Analysis of snail assemblages and related conditions of Tenderfoot and Brown Lakes

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Abstract

Disturbances can affect community diversity. I compared Tenderfoot Lake, a public use lake, to Brown Lake, a private use lake to evaluate how disturbances affect the native snail assemblages. I evaluated the statistical differences in orthophosphate levels between plots within lakes and between Brown and Tenderfoot Lakes along with the Berger-Parker Index to examine the effect that frequent disturbance related to high use would have in comparison to infrequent, low use. I found that private docks located on the southern Tenderfoot shore did not permit aquatic plant growth because of large rocks, and no snails were found to reside within the site. Orthophosphate levels were also significantly lower at this site as compared to sites on both lakes with snails present. I also found that there was no significant difference in adjusted net phosphorus uptake between *Bellamya chinensis*, an invasive species, and *Lymnea stagnalis*, a native species.

Introduction

Disturbances can alter the trajectory of an ecosystem (Turner 2010), and biological invasions are often considered processes of disturbance as their presence can be a driving factor for ecological change (Gilloli et al. 2017). It is important to assess a species environmental impacts through environmental risk assessment as the effects could be undesirable (Gilloli et al. 2017). The introduction of invasive species can also cause a loss in diversity and should be avoided (Wright 2011).

The Chinese mystery snail (*Bellamya chinensis*), also known as black snail or trapdoor snail, is a large, freshwater, species of gastropod from the family Viviparidae that is invasive throughout the United States. The length of the shell can reach 65 mm, and its dry tissue mass can reach 1 gram (Solomon, et al. 2009). They can be identified by their globose shells and concentrically marked opercula (Kipp et al. 2017), which seals the opening of the shell to prevent predation and drying - surviving for up to four weeks out of water (Havel 2011). It is native to Southeast Asia, Japan, and eastern Russia (Kipp et al. 2017) and came to the United States as part of the Chinese food market in the late 1800s in San Francisco. It has spread across North America by means of the Niagara River (Solomon et al. 2009). Because *B. chinensis* is ovoviparous and releases live young, dispersal is especially effective; with this and surviving up to four weeks out of water, it is easy for one snail to survive attached to a boat, for example, and introduce over a hundred individuals into a new body of water (Havel 2011).

B. chinensis can be found in the littoral zone of many lakes in the Great Lakes area, at depths reaching a maximum of 3 m (Chaine et al. 2012), in high densities of 38 individuals per square meter (Solomon, et al. 2009). They have been typically found in the silty sand by submerged vegetation (Chaine et al. 2012). They tend to feed on organic and inorganic bottom material, diatoms, and benthic algae in North America (Kipp et al. 2017).

As far as its ecological effects, *B. chinensis* is relatively benign (Kipp et al. 2017). However, it was found to greatly reduce the presence of the native snail species *Lymnea stagnalis* in sampled areas (Solomon et al. 2009). It is important to try to understand the impacts on communities to determine at what level an invasive species like *B. chinensis* changes local environments (Solomon et al. 2009).

This study was conducted at the University of Notre Dame Environmental Research Center, a University of Notre Dame property that encompasses over 7500 acres on the border of Michigan and Wisconsin. Tenderfoot Lake lies on the southern border of the UNDERC property with an area of 194.24 hectares and 5.87 miles of shores surrounding the lake (Figure 1). Brown Lake lies on the southeast portion of the UNDERC property with an area of 32.87 hectares and 8205 ft of shores surrounding the lake (Figure 2). These two lakes are suitable for comparison because Tenderfoot has significantly more boat traffic than Brown because it is a public lake; this means that it has had more opportunities for the invasive gastropod to be introduced, so the populations were expected to vary between the two lakes.

As of yet, there are no existing control methods for the invasive populations of *B. chinensis* (Kipp et al. 2017), so learning more about the conditions in which they live may shed light on possible solutions to this ecological problem. Based on the work of Johnson, phosphate uptake levels in native and non-native snails may differ significantly (2009), so I also hoped to see if the orthophosphate levels in lakes were an indicator of the populations of native or non-native snails as *B. chinensis* has been seen to be a sink for this nutrient (Johnson et al. 2009). Orthophosphate (Figure 3) was measured rather than phosphorus because orthophosphate levels can be measured directly while elemental phosphorus does not exist by itself in water (Dabrowski and White 2015). Orthophosphate concentration can be tested for, and variations in levels can be considered biological change caused by a disturbance.

Materials and Methods

Data Collection

For this experiment, I investigated 4 sites on Tenderfoot Lake and Brown Lake on the UNDERC property in habitats that *B. chinensis* was expected to be present in, based on Chaine, N.M. et al. and Lam (2012). I chose one five meters by two meters transect in each of the north, south, east, and west quadrants of each lake and sampled four 1x1 meter plots within each transect randomly by tossing a 1x1 m^2 square made from PVC pipe within the transect. To collect snails within each plot, a partner and I used small, mesh nets to search the plot for ten minutes, similar to methods described in Chaine, N.M. et al. 2012. I recorded the coordinates of the four corners of each transect using a handheld GPS device and for the centers of each plot.

After collecting the snails from each transect, I transported the containers to the Hank Lab to identify and count the collected snails. I measured the lengths of the *B. chinensis* shells from tip to tip, the length of the aperture, and the wet masses for each specimen. I decided to also measure the lengths and apertures of vacant snail shells without measuring a mass. After measurements were taken, all *B. chinensis* were placed into a Ziploc bag and frozen. Concerning the native species, they were stored in containers with lake water and vegetation in the Hank Lab overnight before being returned to the sites from which they were collected.

Along with collecting the gastropods in each plot, I recorded some of the conditions present in each of the plots. I recorded current weather conditions and if the site was in close proximity (100 meters) to a boat dock. I also examined the types of present vegetation (submergent, emergent, and floating), the amount of chlorophyll A present, if the bottom could be seen, pH levels, and orthophosphate levels. I did not filter the water samples before testing for orthophosphate, but I allowed the particulate to settle and did not homogenize the water samples before testing. Therefore, levels of orthophosphate included both the dissolved and highly particulate forms based on this method. The process for testing was the Hach Total Ortho-/Meta-Phosphate Test Kit as described by B. Dabkowski and M. White 2015.

I used a chlorophyll content meter to measure chlorophyll A levels, the 1 m^2 PVC square to determine if I could see the bottom, and a pH meter to measure the pH at each of the four plots within the transects. Recording these data has revealed a better picture of the conditions in which *B. chinensis* succeeds in.

In another experiment, I set up forty-five tanks to study the difference in phosphorus digestion between *B. chinensis* and *L. stagnalis* as both are visibly present in Brown Lake. The tanks were filled with 1.5 L of water from Tenderfoot Lake, and I fertilized the water with appropriate amounts of phosphorus to attain three different starting levels of concentration within the tanks: 0 mg/L, 2mg/L, and 10 mg/L. Of the fifteen tanks with each phosphorus treatment, three contained no snails, three contained approximately five grams of *L. stagnalis*, three contained approximately ten grams of *L. stagnalis*, three contained approximately five grams of *B. chinensis*, and three contained approximately ten grams of *B. chinensis.* After allowing the tanks an acclimation period of about sixteen hours, I took water samples to the lab to measure the initial concentrations of orthophosphate with the Hach Total Ortho-/Meta- Phosphate Test Kit. Throughout the week, I checked on the snails periodically to monitor their health. After one week, I, again, took water samples to the lab to measure the final concentrations of orthophosphate with the same Hach kit.

Statistical Analysis

To analyze the relationships between the *B. chinensis* populations and other conditions, I used R 3.5.1 for Windows. I conducted t-tests to examine relationships of the northern, southern, eastern, and western sites on Tenderfoot and Brown Lakes. I also conducted 1-way ANOVA tests to determine variation within the four sites on both lakes. The inverse of the Berger-Parker Index, an index that measures the difference between populations of the dominant species and other species found in the same area, was used to compare the diversity of sites within lakes and between lakes using unpaired t-tests and 1-way ANOVA tests. A Tukey Post-HOC test was used to determine variations within sites on Tenderfoot Lake.

For the experimental orthophosphate values, the net phosphorus uptake was compared between species and the treatments using a series of 1-way ANOVA tests. Tukey Post-HOC tests were taken to compare the mass of the snails to their net phosphorus uptake.

Results

I failed to reject the null hypothesis that there was no difference between plots on Brown and Tenderfoot Lakes based on the inverse of the Berger-Parker Index in diversity for the northern (unpaired T-Test, R^2: 0.2802, p-value: 0.1773, F-statistic: 2.336, df: 6), eastern (unpaired T-Test, R^2: 0.207, p-value: 0.2574, F-statistic: 1.566, df: 6), and western (unpaired T-Test, R^2: 0.08565, p-value: 0.4818, F-statistic: 0.562, df: 6) plots on each lake. There was, however, statistical significance showing a difference between the southern plots of the lakes (unpaired T-Test, R^2: 0.9994, p-value: 6.349×10^{-11}, F-statistic: 1.02×10^4, df: 6).

When testing for significance in the differences between the two different masses of snails, five and ten grams, that were used in the phosphorus uptake experiment, I found that for *B. chinensis* at the 0 mg/L treatment, the mass of the snails did not affect the uptake (1-way ANOVA, df: 1, F-statistic: 3.359, p-value: 0.141). This was also found for *B. chinensis* at the 2 mg/L treatment (1-way ANOVA, df: 1, F-statistic: 0.66, p-value: 0.462) and the 10 mg/L treatment (1-way ANOVA, df: 1, F-statistic: 6.502, p-value: 0.0633). The 0 mg/L treatment for *L. stagnalis* (1-way ANOVA, df: 1, F-statistic: 5.918, p-value: 0.0718) and the 10 mg/L treatment for *L. stagnalis* (1-way ANOVA, df: 1, F-statistic: 0.014, p-value: 0.913) also had uptake that was not affected by the mass of the snails in the treatments. For *L. stagnalis* at 2 mg/L, the mass did affect the uptake of the phosphorus (1-way ANOVA, df: 1, F-statistic: 26.35, p-value: 0.00682).

I ran a Tukey Post-HOC test on the explanatory variable, net phosphorus consumed, in terms of mass of the snails (Tukey, df: 1, F-statistic: 0.817, p-value: 0.373) and treatment (Tukey, df: 2, F-statistic: 1.145, and p-value: 0.331) and found no statistical difference in either regard (Figure 4).

For the orthophosphate tests taken from the lakes at each subplot within the four transects of each lake, the data was normal based on the Q Plot (Figure 5). For these data, there was a statistically significant difference between lakes (1-way ANOVA, df: 1, F-statistic: 5, p-value: 0.0329).

On Tenderfoot lake, there was a significant difference between orthophosphate levels at the four different sites (1-way ANOVA, df: 3, F-statistic: 13.86, p-value: 0.000333).

Between the four different sites on Tenderfoot, normal data was obtained by transforming the inverse of the Berger-Parker Index plus two, squared. There was a significant difference between sites (1-way ANOVA, df: 3, F-statistic: 10.803, p-value: 0.001001). The Tukey Post-HOC test showed significant differences between the west and north sites on Tenderfoot (p-value: 0.0063714) and the west and south sites (p-value: 0.0007975). There were no significant differences between the north and east quadrants (p-value: 0.4580967), the south and east quadrants (p-value: 0.0708191), the west and east quadrants (p-value: 0.0885519), and the south and north quadrants, (p-value: 0.6097928).

On Brown Lake, there was no significant difference between the four sites (1-way ANOVA, df: 3, F-statistic: 0.6971, p-value: 0.5715).

Discussion

We can infer that the cause of this may be related to their land use because the southern plots on each lake were statistically different from each other in every way tested. On Tenderfoot Lake, the southern part of the lake is reserved for private docks, and the shoreline has been modified with large rocks and boulders. My data could suggest that this land development is negatively affecting snail populations, leading to their lack of appearance in this area. There is also more activity occurring from the private docks on Tenderfoot than the single, private dock that UNDERC has on the southern side of Brown Lake, where there was no significant difference between sites within the lake.

Disturbances in the past of Brown Lake may have made it more vulnerable to colonization of *B. chinensis*, before it was closed to public fishing. Currently, Brown Lake is a navigable inland lake, where all shoreline is owned by UNDERC. It can only be accessed through a navigable inland stream, such as Brown Creek (Michigan Constitution, Art IV, §52), meaning potential fishers must travel longer distances and may not have the same resources to clean boats afterward, possibly aiding the spread of *B. chinensis.* It is possible that the low level of disturbance that exists in the lake today has allowed *B. chinensis* to thrive in Brown Lake and not Tenderfoot Lake, a high use public lake. In a previous study, weak evidence was found to support that *B. chinensis* was more likely to be present in lakes with improved boat landings than in lakes with restricted public access (Solomon et al. 2009). However, my evidence appears to reject that there is a link at all.

In Figure 4, we can see that there is no statistically significant difference in the adjusted phosphorus uptake (found by subtracting the mean of the control treatments from the net phosphorus uptake) between *B. chinensis* and *L. stagnalis* as shown by the overlap in the dots. The two far outliers that occurred in *L. stagnalis* occurred in the same treatment, 10 mg/L and 10 grams of snails. These outliers may have had an affect on the overall dataset and may be why there was no significant difference between the two populations, though there was a differential trend seen between phosphorous uptake in the two species. It is likely that these outliers occurred because of the deaths of snails during the one-week experiment. Even with checking frequently on the well being of the snails, it is difficult to tell when they die because they remain in their shells, and the snails appeared heathy until late on day 6 of the experiment. Thus, they were not able to be replaced. In the future, this experiment could be better replicated with more replicates to gather more data and with other ways of checking for life-signs in experimental snails used.

In the observational study, the orthophosphate levels in Brown and Tenderfoot Lakes were significantly different which was likely related to the significant difference between the southern plots on Tenderfoot and Brown Lakes, as the southern plot in Tenderfoot Lake had a significantly different orthophosphate concentration than all other sites sampled. This relates to the idea that this southern site on Tenderfoot displays the negative affects on snail communities due to shore development and frequent disturbance because it is used for many private boat launches and hosts no snails due to the large rocks present. The large rocks are not conducive to vegetative growth, and aquatic plants have important ecological roles as nutritional sources and habitat for invertebrates (Frost and Hicks 2012). Therefore, this data may suggest that human activity has been detrimental to the snail communities present on the southside of Tenderfoot Lake. However, without historical data to show pre-development snail populations in the area, we cannot fully know if development removed the existing snail populations on the south side of Tenderfoot Lake.

Brown and Tenderfoot Lakes have similar inflow and outflow patterns of creeks entering near the south and exiting towards the north, making them more comparable. The differences between sites on Tenderfoot Lake in diversity based on the inverse of the Berger-Parker Index alludes to a possible sedimentation gradient that promotes higher diversity in the western plot due to finer sedimentation, more vegetation, and smaller rocks present as compared to the southern site on Tenderfoot. It is also possible, since the eastern plot on Tenderfoot lake is less diverse than the western plot, that the dock for the UNDERC Wet Lab may cause enough frequent disturbance to hinder a more diverse snail community. There have been instances of *B. chinensis* in the northern part of Tenderfoot Creek where water flow slows, and the soil is rich in particulate organic matter, similar to the soil found at Brown Lake where *B. chinensis* was found at every site. Therefore, it is possible that the water flow through Tenderfoot Lake and the rocky and sandy soil present on most of the lake's shores is a factor in hindering the colonization of *B. chinensis*. In China, the snail is commonly found in rice paddies, and because some research suggests the snail ingests sludge, it is called the Mud Snail (Kurihara et al. 1986).

Though this study did not have a very large sample size, it does suggest that there could be a trend of shoreline development negatively impacting snail populations. These snail assemblages are of conservation importance because they are highly threatened and diverse (Solomon et al. 2009). These snails are important prey for other organisms like crayfish, and their loss could potentially change community diversity in a lake over time (Johnson et al. 2008).

With the lack of diversity appearing at sites with increased human use, it may be possible that humans are negatively affecting aquatic plant communities and the subsequent communities that are supported by aquatic plants through frequent use and modification.

Figures

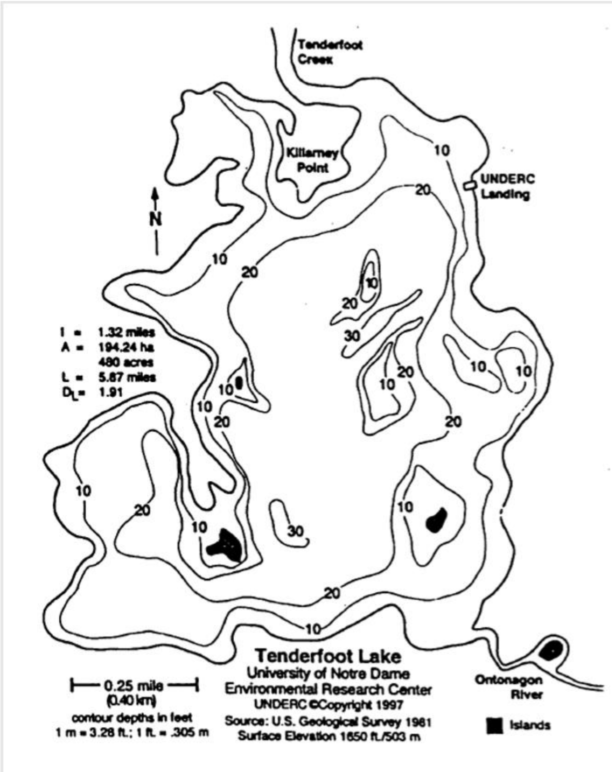

Figure 1: A bathymetric map of Tenderfoot Lake.

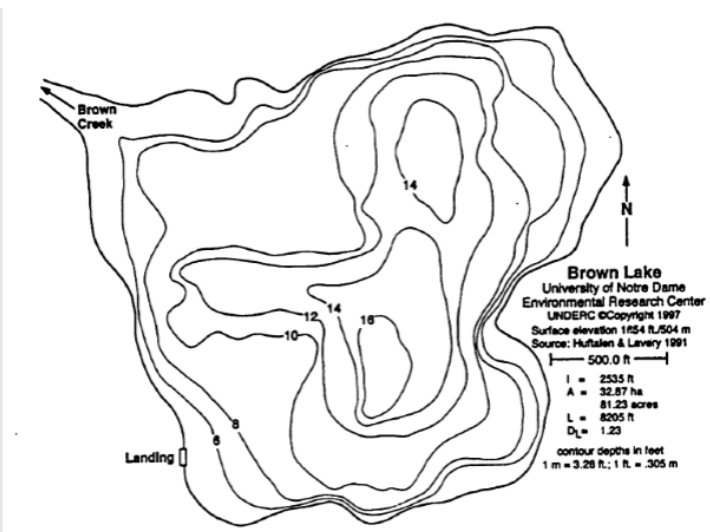

Figure 2: A bathymetric map of Brown Lake.

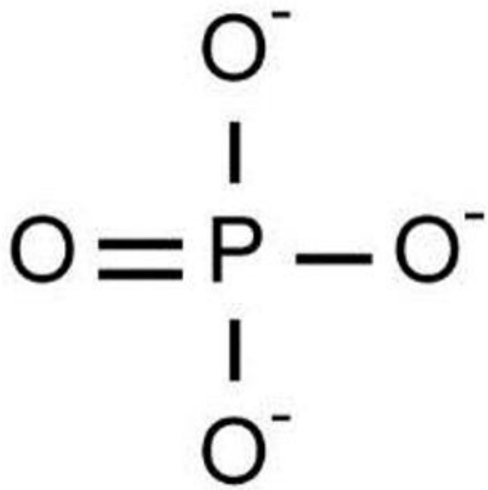

Figure 3: Orthophosphate structure.

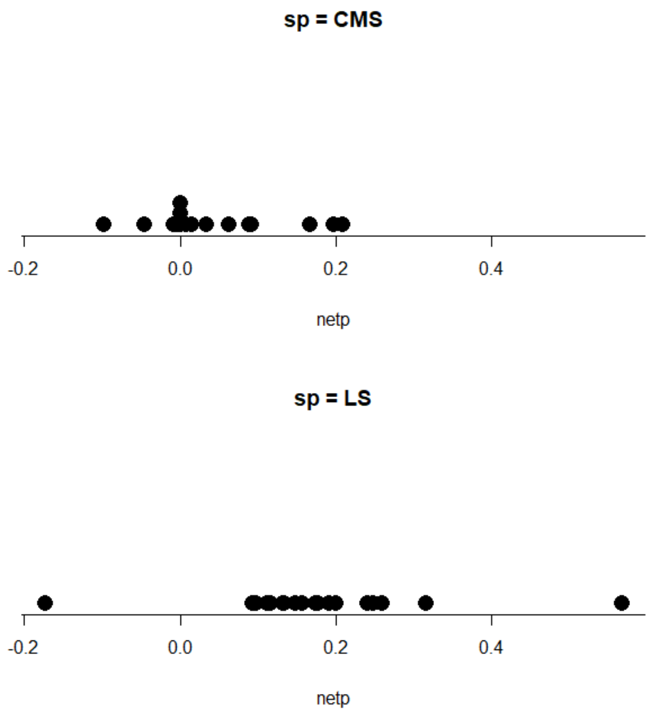

Figure 4: A dot plot of the two species *B. chinensis* (CMS) and *L. stagnalis* (LS) and the occurrences of the net phosphorus uptake for the three treatments. Outliers in the LS plot (seen at -0.17 and 0.57) may have obscured trends of difference in net phosphorous uptake.

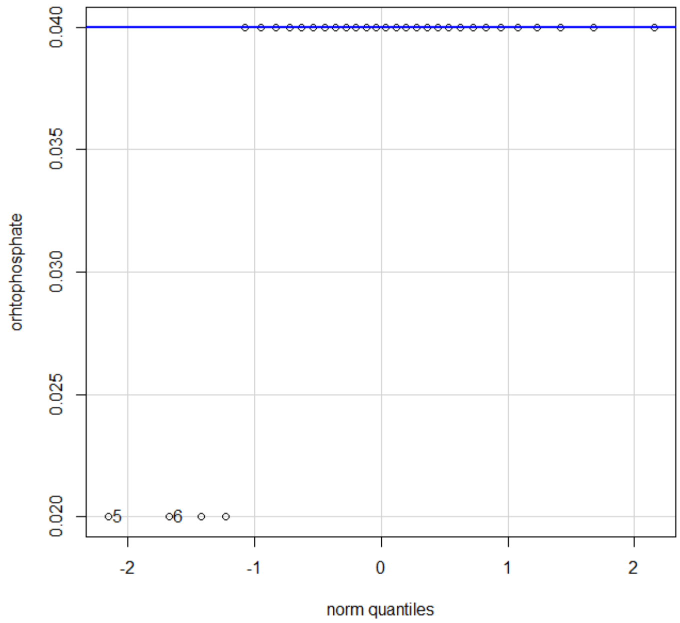

Figure 5: Q Plot of the orthophosphate levels in the four subplots of each of the four sites on Tenderfoot and Brown Lakes (w=0.39059, p-value=2.014×10^{10}).

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