Recolonization Rate and Preference of Zebra Mussels (*Dreissena polymorpha*) on Living and Non-Living Material

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Abstract

Zebra mussels (*Dreissena polymorpha*) are an invasive species in Douglas Lake that have disrupted the aquatic community, such as decreasing the abundance of food for native bivalves. They have major consequences on the population of native mussels and fish species, such as decreased abundance and possibly extinction. To understand how to control the increasing population of zebra mussels, the preference of substrate for colonization and the recolonization rate of the species must be determined. To determine this rate we measured, marked, and removed zebra mussels off of live and dead native mussels. In addition, we introduced clean rocks to observe the colonization rate of zebra mussels on them. We then recollected the substrates and determined recolonization. Our results showed that zebra mussels have no preference for the substrate that they recolonize. The average recolonization rate of each substrate was 0.08 mussels per day. From our observations, we suggest a protocol for controling the population of zebra mussels and the impacts that they cause on the aquatic community. The protocol may help us create a refuge for native mussels to sustain the population in Douglas Lake.

Introduction

Introduced species can have a profound effect on native species, often leading to extinction (Schneider et al., 1997). In 1985, zebra mussels (*Dreissena polymorpha*) were introduced to freshwater lakes in North America from Europe. Their introduction has lead to ecological disturbance in numerous freshwater lakes. Due to their high abundance and strong attachment threads, zebra mussels attach to solid or stable substrates in freshwater lakes (Johnson et al., 1996), such as substrate in Douglas Lake. Since 2001, zebra mussels have had a direct impact on native freshwater bivalve populations. Zebra mussels attach to any portion of the bivalve's shell, but have been observed to benefit through increasing their growth rate, by attaching to the native bivalve inhalant/exhalent siphon (Hormann et al., 2006). Zebra mussels attached to this portion of the shell to injest food particles that are provided by the filter current of the native bivalve. Since their introduction, the population of native freshwater bivalves has decreased as a result of inhibition of feeding, movement, and excretion (Zanatta et al., 2002). In addition, the increase consumption of suspended particles by zebra mussels has limited the food for native bivalves and fish species. If zebra mussels are not controlled, it is suggested that they will ultimately cause the extinction of ~60 native North American freshwater bivalves (Strayer et al, 2007).

To control the population of zebra mussels and create a refuge for native bivalves, the recolonization rate, which is influenced by the growth rate, must be anylayzed. Abiotic factors in the environment, like water temperature, trophic conditions, seasonality and substrate, influence the growth rate of zebra mussels. Optimal growth in zebra mussels occurs in waters that range in temperature from 10°C and 12°C with eutrophic

conditions (Karatayev et al., 2006). For seasonality, the spring and fall turnover are the periods of maximum growth in zebra mussels due to the availability of high levels of phytoplankton and optimal temperature range. Although Douglas Lake is mesoligotrophic, it has all the other abiotic features that ensure continual growth of the zebra mussel population.

Another abiotic factor that affects the growth rate of zebra mussels is the location of the substrate that is colonized. The ideal position for zebra mussel growth was found in shallow depths of lakes in the water column just above the substrate (Karatayev et al., 2006). Hormann (2006) showed that zebra mussels' colonization were higher on complex surfaces than flat surfaces. Allow they did not find any preference for living or non-living substrate that was colonized, they observed that zebra mussels benefited from colonizing bivalves. Zebra mussels grew faster on bivalves than on stones. Substrate influences the growth rate and determines the recolonization rate which will control the population of zebra mussels.

The purpose of this study was to determine the recolonization rate of zebra mussels and the preferences they had to colonize living versus non-living substrate. From the knowledge of seasonal growth rate in zebra mussels (Karatayev et al., 2006) and our own experience, we predict the recolonization rate of non-living and living material should be seven days and that they will have prefered substrate (Hormann et al., 2006). In addition, the number of colonized zebra mussels will be positively correlated to the length and width of the native bivalve. Wider and longer native bivalves will have more zebra mussels attached to their shells. To test our hypotheses, zebra mussels were removed from live and dead native bivalves and rocks, measured, and marked off the

shore of Douglas Lake in Cheboygan County, MI. The marked mussels were then left for a period of time to allow for recolonization. After the time elapsed, the native mussels and rocks were reexamined to determine rate of recolonization. By determining this rate, a protocol can be developed to create a refuge for native mussels. In the future, the protocol will be used to control the population of zebra mussels in Douglas Lake and the impacts it causes the aquatic community.

Methods and Materials

Experimental Design

The study was conducted from July 24th to July 31st 2007 on the South Fishtail Bay shoreline of Douglas Lake. The substrate of the shoreline consisted of fine sediment with a small amount of cobble and woody debris. The marked plot consisted of five transects, four transects of native mussels and one transect of 10 clean, introduced rocks. The 10-meter transects were 10 meters from the shoreline and spaced 3 meters apart. Transects were anchored by tying rope on plastic jugs of sand that were buried in the sediment. Each transect was snorkeled to collect dead and living native bivalves. Mussels were assumed to be in the transect it they were within a 1 meter distance from each side of the line. To ensure accuracy, a meter stick was carried during the process to assess if the native mussel was in the transect.

Measurements of the native mussels were collected through the use of Vernier Calipers. Native mussels lengths', widths', and number of zebra mussels that were attached to the shell were documented. After the measurements were completed, the native mussels were painted with either a paint marker or nail polish. The marked native

mussels were then placed back into their original position in the transect. The zebra mussels were collected in a bucket and disposed away from the shoreline.

After waiting seven days for recolonization, we snorkeled again and measured each marked living and non-living native mussel along each transect. We measured the length and width of each native mussel, and counted the number of attached zebra mussels on its shell. In the rock transect, we counted for any zebra mussels that had attached to them. After compiling our initial and final data about the native mussels and rocks, we replaced all of our specimens to their original positions in the lake.

Statistical Analyses

Statistical analyses were conducted to determine the relationship between the status of the native mussels, their body size, and the number of attached zebra mussels on their shell. T-tests, regression analyses, and the recolonization rate were used to determine the relationship or difference between native bivalves and zebra mussels. The t-tests determined if there was a significant difference a) in length and width between the live and dead native bivalves, b) the number of zebra mussels on dead and live native bivalves in a square millimeter, and c) the initial and final average number of zebra mussels on live versus dead native bivalves. The regression analyses determined if there were relationships between native mussel length or width and the number of attached zebra mussels. To create the protocol, we had to determine the recolonization rate in the reattachment of zebra mussels on all substrates. This rate was found by calculating the average number of zebra mussels that colonized per day.

Results

After measuring length and width of the shucked bivalves, we found that there is no difference between length and width of dead versus live native bivalves (t-test, t =0.40, df =5, p = 0.70; t-test, t = -0.34, df = 5, p = 0.75, respectively) (Table 1). The average length and width for live native mussels was 32.29 mm and 55.29 mm respectively. While the average length and width for dead native mussels was 30.26 mm and 53.11 mm respectively (Fig 1).

To determine if the number of zebra mussels attached to native bivalves increased with length and/or width, we conducted a regression analysis. For live native bivalves, we observed that the number of attached zebra mussels did not depend on their lengths (regression, p = 0.35, R^2 = 0.22) (Fig 2). Additionally, the widths of the live native bivalves had no correlation to the number of attached zebra mussels (regression, p = 0.69, R^2 = 0.04). After regression analysis was conducted for dead native bivalves, we found that the number of attached zebra mussels was not dependent on their lengths (regression, p = 0.04, R^2 = 0.08) or widths (regression, p = 0.02, R^2 = 0.11).

Before removing zebra mussels from the live and dead native mussels, we observed that there was significance in the substrate that zebra mussels colonized (t-test, t = -3.56, df = 45, p = 0.00090) (Table 2). Zebra mussels preferred to colonize dead native mussels over all other substrates. However, after recolonization occurred, we found no significance in the number of attached zebra mussels to each substrate and no preferred substrate for recolonization (t-test, p = 0.97, df = 14, t =0.04).

Also, we found that number of zebra mussels that colonized per millimeter squared did not depend upon their substrate being live or dead native bivalves was not

significant (t-test, t = 0.40, df = 21, and p = 0.69). The average amount of zebra mussels on a dead native bivalve was per square millimeter was 0.02 zebra mussels/clam and the average amount of zebra mussels on a live native bivalve was 0.01 zebra mussels/clam (Fig 3).

The average recolonization rate for dead native bivalves was higher than the rates for live native mussels and rocks (Fig 4). For dead native bivalves, the average recolonization of zebra mussels was 0.63 zebra mussels/seven days while the average rate for one live native bivalve was 0.57 zebra mussels/seven days On average the colonization rate for a rock, 0.5 zebra mussels/seven days, was lower than the recolonization for live and dead native bivalves. However, since there was found to be no significance in the type of substrate that zebra mussels colonized, we compiled all the substrates together to calculate the recolonization rate per day (t-test, p = 0.97, df = 14, t =0.04). The rate of recolonization to all substrate was observed to be 0.08 zebra mussels/day.

Discussion

From this study, we have concluded that zebra mussels are not dependent on the length or the width of the substrate that they colonize. Zebra mussels abundance does not increase due to the amount width and/or length of the live and dead native bivalves. This data is consistent with a study done by Hormann (2006) suggesting that there is no correlation between size of substrate and colonization. The explanation for these finding is uncertain, but could be that zebra mussels recolonize any substrate, no matter its size, as quickly as possible. The recolonization rate that was calculated during the study could

be used as evidence for this explanation , 0.08 zebra mussels/day. To build on the explaination, Hormann (2006) has stated that zebra mussels can change their position and substrates during the year, suggesting that if resource availability on one substrate is not enough that zebra mussels will move to a different substrate.

In addition, we found that zebra mussels have no preference on the type of substrate they recolonize. Zanatta (2002) has stated that zebra mussels attach to any hard surface; including rocks, wood, boats, other zebra mussels and native bivalves. However, there has been data collected on how zebra mussels benefit when they colonize on bivalves compared to regular substrate (Hormann, 2006). Although the data on the preference of substrate for zebra mussels is in its elementary stages, there is significant need to research documenting the benefits of colonization on inanimate versus living substrate.

To control the population of zebra mussels we must create a refuge for native bivalves. Several studies have proposed ideas for maintaining the native bivalve population, such as relocating native bivalves to zebra mussel-free sites (Schloesser et al., 2000). Another study suggested that native bivalves with no colonization and previously cleaned native bivalves should be quarantined to survive and reproduce (Hallac et al., 2001). Further study of zebra mussels and native bivalves suggested that removal of zebra mussels from native bivalve shells may be a viable way of reducing the impacts of the invasive species (Schloesser et al. 2000). A study has observed that species of native bivalves had higher survival and increased energetic stores after cleaning (Hallac et al., 2001). However, one study suggested that removal of zebra mussels on bivalves is does not increase the native bivalves' survival and is not an effective management tool for

their conservation. Depending on the study, these methods may or may not have been viable methods to retain native bivalve populations and further study to determine the correct method should be done.

Assuming that periodic cleaning of native bivalves enhances their survival, the recolonization rate of zebra mussels is an asset for creating a refuge. If zebra mussels recolonize on substrates like we have observed, then we can predict that in a period of 90 days that 6.72 mussels will have accumulated on any substrate. The initial colonization data observed that live native bivalves survived with an average of twenty-four zebra mussels/ bivalve. From this data, we can conclude that this amount of recolonization, 6.72 mussels/90 days, would not have a significant affect on the substrate that it would recolonize. From the observation of the recolonization rate on all substrates, 0.08 zebra mussels/day, we may be able to create a protocol to control the population in Douglas Lake.

However, the recolonization rate could be an underestimation of the attachment per day. Since the study was conducted in July, during the middle of the growing season that lasts from early spring to the winter, the seasonality has an impact on our observed recolonization rate. From previous literature, the growth for zebra mussels has been determined to be maximized during the spring and fall turnover and conducting the experiment in July may make our recolonization rate less accurate (Karatayev et al., 2006). On the other hand, this rate could represent the average recolonization that occurs through the growing season since it was conducted during average growth of the zebra mussels. Overall, the recolonization rate is a critical part of sustaining the native

population and experiments should be conducted during the maximum growth of zebra mussel to find a more accurate recolonization rate.

The proposed protocol for Douglas Lake would consist of shucking zebra mussels off of all substrates, dead and alive, every 90 days. The shucked zebra mussels should be disposed of out of water to ensure the recolonization does not occur (Zanatta et al, 2002). The protocol would have to be preformed most often during the growing season of zebra mussels from early spring to fall (Karatayev et al., 2006). Since the maximum growth of zebra mussels occurs in depths of 1 to 1.5m, the protocol should be conducted on the shoreline of Douglas Lake at this depth (Karatayev et al., 2006). If the protocol of cleaning native bivalves every 90 days is successful, Douglas Lake could be an example of how to create and maintain a refuge for biota from zebra mussels.

There are several aspects of our research project that can be improved for future research on preference and recolonization rate of zebra mussels. It may be beneficial to have a larger amount of samples of substrates and a longer amount of time to observe the recolonization rate. Observing the recolonization by day may help to observe more accurate data in the future. In addition, using markers that last longer, like nail polish, on the biota would help to observe the individual after recolonization. Another aspect that would predict maximum accuracy of growth of zebra mussels is to have average plot depth at 1 to 1.5 meters in the lake. An aspect that is hard to control but could be improved upon is weather and human disturbance of the plots. Our data could be more accurate if outsiders did not shuck zebra mussels off of clams in our plot. Enclosing the area of the plot could improve the overall project since a majority of our substrate was

lost due to a storm. If these aspects of our project our modified, the overall results will be more accurate and beneficial to create a refuge in Douglas Lake.

From our project, we have observed that zebra mussels have no preference to the substrate that they inhabit. Also, the average recolonization rate for any substrate is 0.08 zebra mussels/day. From our data, we can predict that a protocol of shucking zebra mussels every 90 days during their growing season could help control the population in Douglas Lake. If this protocol is conducted consistently, food sources for native bivalves and fish will not diminish and zebra mussels will have less of an impact on the biota that occupies the lake. In conclusion, by conducting this protocol we may decrease the abundance of zebra mussels in Douglas Lake and create a refuge for the native bivalve population.

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LENGTH
t-Test: Two-Sample Assuming Unequal Variances

	Live	*Dead*
Mean	47.75926	44.8
Variance	236.7523	249.7
Observations	54	5
Hypothesized Mean Difference	0	
df	5	
t Stat	0.4015	
P(T<=t) one-tail	0.352319	
t Critical one-tail	2.015048	
P(T<=t) two-tail	0.704637	
t Critical two-tail	2.570582	

WIDTH
t-Test: Two-Sample Assuming Unequal Variances

	Live	*Dead*
Mean	25.57407407	27
Variance	94.02271139	80.5
Observations	54	5
Hypothesized Mean Difference	0	
df	5	
t Stat	-0.33758676	
P(T<=t) one-tail	0.37469295	
t Critical one-tail	2.015048372	
P(T<=t) two-tail	0.7493859	
t Critical two-tail	2.570581835	

Table 1: No significance difference between length and width of dead versus live native bivalves (variable 1 = live, variable 2 = dead).

Zebra Mussels Alive Vs. Dead (before recolonization)
t-Test: Two-Sample Assuming Unequal Variances

	Live	*Dead*
Mean	18.2	28.66667
Variance	8.7	373.6604
Observations	5	54
Hypothesized Mean Difference	0	
df	45	
t Stat	-3.55679	
P(T<=t) one-tail	0.000449	
t Critical one-tail	1.679427	
P(T<=t) two-tail	0.000898	
t Critical two-tail	2.014103	

Zebra Mussels Dead Vs. Alive (after recolonization)
t-Test: Two-Sample Assuming Unequal Variances

	Live	*Dead*
Mean	0.586206897	0.571428571
Variance	1.465517241	0.619047619
Observations	29	7
Hypothesized Mean Difference	0	
df	14	
t Stat	0.039642769	
P(T<=t) one-tail	0.484468855	
t Critical one-tail	1.761310115	
P(T<=t) two-tail	0.968937709	
t Critical two-tail	2.144786681	

Table 2: Before recolonization, significant difference in number of attached zebra mussels for live and dead bivalves. After recolonization, no significant difference in number of attached zebra mussels for live and dead native bivalves.

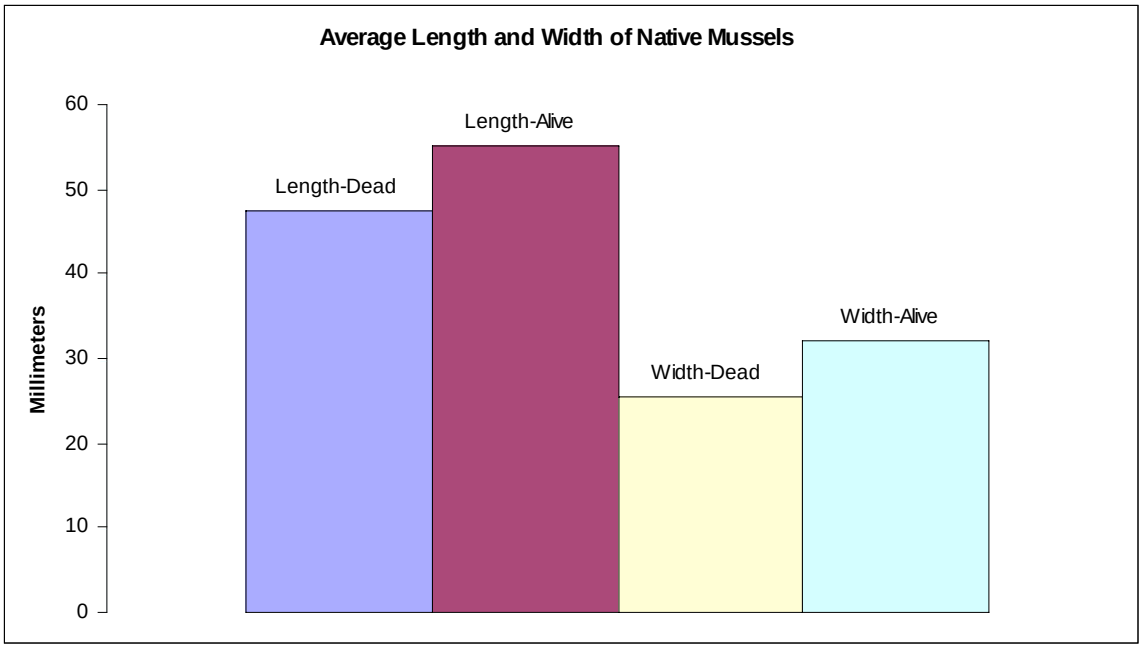
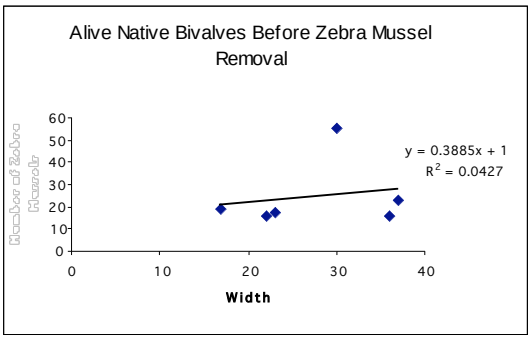
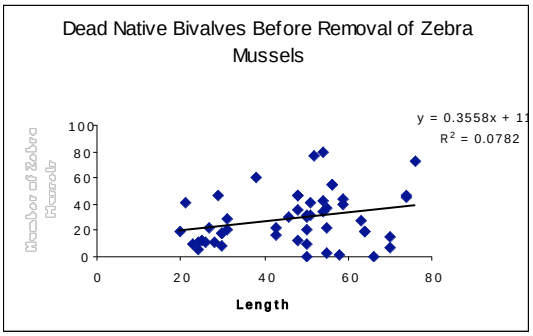
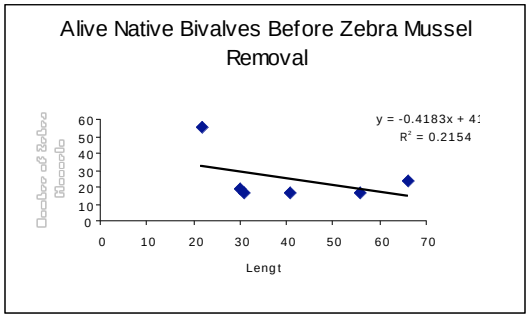


Figure 1: No significant variation in the colonization of zebra mussels on average length and width of dead and live native bivalves.







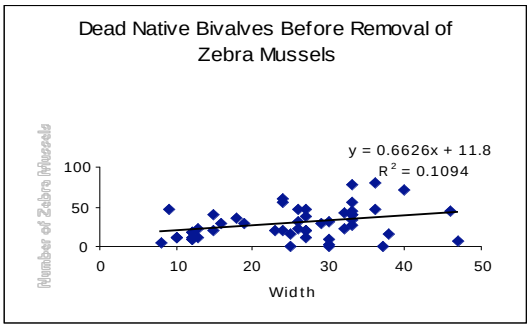


Figure 2: No relationship between the number of zebra mussels attached and length and width living and dead native bivalves

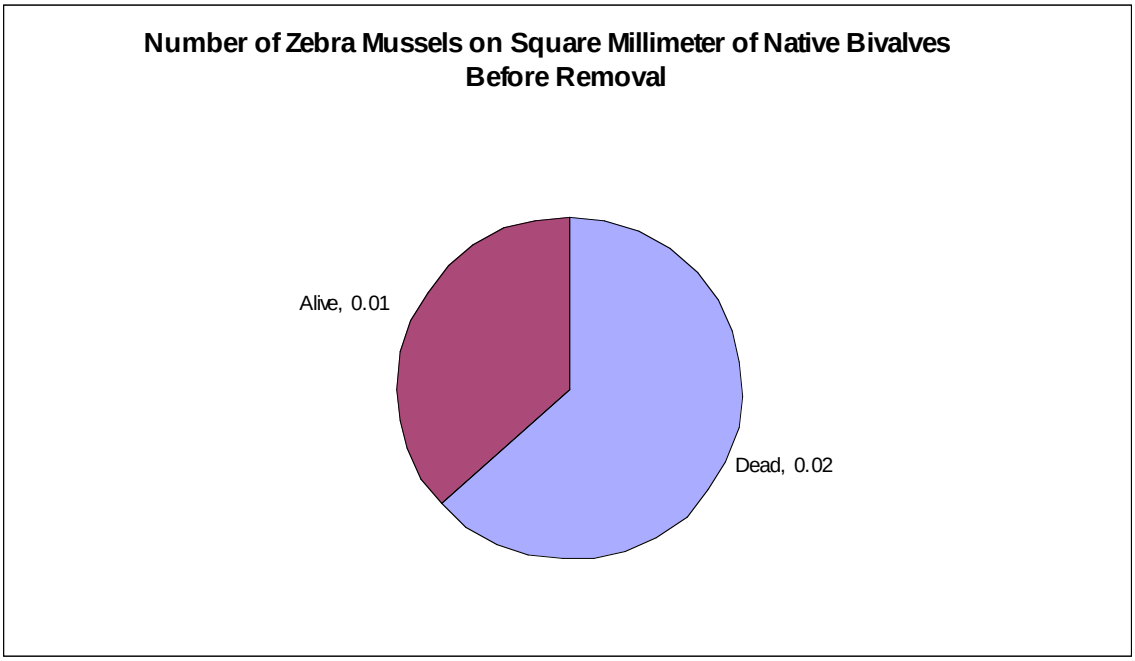


Figure 3: Abundance of colonized dead native bivalves is greater than colonized live native bivalves before zebra mussel removal.

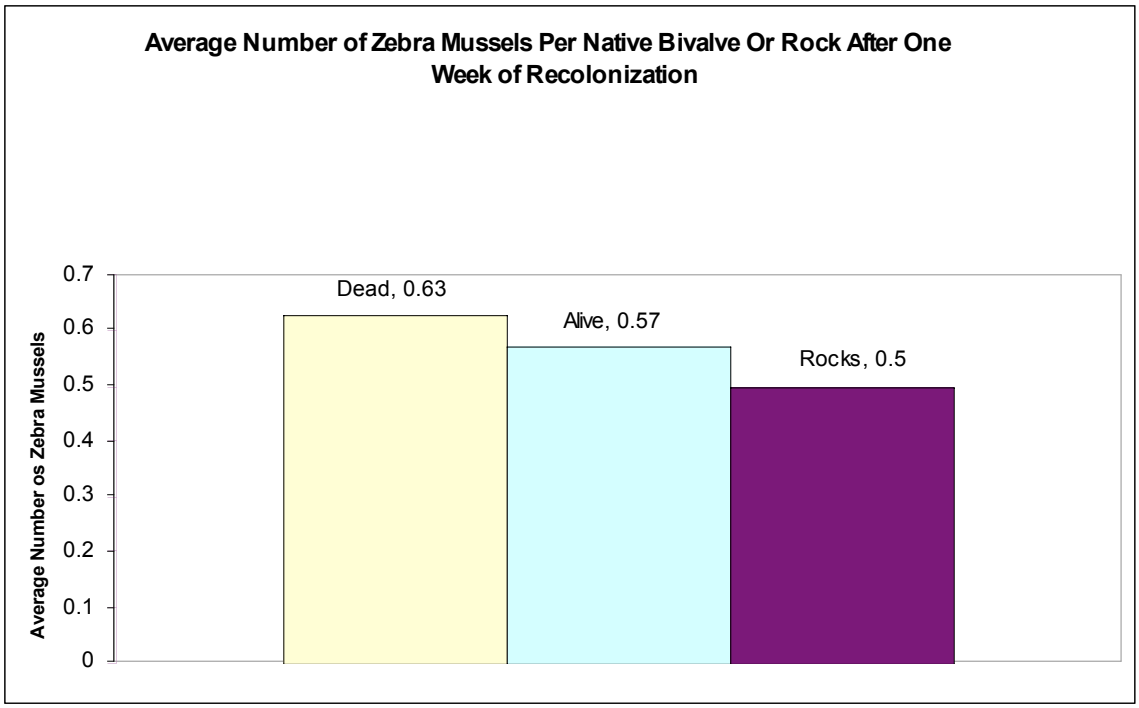


Figure 4: No significant difference in the average number of zebra mussels on the recolonized dead, native bivalves, live native bivalves, and rocks