different even though they have the same name, which is why they show us different data. Graph 4 shows the average percentage of live diatoms in each sample for each genus of benthos compared to snail feces. Graph 6 shows us the total percentage of live diatoms for each genus in each type of substrate when all the benthic samples are summed together and all the feces samples are summed together.



Discussion

For our study, we wanted to figure out if there was a difference in the benthic algae that made up the benthic sediment at the Gourley site, which was primarily sand, and at the Hayo-Went-Ha site, which was primarily cobble. It is important to note the difference in the benthic algae between the two substrates so that we know that the snails at the two locations had different amounts of each type of algae available. We did not find a big statistical difference between the two substrate types (Graph 1). However, we did discover that the cobble substrate had a higher average relative abundance of fragilaria diatoms in the benthic algae than in the sand substrate. The sand substrate also had a higher average relative abundance of *Epithemia* diatoms in the benthic algae than the cobble substrate, which demonstrates that the two substrates did have subtle differences. We also wanted to study both species of snails, the *Pleurocera* and the *Physa*, to see if there was any difference in the types of algae that they ate. In the end, both the *Physa* and *Pleurocera* had no significant difference in the relative abundance of the benthic algae in the fecal matter (Graph 2) , which shows that there is no significant difference in the types of algae that they chose to eat. This information allows us to infer that both types of snails have the same diet despite being two different types of snail.

It is worth noting that we did not find a lot of snails at either the sand or the cobble site (we imported the snails from elsewhere), and many of the snails we imported escaped before we could recollect them, so it is possible that had we had a larger group of data our numbers could have been slightly different.

We also chose to look specifically at the data to see if the snails could digest benthic algae. At both of the sites and in both of the species, the percentage alive of the benthic algae was very similar to the percentages alive of the benthic algae in the fecal samples (Graph 4). This suggests that the majority of the algae types are not actually digested when they are eaten by the snails. In fact the *Diatoma* diatoms is the only algae that seemed to have a significant percentage digested by the snails. In the benthic sediment, an average of 80% of the *Diatoma* diatoms was alive in the benthic sediment compared to 49% of the *Diatoma* in the feces samples, which means that about half of the *Diatoma* could be digested (graph 6). It is significant that most of the algae types are not digested by the snails, because this suggests that the snails may not be eating the algae because of the algae itself, but maybe because of the mucilage that surrounds the algae. Since most of the algae is not digested by the snails, the snails do not likely play a large role in controlling the algae population in the lake.

Conclusion

Upon analysis of the data, it was discovered that there was no clearly discernible difference between the two species of snail and/or the type of substrate they grazed upon. Therefore, we combined all corresponding data sets together from the variables that are the snail and substrate types, respectively. As noted in figures 1 and 2, however, there is a statistically significant difference of $p < .003$ by the student's T-test between the number of live *Diatoma* diatoms found in the benthic sample and those found alive in the snail feces, thus making them highly available to the snails. This trend was followed less obviously through all species of diatom, with the exception being that of the *Centrics*, where more live diatoms were found in the snail feces than the benthos. One possible explanation for this bizzare finding is that the snails were directly or indirectly grazing for the *Centrics*, covering a larger expanse than the relatively small benthic sample collected by the team, and that all other consumed diatoms were merely bycatch. It was hypothesized by Dr. Stevenson that the *Centrics* may be a large part of the photosynthesizing power that we can see on the lakebottom, producing the golden brown color that rests in large quantities on top of the algae mats. This is supported by the fact that a large percentage of all consumed diatoms were found alive, especially the *Centrics*, which led us to infer that they did not break down to provide nutritional value to the snails, thus solidifying that the snails are most likely not pointedly aiming to consume the *Centrics* as they provided little to no nutritional benefit in our study.

We would propose a future study examining the snail feces further for bacteria that may be indicative of what they truly are grazing for and its connection to the Golden Brown Algae. As an extension of the aforementioned, we hypothesize at this time that the bonding mucilages and other byproducts of the Golden Brown Algae are what nutritionally fuel the snails rather than the diatoms that are the algae themselves. Through the analysis of these two statistically differentiated diatoms (*Diatoma* and *Centric*) that pointed to separate conclusions about the eating patterns of the snails, as opposed to the other diatoms (*Cybelloids*, *Fragilaria*, *Epithemia*, and other) that consistently followed similar patterns when analyzed relatively, our team concluded that there is no true trend in the eating habits of the snails that would point to their intentional consumption of the diatoms that make up the Golden-Brown algae.

Bibliography

Personal communication: Dr. Jan Stevenson

Academic Article: Christopher G. Peterson

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