The Effect of Nitrogen, Phosphorus, and Calcium Concentrations on *Dreissena polymorpha* Population Densities in Douglas Lake

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Abstract

The zebra mussel *(Dreissena polymorpha),* a highly invasive species in the Great Lakes region, depletes native unionidae of their necessary environmental components, such as phytoplankton. We hypothesized that the concentration of nitrogen, phosphorus, and calcium, as necessary components for plankton and mussel growth, will be limiting factors in *D. polymorpha* population densities. Our study involved the sampling of various sites around Douglas Lake. *Dreissena polymorpha* showed no preference for calcium or phosphorus concentrations, but showed a trend between increasing population densities and decreasing nitrogen concentrations. There seems to be a strong effect between population densities of *D. polymorpha* and human activity. Understanding how *D. polymorpha* use nutrients may be a key factor in decreasing their negative effects on our freshwater ecosystems.

Introduction

Many invasive species cause adverse effects upon the habitats they invade. One such invasive species includes the zebra mussel *(Dreissena polymorpha)*. Originating from the Black, Caspian, and Azov Seas in Central Asia, they were first spotted in Lake St. Clair, Michigan in 1988 and have continued to spread throughout the Great Lakes region. Their dispersal throughout these areas occurred through accidental collection and transport of their larvae and adult individuals in the ballast water of many barges and trade ships, as well as the unhygienic treatment of recreational boats. These mussels fill in quickly and densely, with densities recorded up to 700,000/m^2 in a water intake valve of a Michigan power plant (Benson and Raikow 2010).

Dreissena polymorpha pose a problem both economically and ecologically. The mussels have been known to infect many water supply pipes of nuclear power plants, public water supply plants and industrial facilities, impairing the delivery of water to these facilities' users. They have caused large disturbances to the ecosystems that they have invaded. These mussels filter suspended clay, silt, bacteria, phytoplankton and zooplankton from the water around them (MacIsaac 1996). Not only does this increase competition between other native plankton consuming organisms, but it also increases the water clarity in these areas (Benson and Raikow 2010). Increased water clarity allows increased light transmittance in the water column, in effect increasing water temperature and the growth of benthic plants. This shifts the biomass production in shallow regions from pelagic to benthic food-webs, creating an alternative ecosystem (MacIsaac 1996).

Calcium, being a main component of *D. polymorpha* metabolic rates and the structure of their shell, may be a key factor affecting their distribution (Whittier *et al*

2008). It has been shown that mussels in different areas have different calcium thresholds, with North American mussels having a lower threshold than those of Europe. These varying thresholds have been correlated to the mussels' ability to reproduce. Calcium levels can be highly variable in some bodies of water between different depths and locations (Cohen 2001).

Along with calcium, abundance of their food source, phytoplankton, may act as a limiting factor on the growth and proliferation of *D. polymorpha* (Benson and Raikow 2010). Phytoplankton are the autotrophic component of the plankton group. They are the foundational food source for much aquatic life, such as different unionidae and many other primary consumers. While there are many different species of phytoplankton that vary in shape and size, they all depend on similar macronutrients, such as nitrates and phosphates, to grow (Herring 2010). Thus, it can be assumed that higher concentrations of these nutrients will generate higher concentrations of phytoplankton.

This study focuses on the population densities of *D. polymorpha* as a result of varying concentrations of nitrogen, phosphorus and calcium. Because phytoplankton are the main food source of *D. polymorpha* and rely heavily upon nitrates and phosphates for their growth, higher concentrations of these macronutrients should yield higher concentrations of phytoplankton. Similarly, higher concentrations of necessary components for *D. polymorpha's* growth, such as calcium and phytoplankton, should yield higher population densities of these mussels. Therefore, we hypothesized that higher population densities of *D. polymorpha* will be found in areas with higher levels of nitrogen, phosphorus, and calcium.

Materials and Methods

We selected five different locations around Douglas Lake to survey. These included two populated locations: Silver Strand Beach and the shore near Pell's Island, in anticipation of higher levels of nitrogen and phosphorus, possibly due to pollution. We also surveyed two locations in South Fishtail Bay: Sandy Shoal and the University of Michigan Biological Station (UMBS) Beach, where more water mixing occurs due to wind prevalence. The last location was a relatively undisturbed beach in North Fishtail Bay (Figure 1). Using a transect tape, we measured three 9 m transects at a water depth of 0.4 m at each site, randomly selecting three points along each transect (Figure 2). We counted the living zebra mussels within one square meter sampling plots at each of the three points along each transect using square meter PVC pipes. At each site, we took two one-liter water samples, one in the afternoon of May 29, 2010 and one in the afternoon of May 30, 2010, to be analyzed for calcium concentrations. We also took two 100 mL water samples on the same days to be analyzed for nitrate and phosphorous concentrations. Water temperature and pH, measured with a Fisher Scientific AP 10 meter, were taken on two different days in order to obtain an accurate average of the data. A Garmin GPS 60 was used to record the latitudinal and longitudinal coordinates of each site.

Statistical Analysis

A Kruskal-Wallis Analysis of Variance was used to test for statistical significance between the population densities of *D. polymorpha* among our five different sampling sites. A Mann-Whitney U Rank Sum Test was then used to compare the densities of *D. polymorpha* between two sites. We ran ten Mann-Whitney tests to compare each site to all four other sites. Average nitrogen, phosphorus, and alkalinity ($CaCO_3$) levels were found using the two water samples from each location. In order to compare, we made a separate bar graph for each nutrient in order to demonstrate its varying levels at each site, as well as a bar graph to compare *D. polymorpha* population densities at each site (Figures 3-7). Because the site near Pell's Island contained significantly more zebra mussels than any of the other sites, we excluded its data from one of the population density graphs in order to better compare trends among the rest of the sites (Figure 4). A significance level of 0.05 was used for all statistical tests.

Results

We found statistically significant differences in the mean number of *D. polymorpha* between some sampling plots compared to others (Figure 3). Significant differences were found between the following: UMBS beach and Pell's Island ($p < 0.001$), UMBS beach and North Fishtail ($p = 0.017$), North Fishtail and Pell's Island ($p < 0.001$), North Fishtail and Silver Strand Beach ($p < 0.001$), Pell's Island and Sandy Shoal ($p < 0.001$), and Pell's Island and Silver Strand Beach ($p < 0.001$) (Figure 3).

Nutrient samples showed that while alkalinity varied little between locations on both days one and two, phosphorous and nitrogen levels did vary. Nitrogen was highest at Silver Strand Beach, and remained at a relatively low level at other locations (Figure 5). Phosphorous was highest at the two locations nearest houses: Pell's Island and Silver Strand Beach (Figure 6).

Using multiple bar graphs to look for general trends, we did not see a trend between phosphorous levels or alkalinity with *D. polymorpha* population densities at any site (Figures 3, 6, 7). However, there appears to be a general trend between nitrogen levels and population densities (Figures 4, 5). In removing data from Pell's Island, we detected a decrease in the mean densities of zebra mussels as nitrogen levels increase.

Discussion

When analyzing our data, statistical tests could only be run to compare population densities of *D. polymorpha* at each site compared to every other site. No statistical comparisons could be made between the population densities and the nutrient concentrations at each of the sites due to the type of data collected, as well as an insufficient amount of water samples. However, there were some general trends that could be observed in the bar graphs between population densities of *D. polymorpha* and nutrient concentrations. Although we were able to see such trends, we could neither support nor reject our hypothesis that increasing population densities of *D. polymorpha* correlate with increasing nutrient concentrations without adequate statistical support. Even when considering other studies, it is hard to support or reject our hypothesis because there are many contradictory studies that relating higher nutrient concentrations to both higher and lower population densities of *D. polymorpha.*

One result was that the two most developed areas, Silver Strand Beach and Pell's Island, contained the highest concentrations of both nitrogen and phosphorus (Figures 5, 6). This may indicate that byproducts of human development, such as septic tank leaching, increase nitrogen and phosphorus concentrations in the water. These varying concentrations between developed and undeveloped sites seem to have some interesting effects on the population densities of *D. polymorpha.*

In comparing nitrogen concentrations, Silver Strand Beach had the highest concentration of nitrogen by 27%, or .1305 mg N/L, over Pell's Island (Figure 5). Silver

Strand Beach also had the highest concentration of phosphorus by 5.2%, or 0.7 ug P/L, over Pell's Island (Figure 6). The combination of these higher concentrations is a possible explanation as to why Silver Strand Beach had the lowest population density of *D. polymorpha* while Pell's Island had the highest population density. These higher concentrations could indicate that eutrophication, a large increase in chemical nutrients such as nitrogen and phosphorus, had a detrimental effect upon the development of *D. polymorpha,* as one study suggests (Stancykowska 1983). Further experimentation is necessary to test whether the individual components of higher concentrations of nitrogen or phosphorus have an effect on the development of *D. polymorpha.*

In comparing population densities of D. *polymorpha* and nitrogen concentrations, with the exception of Pell's Island, we noticed a trend in decreasing population densities of *D. polymorpha* with increasing concentrations of nitrogen (Figure 4, 5). This is contrary to our hypothesis and many studies, including Johegen et al. (1995) study, which have suggested that an increasing number of *D. polymorpha* should cause an increase in the concentration of nitrogen. We believe that our results do not reflect a process that the *D. polymorpha* are causing; rather they possibly reflect the level of human activity around our testing sites. It seems that the level of human activity affects the level of nitrogen in the water, and generally, *D. polymorpha* population densities seem to be restricted by high levels of eutrophication in the water that they are attempting to infest (Stancykowska 1983).

Our results did not show a notable difference or trend in population densities of *D. polymorpha* between areas of varying calcium concentrations (Figure 7). However, a previous study by Stanczykowska (1964) has shown a weak relationship between calcium concentration and *Dreissena* densities. Similarly, it has been noted that both fertilization success in eggs and survivorship of *Dreissena* embryos were enhanced by calcium concentrations above 47 mg/L (Sprung 1987). Our results showed a minimum alkalinity concentration of 131.59 mg/L, well above the 47 mg/L threshold (Figure 7). Since population densities of *D. polymorpha* varied drastically between sites and alkalinity varied only slightly, it seems that differing concentrations of calcium above this threshold have no effect upon mussel growth. Therefore, because Douglas Lake contains approximately similar calcium concentrations throughout, varying levels of this nutrient above the threshold may not be the limiting factor in *D. polymorpha* densities. Future studies should look at mussel densities between lakes that differ in water chemistry to determine if a correlation does exist.

We noticed that areas containing lower mussel densities are those regularly visited by beach goers. The Mann-Whitney U tests did not indicate statistical significance of this, but definite trends may be seen. For example, the site near Pell's Island may directly demonstrate this. The transect that was along the beach at this site contained notably fewer mussels than the transects located further away from the beach, with the furthest showing the highest density of mussels (Figure 8). Similarly, the Silver Strand Beach site, which had the lowest *D. polymorpha* population density, was located directly in front of a family's house. The UMBS beach site, with the second lowest population density, is a popular bathing site for University of Michigan students and staff (Figure 3). There are two possible explanations for this: the first being as simple as people removing zebra mussels in these popular sites in order to recreate in safer substrates, and the second being the Intermediate Disturbance Hypothesis.

The Intermediate Disturbance Hypothesis is described as an intermediate level of disturbance that maximizes biodiversity by preventing any species from reaching its carrying capacity and outcompeting other species (Grime 1973, Horn 1975, Connell 1978). This specifically relates to the differing population densities of *D. polymorpha* because some of the sites we sampled seem to have had more human activity than the other sites we used. Human activity could be considered an intermediate disturbance when it includes disturbing sediments, moving substrates and mixing the water, perhaps making it more difficult for *D. polymorpha* to proliferate. However, we were not able to determine if human disturbance could have caused *D. polymorpha* from reaching carrying capacity, because we do not know the carrying capacity of these specific plots. The level and frequency of disturbance, rather than nutrient availability alone, could act as a factor preventing *D. polymorpha* from reaching their carrying capacity.

There are multiple improvements that could be made in the future for a more practical and conclusive study on the relationship between population densities of *D. polymorpha* and nutrient concentrations. A more extensive experiment should be conducted in which nutrient levels at these sites are looked at over a period of several years, or even a controlled experiment in order to isolate variables, to fully understand the role of nutrients in *D. polymorpha* growth. Furthermore, concentrations of nutrients such as chloride and silica, as well as more variance in calcium concentrations, may provide additional insight into varying *D. polymorpha* densities around similar habitats. Our results suggest that factors other than nutrient availability may affect *D. polymorpha* densities, such as human presence and other disturbances. It would be interesting to study these factors further in order to gain an understanding of how they might affect *D. polymorpha* population densities in Douglas Lake and in the greater Great Lakes region.

While our study did not bring about conclusive evidence to support or reject our hypothesis, or to fully demonstrate the impact of nutrient concentrations on the population densities of *D. polymorpha,* we were able to observe some trends that could set the basis for understanding this relationship. Given this, *D. polymorpha* warrants intensive further study, especially with its rank as the ninth worst invasive species in the world (Fetini 2010). With as much ecological and environmental damage as it causes to species richness and humans, *D. polymorpha* should continue to be studied in order to understand what makes certain bodies of water more susceptible to its growth, and how to control or eradicate infested waters in an attempt to restore native habitats.

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Figure 1: *Dreissena polymorpha* populations were recorded at 5 different sites: Silver Strand Beach (GPS: N: 45°35'11.2"; W: 84°43'47.1"), Pell's Island (GPS: N: 45°34'19.9"; W: 84°32'48.4"), UMBS beach (GPS: N: 45°33'36.4"; W: 84°40'27.7"), Sandy Shoal (GPS: N: 45°33'43.6"; W: 84°40'2.1"), and North Fishtail Bay (GPS: N: 45°35'10.1"; W: 84°39'47.4").

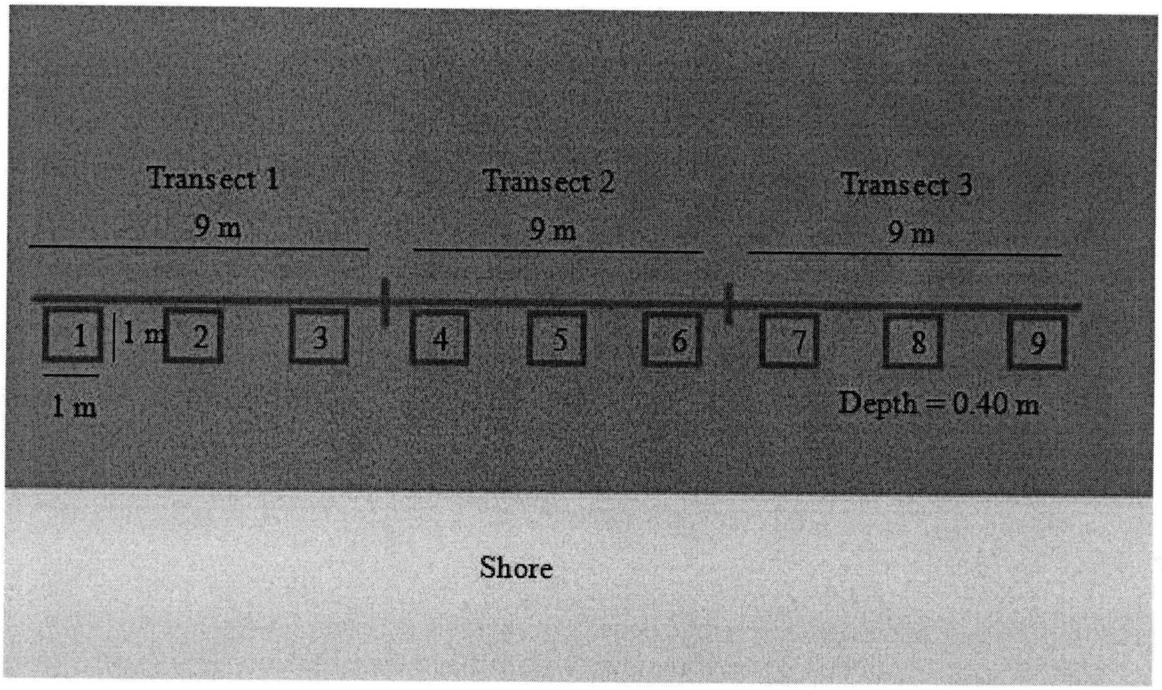

Figure 2: At each site, three 9 m transects were taken at a depth of 0.40 m, each containing three randomly spaced 1 m^2 sampling spots. *Dreissena polymorpha* populations were observed and recorded from each of the sampling spots and water samples were collected from each site.

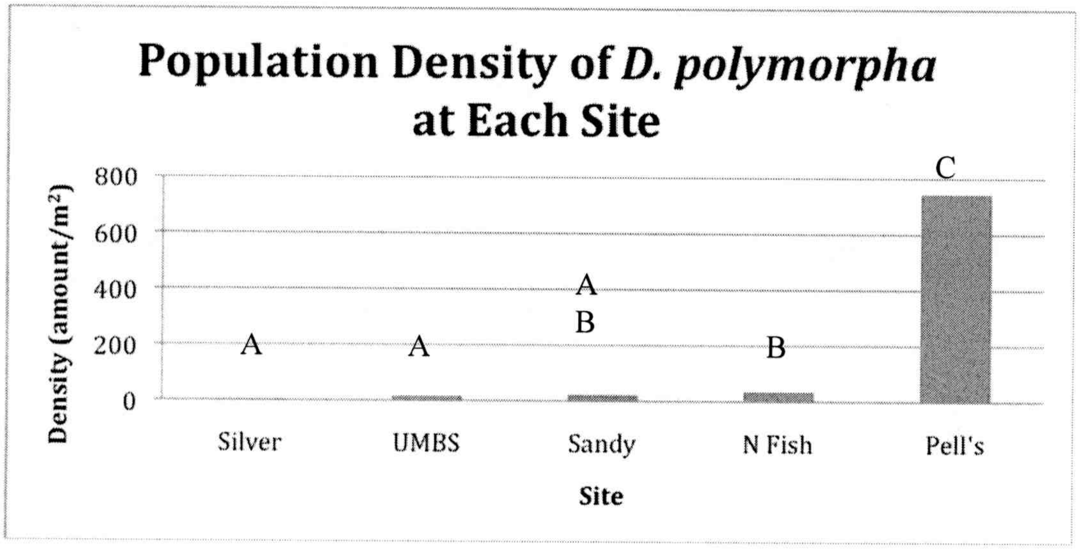

Figure 3: The average density of mussels per sampling site. It may be seen that Pell's Island contained an overwhelmingly higher density than the other sites.

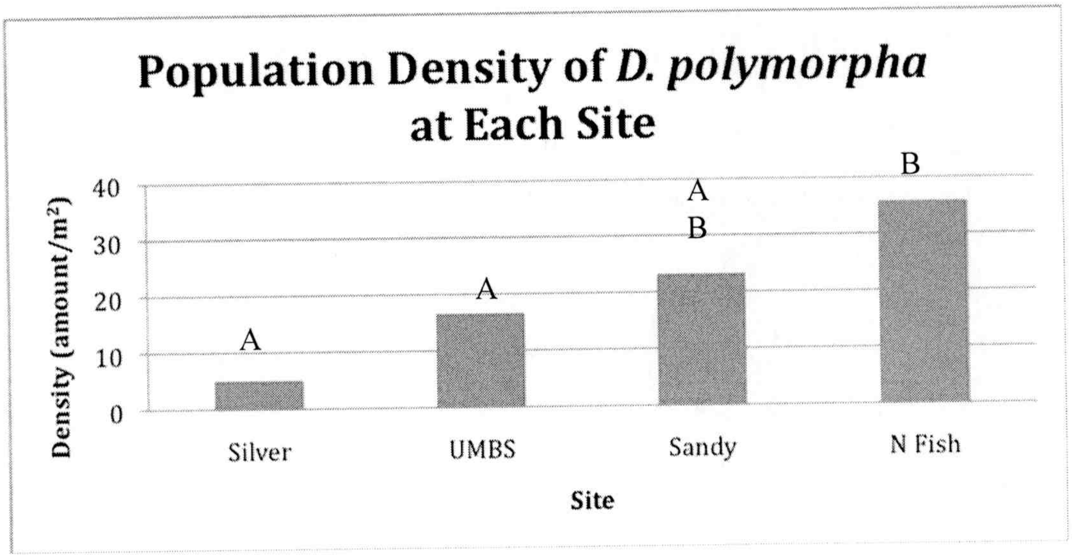

Figure 4: The average density of mussels per sampling site, with Pell's Island removed to better see the population density of *D. polymorpha* in lower density sites.

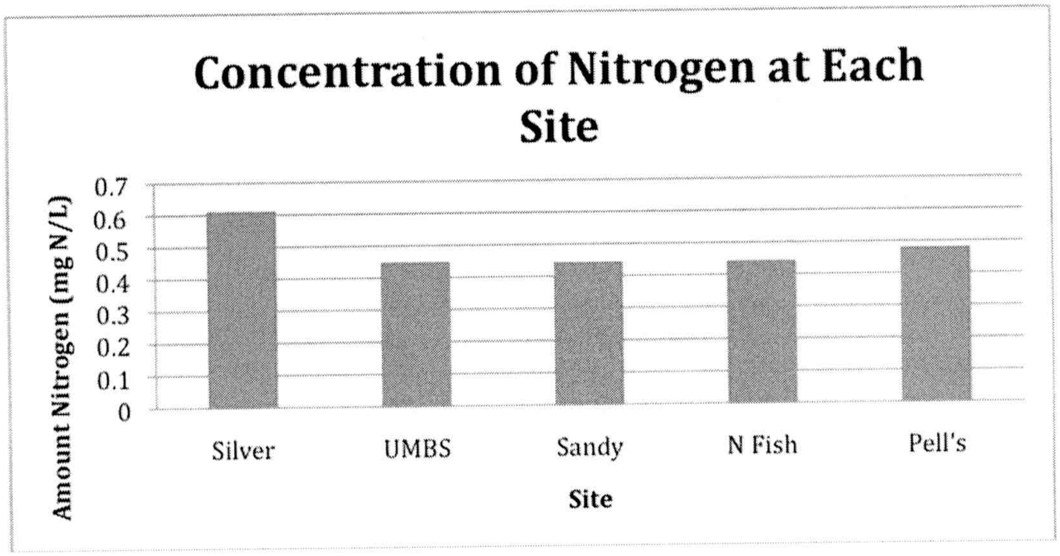

Figure 5: The average concentrations of nitrogen found in the different sampling sites. The nitrogen levels range from lowest to highest, corresponding to North Fishtail, Sandy Shoal, UMBS beach, Pell's Island and Silver Strand Beach, respectively.

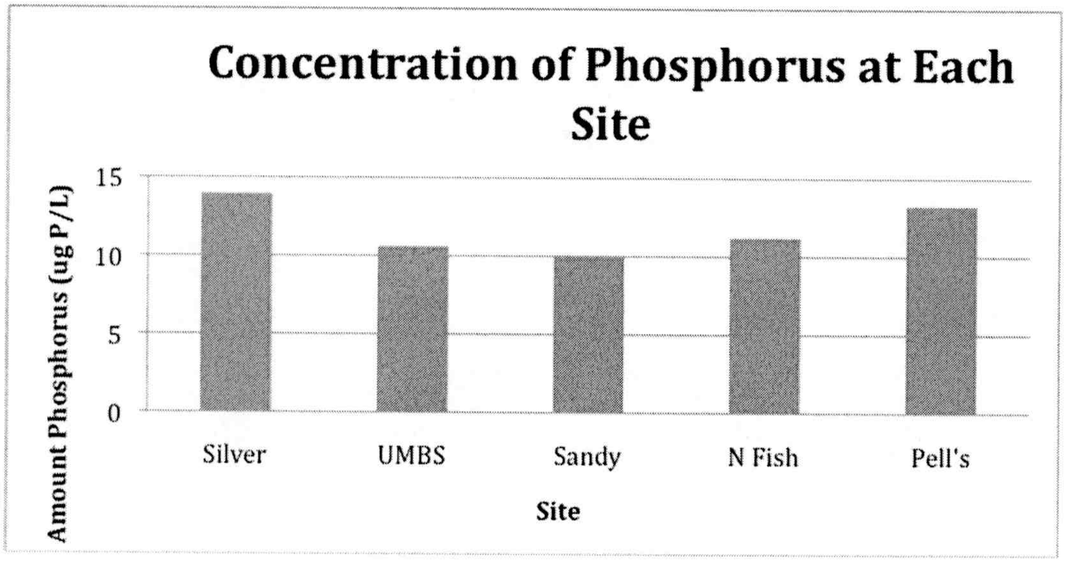

Figure 6: The average concentration of phosphorus found in the different sampling sites. The phosphorus levels range from lowest to highest, corresponding to Sandy Shoal, UMBS beach, North Fishtail, Pell's Island and Silver Strand Beach, respectively. No trend may be seen.

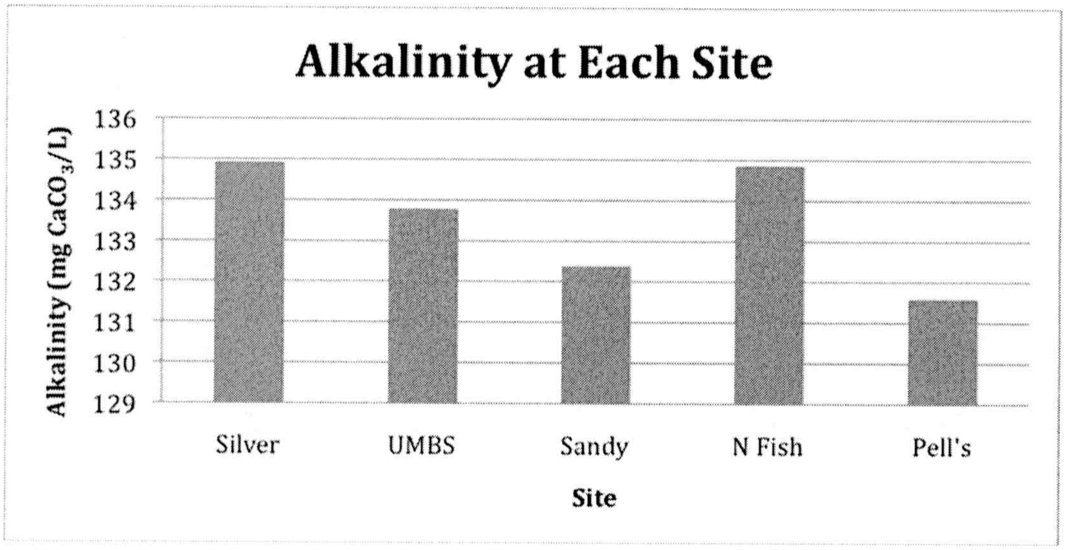

Figure 7: The average concentrations of $CaCO_3$ found in the different sampling sites. The alkalinity ranges from lowest to highest, corresponding to Pell's Island, Sandy Shoal, UMBS beach, North Fishtail and Silver Strand Beach, respectively. No trend may be seen.

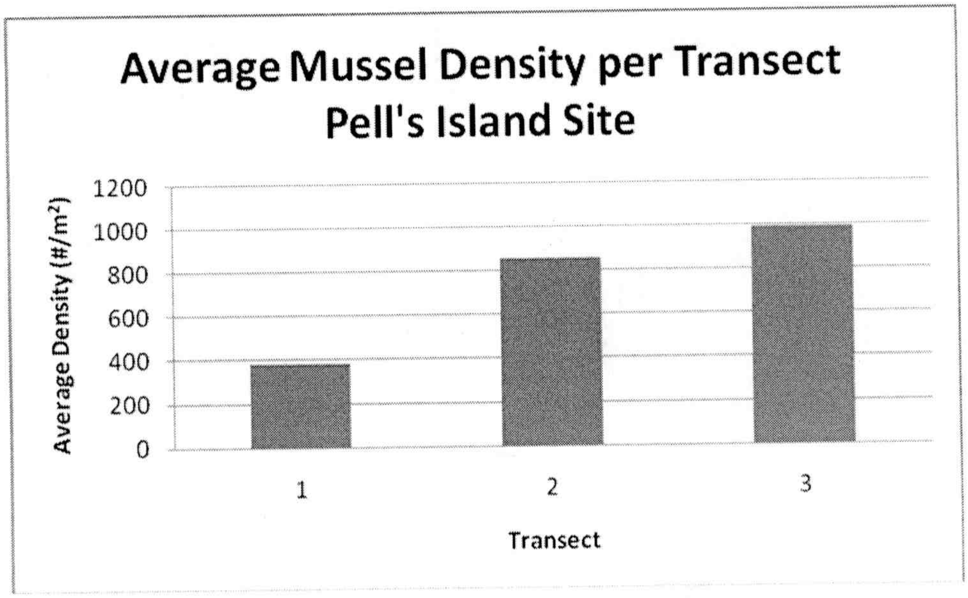

Figure 8: The average zebra mussel density per transect at Pell's Island. A trend may be seen in increasing density of mussels as distance from the beach increases, with transect 1 being the closest to the beach.