Population change of invasive *Dreissena polymorpha* in Douglas Lake from 2003 to 2009

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Abstract

Field observations led us to believe that populations of *Dreissena polymorpha* in Douglas Lake were decreasing. We hypothesized that this change was caused by environmental changes such as a change in water level, population dynamics including an approach to carrying capacity, or the reduction of resources such as suitable substrate and phytoplankton supply. Our study followed many of the methods of the Galligan (2005) survey for the best comparison between years. Sampling gave a greatly reduced number from the past survey; we found a total of 358 *D. polymorpha* in which 213 were living. While in 2005, Galligan found a total of 3437 *D. polymorpha*. Biomass was calculated from this sampling and was compared with phytoplankton densities; however there were no significant results. The survey for *D. polymorpha* veligers was also inconclusive as it produced only one veliger. The decrease in population may be a result of biotic environmental changes or an approach to carrying capacity but further specific research would have to be done to pinpoint a specific mechanism for this decrease. Our research provides valuable knowledge of an invasive species that could contribute to the eventual control of *Dreissena polymorpha* by natural means.

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Introduction

Invasive zebra mussels (*Dreissena polymorpha*) have negatively affected many of the waterways in the United States. *D. polymorpha*'s original habitat is the Caspian Sea (Williamson 1996). This species spread throughout Europe and the United States by commercial and recreational ship traffic as a primary vehicle for transport. The planktonic free-swimming larval form, veligers are commonly taken in with ship ballast water and can be transported long distances (Williamson 1996). *D. polymorpha* were first observed in Lake St. Clair in 1988 and spread shortly after to Lakes Michigan and Erie (Williamson 1996). They have rapidly expanded their distribution into many lakes and rivers in the eastern United States and Canada (USGS 2008).

D. polymorpha are highly invasive species that possess traits with negative effects on freshwater ecosystems. Adult D. polymorpha can produce up to one million eggs or ten billion sperm per season, which are released into the water for external fertilization. The first stage is a transformation from trochaphore larvae to free-swimming veligers. The veligers remain in this stage for eighteen to ninety days before they develop byssal threads to attach to hard surfaces. Finally, they become adult plantigrades, firmly attached to a substrate (Dobson and Frid 2009). D. polymorpha out compete native unionid populations due to their high fecundity, ability to colonize native mussels, and their high rate of filtering one liter of water per day (USGS 2008).

In habitats like Douglas Lake, *D. polymorpha* have the ability to reduce native populations of unionids, zooplankton, and fish because of interspecific competition over limited resources and habitat. *D. polymorpha* filter so much phytoplankton that native zooplankton and most fish communities suffer due to limiting food supply. *D. polymorpha* improve water clarity

and allow plant growth, which is beneficial to yellow perch and northern pike. However in combination with limited food supply it has not been an observed positive effect (USGS 2008).

D. polymorpha were first observed in Douglas Lake in 2001. Rapid expansion of their population is directly correlated to the severe diminution of native mussel populations. Although D. polymorpha populations in Douglas Lake have been rising since 2001 (Galligan 2005), our initial field observations led us to believe that their populations have declined. This may be due to a lack of substrate such as native unionids or limited food supply. Therefore, we hypothesize that D. polymorpha populations in Douglas Lake have declined since 2005. Certain aspects to look at are environmental changes such as change in water level. We expect to see D. polymorpha closer to the shoreline with greater substrate presence. Another aspect is population dynamics such as an approach to carrying capacity. With a greater D. polymorpha presence, we expect to see lower phytoplankton availability because of limiting resources due to intraspecific competition.

Methods

We surveyed Douglas Lake for *D. polymorpha* and tested for factors related to population decline. The six sites selected were North Fishtail Bay, Stony Point, West Pell's Island, Bryant's Bay, Grapevine Point, and South Fishtail Bay. We established four linear transects at each site using ~1.5m PVC pipe posts equally spaced. Each transect was one meter wide and spaced one meter apart. In each transect, there were three sampling spots, giving twelve sampling spots per site, 72 sampling spots total. The sampling spots started at 0.5m, 2m, and 3.5m from the shoreline (Figure 3). Observations on shoreline environment and prominent vegetation were recorded. Random samples of pH, temperature, sediment type, and three one-liter bottles were

collected for each site on May 23, 25, 30, 2009. We performed tests of water temperature using a field thermometer, depth using a meter stick, and pH using a Fisher Scientific AP 10 pH meter before and after removing all *D. polymorpha* at each site.

We collected *D. polymorpha* on May 25, 2009 between 9a.m. and 2p.m. Sites were surveyed for native unionids and *D. polymorpha* by placing m² PVC quadrants at each sampling spot (0.5m, 2m, and 3.5m from the shoreline). We surveyed each location using glass bottom buckets and mussels were collected by hand and three pronged hand rakes. We included notes on mussel condition and substrate type. Mussels were sorted by depth at each site and placed into labeled plastic bags. To preserve any live *D. polymorpha*, we added 70% ethyl alcohol and all samples were refrigerated.

We chose one bottle at random out of three from each site for phytoplankton presence. We shook the bottles and filtered 120cc of water through a 0.45 µm chlorophyll filter using a filtration rig. These samples were preserved and taken to UMBS staff for chlorophyll analysis. We tested the other two one-liter bottle samples for veliger presence, which were filtered through an 80 µm filter. We examined these condensed samples under a Bausch and Lomb dissecting microscope for veligers. We performed a second method for collecting *D. polymorpha* veligers by using a zooplankton tow rig with a 150 mL bottle attached at the end. We took tows horizontally for thirty seconds at each site and analyzed these condensed samples for veligers in the same way as the previous one-liter samples.

To show population variation of *D. polymorpha*, we compared our data with past surveys from 2003, 2004, and 2005 (Figure 1), using six sites around Douglas Lake (Figure 2). Using Garmin GPS 60, we determined six test sites that closely matched the corresponding sites from

the 2005 survey (Table 1). In order to compare our results to the previous 2005 survey, the methods were standardized from Galligan's 2005 survey (Figure 3). We had difficulty interpreting some of the materials and methods as well as findings of the 2005 survey. Numbers of *D. polymorpha* were given, but it was never indicated whether only live mussels were being collected or the total number of mussels, including shells.

Methods Analysis

Statistical tests run on SPSS 15.0 determined the significance of our findings setting alpha at p=0.05. We compared total living *D. polymorpha* found at the six sites that our study repeated with Westbrook (2003, 2004) Galligan (2005) surveys. We performed a descriptive test to check for normality and then performed a Wilcoxon Signed Ranks Test, assuming dependence of site samples for each year and variance of *D. polymorpha* population. To see if phytoplankton and *D. polymorpha* densities are directly correlated, we compared chlorophyll concentrations with the average and total biomass of *D. polymorpha* by using a linear regression assuming independent water samples at each site and variance in phytoplankton population.

Results

To test our hypothesis of a decrease in *D. polymorpha* population, we observed temperature, pH, habitat, veliger population, unionid and *D. polymorpha* counts, and phytoplankton presence. The observed results for water chemistry (Table 2) include water temperature and pH, which remained relatively constant. Site observations (Table 3) showed variations across sites including characteristics of land, water, and site substrate.

Results were inconclusive for *D. polymorpha* veliger counts. We analyzed twelve oneliter water samples (two from each site) for veligers in each site and none were found. We also analyzed two replicates of concentrated samples taken from the zooplankton tow for each site and found only one veliger in site B on the first replicate.

D. polymorpha counts varied between sites and across survey years. Counts across sites in 2009 (Figure 3) ranged between zero to 213 living *D. polymorpha* and one to 358 total *D. polymorpha*. To see if there was a change in population, we compared our *D. polymorpha* counts per site with Westbrook (2003, 2004) and Galligan (2005) surveys.

We performed a descriptive test for normality and performed a nonparametric paired samples test, the Wilcoxon Signed Ranks Test to compare the results of our survey for total live mussel count with past survey totals in 2003, 2004, and 2005, which generated the following respective p values: 0.173, 0.345, and 0.028. Only the 2005 survey showed a significant difference in mussel count with our survey.

Our survey for native unionids yielded no living specimens. However, total nonliving shell biomass was calculated for each site (Figure 4) and ranged between 0.1g and 16.4g. As well as native unionids, we investigated the biomass of *D. polymorpha* in order to have another perspective on the collected totals instead of looking only at counts. To compare biomass between sites within our 2009 survey, we calculated total living *Dreissena polymorpha* biomass (Figure 6) for each site and then divided by the total number of living individuals for an average biomass of a single *D. polymorpha* across sites (Figure 7).

The concentration of phytoplankton pigments is most conveniently measured as chlorophyll concentration (Bannister 1974) which varied across sites and replicates, ranging from $0.51 \mu g/L$ to $1.96 \mu g/L$ (Table 4). We omitted the results of our first replicate due to improper preservation of chlorophyll filters. The regression between chlorophyll concentration and *D. polymorpha* average biomass showed that there was no significance (R= 0.424, p=0.161). To take into account the number of *D. polymorpha* found at each site, we did a regression between chlorophyll concentration and *D. polymorpha* total biomass at each site, which showed no significant correlation (R=0.404, p=0.175).

Discussion

The observed low veliger count may be the result of many factors. When we analyzed samples for veligers, there were difficulties in identifying the larval D. polymorpha. Other microscopic organisms such as zooplankton and water mites were often mistaken for veligers and had to be studied before being ruled out. The amount of sand in samples also made it difficult to find veligers of roughly the same size as the grains of sand. A microscope of higher magnitude could have helped conclusively identify veligers. Research indicates that well fed D. polymorpha populations prefer to feed on algal foods 50 μ m or smaller while starving D. polymorpha populations feed on diatoms up to 750 μ m (MacIsaac et al. 1991). Average veliger size is 310 μ m (Mills 1995), and mortality may be increased by predation by adult D. polymorpha, if their normal food source is currently low (MacIsaac et al. 1991). Veliger mortality rates can be \geq 99% due to a lack of suitable substrate as well as predation. Many of the sites sampled in Douglas Lake had sandy or silty substrates (Table 3) that may have contributed to low veliger counts. The first colonizers demonstrate a preference for hard irregular surfaces

that are out of direct sunlight, that is, either deep, vertical, or shaded surfaces. Once these surfaces are highly colonized, mussels spread to less desirable areas to smooth bedrock and sunlit surfaces. Only after hard substrates are fully colonized would mussels tend to spread to sand and mud substrates (Marsden and Landsky 2000). We saw that *D. polymorpha* preferred substrates as we found 193 *D. polymorpha* attached to a substrate out 385 total *D. polymorpha* collected (Figure 8). The lack of veligers could be explained by environmental factors such as time of year. The temperature of Douglas Lake during our experiment was within range of the preferred spawning temperature of *D. polymorpha* which is 14-16 degrees Celsius, however, a water temperature of 20-22 degrees Celsius is needed for veliger development (Strayer, 2009). A higher consistent lake temperature is necessary for the densities observed in previous years of *D. polymorpha* spawning. Rehmann (2003) suggests that strong turbulence increases larval mortality by 45% for 101µm mussels and 35% for 126µm mussels. Strong winds led to high turbulence at our sites during sampling, and may be another explanation for our low veliger counts.

Our survey of D. polymorpha showed a significant decrease in population since the last survey in 2005, there are biotic and abiotic factors that may explain this change. We conducted brief post-collection surveys to further search the area surrounding sites for presence of D. polymorpha. Adverse conditions of the lake during two field observing sessions and during three out of four sampling sessions made surveying the lake bottom difficult and prevented us from observing populations further than \approx 5m from shore; on our first calm sampling day we could conduct surveys in deeper water. At site C, the majority of the mussels were 6m from shore, while at sites D and E, there is a greater density \approx 15-20m from shore. The depths at these higher density sites ranged from 50-75cm. We can infer that D. polymorpha are showing a preference

towards deeper water (water ≥50cm deep) because of their preference to low and moderate light levels (Marsden and Landsky 2000) or to escape the high energy environment close to shore. Annual lake level oscillations may have an effect on our results. Between July 2005, when the last survey was taken, levels dropped by 0.299m (pers. com. Vann de Kopple). This water level change possibly resulted in testing higher in the littoral zone and may contribute to the lack of *D. polymorpha* in our shallow water testing sites. *D. polymorpha* close to shore in Douglas Lake experience higher predation rates from diverse predators, including crayfish (Green et al. 2008). A decrease of the number of *D. polymorpha* in our survey could be due to an increase in predators near the shoreline in Douglas Lake.

Phytoplankton is a major resource for *D. polymorpha* and a survey of phytoplankton density can be informative of *D. polymorpha* feeding habits. An experiment conducted by Beaupre and Christenson (2006) showed that 5 mussels only filtered 14% of the water in the initial 24 hours. In contrast, 50 *D. polymorpha* would have filtered 147% of this water, explaining the sudden decline in overall density of phytoplankton. We expected to see a decline in phytoplankton density at sites with greater biomass of *D. polymorpha*, we witnessed an insignificant increase. If more replicates were taken and phytoplankton surveys were repeated over time, a significant trend would most likely be visible. Average *D. polymorpha* biomass varied between sites (Figure 4), showing that average size varies across conditions in the lake. The differences correspond with *D. polymorpha* preferences of substrate and resource availability.

The absence of living native mussels could be a result of competition with *D*. *polymorpha* and a corresponding local extinction, or a shift in habitat. Hollandsworth (2006)

suggests that native unionids have shifted their distribution to deeper waters in Douglas Lake and often burrow to escape energetic costs of being colonized by *D. polymorpha*. The possibility of a local extinction is supported by research done by Schloesser and Nalepa (1994); in Lake Erie the proportion of live unionids declined from 53% in September 1989 to 17% in May-June 1990 and to 0% in September 1990: this 100% mortality coincided with heavy infestation by *D. polymorpha*.

Our observations of decreased numbers of *D. polymorpha* coupled with the low veliger populations suggest that zebra mussel populations in Douglas Lake have met or are approaching their carrying capacity. Populations of organisms can grow exponentially when their resources are unlimited (pers. comm. Nadelhoffer). As a successful invasive species, *D. polymorpha* is usually the dominant competitior in exploitative or interference competition. Usually a predator or a non-dominant position in a competition interaction would control a population. It is possible that *D. polymorpha*, may be demonstrating a "boom and bust" population cycle (pers. comm. Nadelhoffer). The population could be leveling off as it reaches carrying capacity or it may have passed carrying capacity and is now declining. In a lake of small size, such as Douglas Lake, resources may be depleted more quickly than in a lake of larger size and result in the observed population decrease.

Areas for Further Study

A number of things could be done to expand upon our experiment. Lab tests or field surveys could be run to test specific factors that may have a negative effect on *D. polymorpha* populations such as high turbidity and low temperature that may decrease the number of veligers. Further comparisons of biomass and phytoplankton may give a better picture of the correlation

between phytoplankton consumed and biomass of individuals or populations. Tests could also be run to determine what conditions must arise for invasive populations to reach carrying capacity, such as phytoplankton availability and predation on D. polymorpha. In the 2003, 2004, and 2005 surveys, students had the time to conduct sampling across many more sites than our own. It would be very informative to continue surveying as many sites as possible for D. polymorpha using the same, or similar methods as the 2005 and 2009 surveys. Our results suggest that the best time to perform these surveys would most likely be during the summer months, when lake levels are more likely to be lower, and when conditions are calmer. At the University of Michigan Biological Station, we were allotted one month for our survey during the spring term, while the summer term consists of two months. Running a survey on a longer time scale would be beneficial to test more sites and run more replicates. Further surveys conducted to look at distributions by depth and distance from shore would be helpful in identifying distribution changes of native populations since 2001 when D. polymorpha was introduced to Douglas Lake. Overall, more frequent surveys and greater numbers of testing sites will provide a valuable contribution to the collective knowledge of D. polymorpha and a clearer mechanism for their population decrease

Conclusion

There has been a significant change in *D. polymorpha* population in Douglas Lake since 2005. We only found a total of 358 *D. polymorpha* in which 213 were living, while in 2005, Galligan found a total of 3437 *D. polymorpha* from the six sites surveyed. A decrease in the shoreline populations of *D. polymorpha* are due to various enironmental factors such as increasing lake levels, colder temperatures, and substrate availability. With growing population of

D. polymorpha since 2001, the Douglas Lake community adjusted to the decrease of D. polymorpha due to intraspecific competition for limiting resources and habitat. The possibility of a leveling off or decline in population could mean the beginning to a return of natural conditions to harmed ecosystems. Finding a natural control of D. polymorpha is crucial to invasive species ecology in which Douglas Lake could be a model for and these natural causes could be applied to other waterways with invasive D. polymorpha.

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Coordinates of Sites in Douglas Lake

Site Name	2005 Coordinates (UTM)	2009 Coordinates (UTM)
North Fishtail	682255, 5050632	682264, 5050554
Stony Point	681703, 5050314	681598, 5050038
West Pell's Island	678731, 5050237	678820, 5050063
Bryant's Bay	679035, 5048749	679401, 5048492
Grapevine Point	681185, 5048998	681099, 5048830
South Fishtail	681308, 5048104	681261, 5048120

Table 1. Six of our sites were chosen relative to a past survey from 2005. Some of our sites could not be exact because of a higher shoreline and current experiments nearby.

Water tests run at each site on Douglas Lake

Site	Temp				pН				Depth	Depth	Depth
									1 m	2.5 m	4m
	R1	R2	R3	R4	R1	R2	R3	R4			
Northfish Tail	17°C	13°C	14°C	17°C	7.69	8.23	8.40	8.39	5 cm	14cm	33cm
Stony Point	17°C	14°C	16°C	18°C	7.69	8.23	8.47	8.49	2cm	17cm	35cm
W. Pell's Island	15°C	15°C	14°C	17°C	8.12	8.24	8.36	8.48	9cm	18cm	21cm
Bryant's Bay	17°C	14°C	15°C	18°C	7.94	8.40	8.40	8.39	25cm	30cm	29cm
Grapevine Point	17°C	15°C	14°C	-	8.08	8.44	8.34	8.69	9cm	13cm	23cm
South Fishtail	16°C	16°C	14°C	17°C	7.88	8.33	8.46	8.69	22cm	38cm	53cm

Table 2. R stands for replicate, R1 was taken before mussels were removed, R2 on the same day that the mussels were removed and R3 and R4 afterwards. R1 taken between 1:00 and 5:00pm, R2 taken between 9:30am and 2:00pm, R3 taken between 4:00pm and 6:00pm.

Observations at each tested site on Douglas Lake

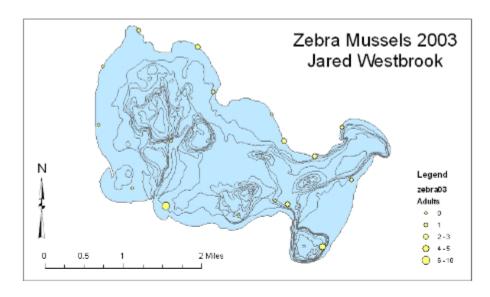
Site	Land	Water	Sediment
Northfish Tail	Small beach, shrubs and small trees	Few snails, calm	Sand, some mud
Stony Point	Woody, swampy	Some dead land plants	Sand and small rocks
W. Pell's Island	grasses, cabin, cedars	Turbulent, many dead unionids	Cobble over sand
Bryant's Bay	No beach, medium trees	Some leaves	Silt and sand
Grapevine Point	Small beach, larger rocks, larger trees	Large rocks, few and scattered	Firm sand and mud, anchored by roots
South Fishtail	Larger trees and sticks, larger rocks	Calm, Large amounts of riparian input, logs, leaves, rocks	Leaf layer over silt and sand

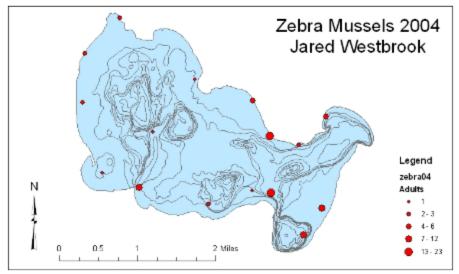
Table 3. Observations between all sites.

Chlorophyll concentration (µg/L) at each Site

Site name	R1 (μg/L)	R2 (μg/L)	R3 (μg/L)
North Fishtail	2.93	0.83	0.68
Stony Point	0.74	1.19	1.64
West Pell's			
Island	11.48	0.9	0.56
Bryant's Bay	1.57	1.96	0.58
Grapevine Point	2.92	1.73	0.51
South Fishtail	0.82	1.38	0.66

Table 4. Chlorophyll samples were taken to see phytoplankton presence, which can be filtered by *D. Polymorpha* between each site and before and after removal of *D. Polymorpha*.





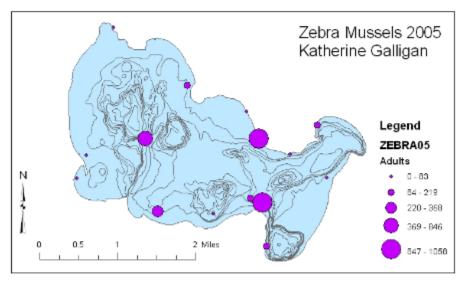
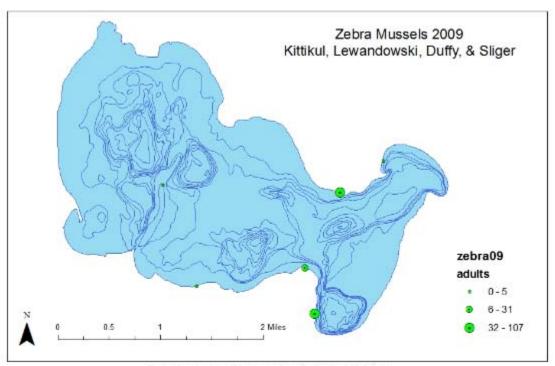


Figure 1. Surveys from 2003, 2004, and 2005 maps show previous sampling sites around Douglas Lake and how many D. polymorpha where collected. However, the methods of Galligan and Westbrook were not standardized.



Douglas Lake, Cheboygan County, Michigan

Figure 2. We found a wide ranging amount of total *D. polymorpha* at each our six sites including North Fishtail Bay (1), Stony Point (143), West Pells Island (13), Bryants Bay (5), Grapvine Point (88), and South Fishtail Bay (108).

Sample Site Layout

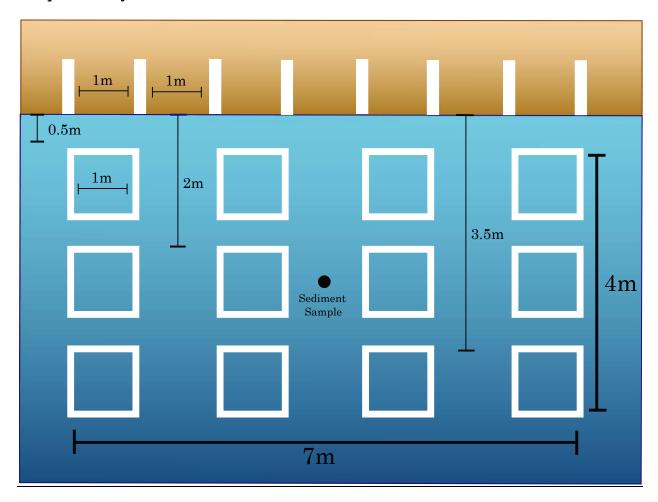


Figure 3.Each of our sampling sites had four transects, which were 1m apart from each other. Sampling spots were laid out with m² PVC pipe at three different points away from the initial spot from the shoreline: 0.5m, 2m, and 3.5m. *D. polymorpha* were observed and taken from each of the twelve sampling sites at each spot. A sediment sample was also taken in the exact middle of the site.

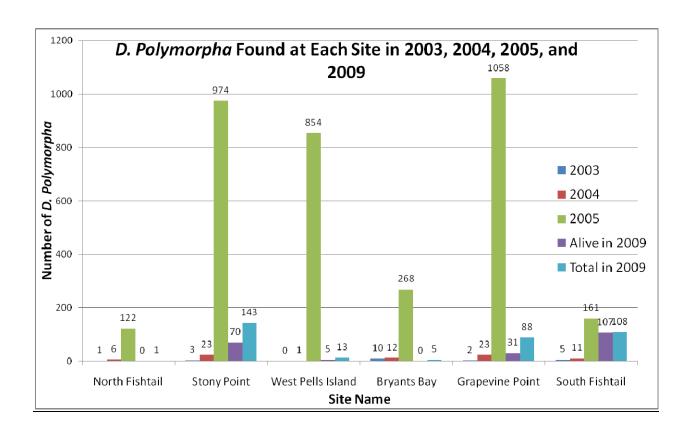


Figure 4. Total number of D. Polymorpha found in Westbrook's survey in 2003 and 2004 and Galligan's survey in 2005 from similar site locations. We took into account the number of alive and dead D. Polymorpha, however, prior surveys did not specify whether individuals counted were alive or dead.

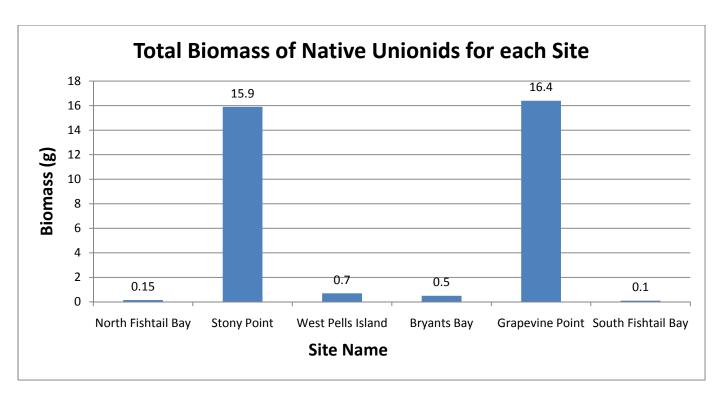


Figure 5. Native Unionids shells and pieces were weighed as potential substrates for *D. polymorpha*.

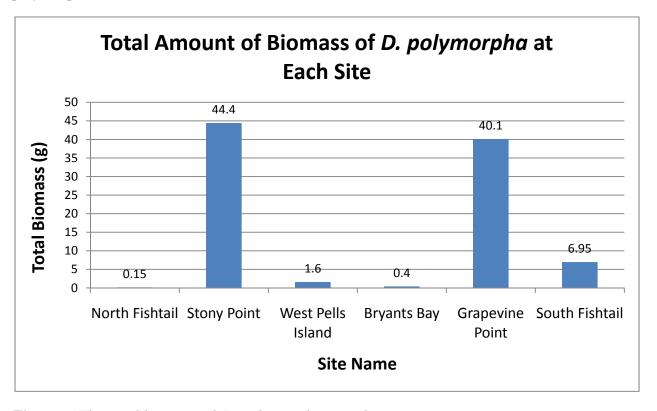


Figure 6. The total biomass of *D. polymorpha* at each site.

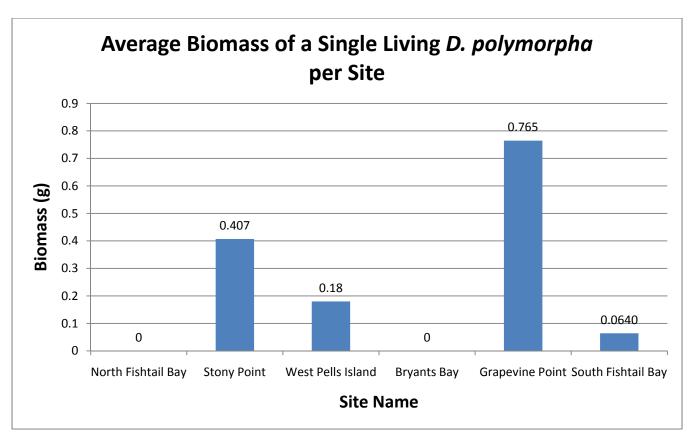


Figure 7. The average biomass for each single *D. polymorpha*

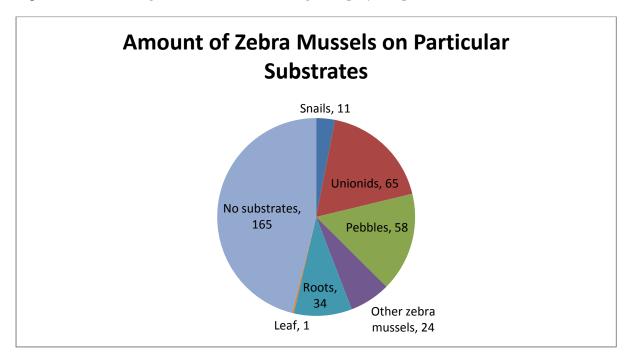


Figure 8. The number and types of substrates total zebra mussels were found on with no substrate taken into consideration.

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