FOOD PREFERENCES AND GROWTH RATES OF JUVENILE *QUADRULA QUADRULA* MUSSELS

By Vikash Kanodia (2003)

Summary

This project studied ways to artificially culture freshwater mussels in order to repopulate endangered mussel species. The data suggest that growth and survival increased when mussels were raised in sediment from the St. Croix River and water from the Mississippi River. Mussels preferred diatoms found in sediment over those suspended in the water. My observations support the theory that juvenile mussels feed with their foot during the first few months of life.

Introduction

The goals of this project were to successfully rear *Q. Quadrula* freshwater mussel juveniles in a laboratory and analyze sediment/water combinations to determine the combination that best promotes *Q. quadrula* juvenile growth and survival. Once the optimal sediment/water condition was determined, the final goal was to analyze algae food sources in the best sediment/water combination and compare that data to algae food sources found in other less successful combinations to determine food preferences of *Q. Quadrula* juveniles.

Freshwater mussels (*Mollusca:Bivalvia*) are one of nature's indicators of water quality. Because freshwater mussels are suspension feeders, they filter unicellular algae, bacteria, zooplankton, and suspended detrital particles from water (1). In doing so, they maintain water quality. However, for this reason they are susceptible to pollution. As a result, freshwater mussel populations are declining at an alarming rate (2).

Of the 297 mussel species that have been identified in the United States, 18 are extinct, 58 are listed as candidates for protection under the Endangered Species Act, and 60 are endangered or threatened. Among these endangered mussels are *Quadrula fragosa*, a close cousin to *Q. quadrula* that were studied in this project (2, 3).

The major threats to freshwater mussels include: habitat loss and degradation, stream-bank erosion and floodplain development, toxic spills, channel modifications, siltation, population isolation, illegal poaching (*e.g.* for pearl production), loss of fish hosts, the introduction of exotic mussels such as the zebra mussel (*Dreissena polymorpha*), and especially pollution (1, 4, 5). Major pollutants that negatively affect mussel growth are ammonia levels, abnormal pH levels, suspended solids, and heavy metals (6).

Studies have shown that one of the best methods of reestablishing mussel populations is through artificial culturing of endangered species in a laboratory. However, information pertaining to growth and survivorship of cultured mussels is scarce and incomplete. In previous mussel-rearing studies, scientists have observed high juvenile mortality rates; anywhere from 50-90% die between the time juveniles switch from pedal feeding to filter feeding (7). A study by Gatenby *et al.* showed that juvenile *Villosa iris* reared in sediment substrates had higher survivorship than those reared with no sediment. They also reported that algae may be a good source of food for juvenile mussels (8).

Juveniles have two sources from which to collect algae, sediment and water. Algae located in sediment are referred to as benthic algae. Algae located in water currents are referred to as planktonic algae. Benthic algae are primary producers in an ecosystem (9). The algae food sources studied in this project were diatoms, which are unicellular organisms of the kingdom *Protista*, characterized by a silica shell. Diatoms are highly ubiquitous and can be found in almost any environment (10).

Mussels undergo two main stages of growth. After being released into the water by the mother, glochidia (undeveloped mussel "babies") must successfully attach to suitable host fish. This parasitic stage can last anywhere from three weeks to nine months. During this period, the internal organs of glochidia grow and develop. After metamorphosis the glochidia are ready to survive on their own, and fall off their host fish. At this point, they are called juveniles, which is where the study reported here began. Once juveniles become sexually mature, they are called adults or mussels. Mussels can live anywhere from 10 to 100 years, so scientists can culture and reintroduce mussels in a relatively short period of their entire lives. (11).

There have been no previous rearing or food-preference studies performed on *Q. fragosa* or on species closely related to *Q. fragosa,* such as *Q. quadrula*. The project reported here applied and expanded knowledge about juvenile mussel culturing to *Q. quadrula* mussels. By determining optimal conditions for *Q. quadrula,* environmentalists may experience more success in rearing and reintroducing endangered *Q. fragosa*.

Methods

Juvenile *Q. quadrula* were obtained from Mark Hove, Department of Fisheries, Wildlife and Conservation Biology at the University of Minnesota. Two 10-gallon juvenile holding aquaria were prepared. Two shelves were suspended in each tank. These shelves were made from PVC pipes with a mesh bottom.

Water for the experiment was collected from the Mississippi River near the damn in Coon Rapids, MN and at the St. Croix River just upstream of Stillwater, MN. Water was collected using 5-gallon buckets and placed into a portable water-holding tank. The water was then transported back to the laboratory where it was siphoned and sieved through a 125-micron filter into 50-gallon holding aquaria. Silt was collected weekly at both sites and sieved through a 125-micron filter. A third silt called "lab silt" was comprised of calcium carbonate crystals that had formed on the walls of holding tanks in the laboratory.

Petri dishes were obtained and numbered; 15 juveniles were placed into each of 29 petri dishes. Due to natural mortality, only five juveniles were placed in the 30th dish. Each dish was appointed with one of four sediment types: Mississippi silt, St. Croix silt, no silt, and "lab silt." Half of the dishes were placed in tanks holding St. Croix water and the other half in tanks holding Mississippi water. Juvenile holding tanks were located directly above the 50-gallon holding tanks; the 50-gallon tanks supplied the 10-gallon juvenile holding tanks with a steady flow of water using a pump and water-intake tube. An outtake tube was placed a few inches from the top of the tank, so water did not over-flow. An aerator stone in both the 50-gallon and 10-gallon tanks produced a steady current for the purpose of stirring algae for food distribution.

Juvenile growth/size was measured weekly by removing each petri dish from the tanks and sieving the contents through a 125-micron filter, leaving only juveniles behind. Using an optical micrometer zeroed under 5X zoom, the size of juveniles was measured. Juveniles were then placed back into their appropriate tanks.

Algae Identification
A juvenile from each of petri dishes 1-12 and 17-28 was placed into a small test tube filled with 1 mL of water. (Not all petri dishes were sampled to avoid higher mortality rates.) Sprite™ was added, and the tube was capped with a stopper. After 15 minutes, the carbonated water was pipetted out and replaced with 95% ethanol. The tube was recapped, labeled by dish number, and stored. This process was repeated for a total of 24 juveniles. Preserved juveniles were taken to a watershed station in Stillwater, MN for observation and algae identification under high-powered microscopes. First, preserved juveniles were pipetted into a well slide. Their valves were opened using two dissecting needles, and the stomachs were detached. Each stomach was then placed onto a microscope slide in a water mount. These slides were heated to boiling for half-hour. Next a Naphrax mount and cover slip were applied to the gut contents on the slides. Each slide was reheated until the mounting boiled. Finally, the slides were observed under the high-powered microscope to locate diatoms and identify gut contents for each juvenile.

Results

Figure 1: Final avg. size of live juveniles

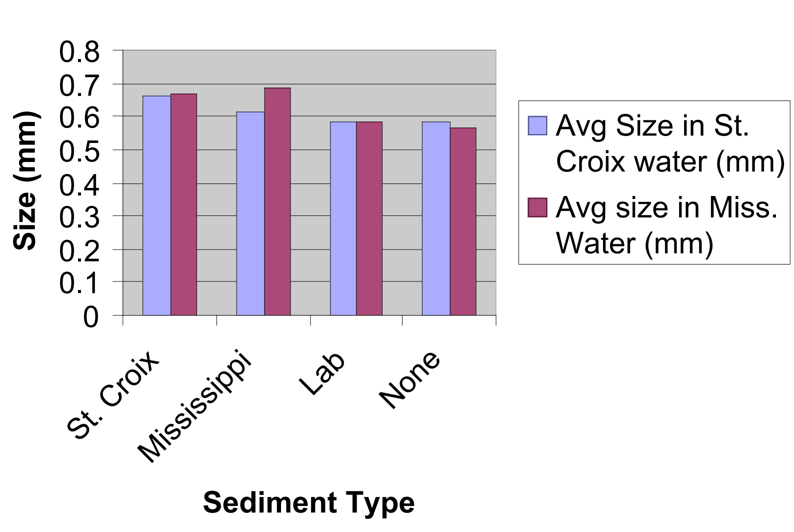


Figure 1 shows the average sizes of live juveniles after a period of four weeks in both St. Croix and Mississippi River water. As seen in Figure 1, the best combination for juvenile growth was Mississippi River water and sediment, with an average juvenile size of 0.688 mm after four weeks. The second best combination for growth was St. Croix River sediment with Mississippi River water where average juvenile size was 0.67 mm after four weeks, a 0.018 mm difference between the best and second best combinations. There was no statistical difference between growth sizes and water type ($p = 0.1762$). There was a statistically significant difference between the growth sizes and sediment type ($p = 0.0003$). All figures for statistics can be seen in Appendix A.

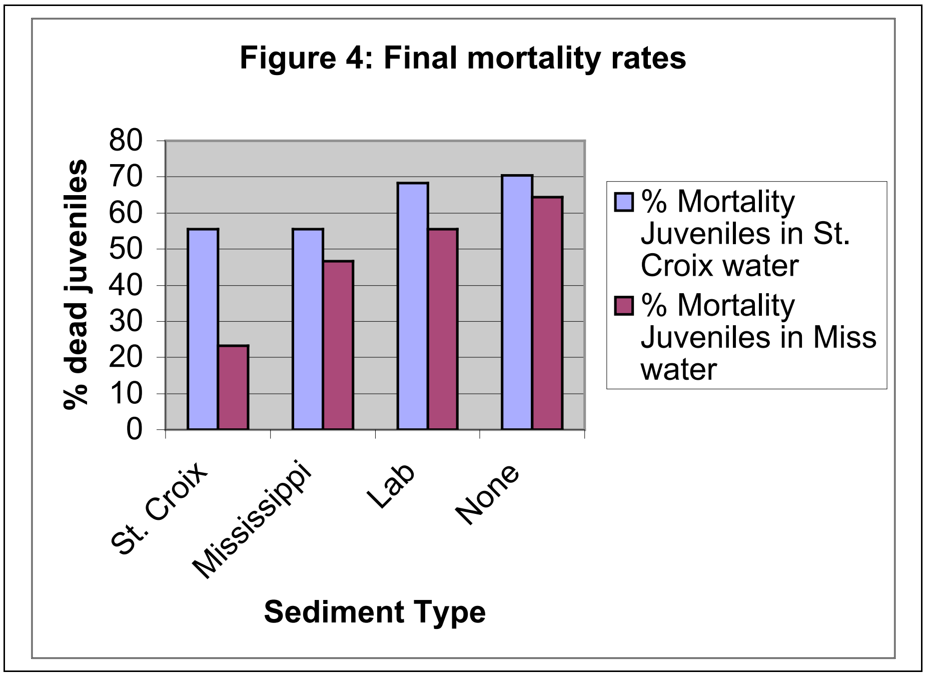


Figure 4 shows the mortality percentage of juveniles in each sediment type and for each water type. The best combination for juvenile survivorship was St. Croix River sediment with Mississippi River water, where the mortality count was 23.3% after four weeks. The second best combination for survivorship was Mississippi River water with Mississippi River sediment, resulting in a 46.7% mortality count. There was a statistically significant difference between mortality and sediments ($p = 0.0257$). There was also a statistically significant difference between mortality and water types ($p = 0.0116$).

Table 1: Benthic diatoms found through gut analysis

Sediment type	*Achnanthes exigua*	*Achnanthes linearis*	*Achnanthes lanceolata*	*Achnanthes minutissima*	*Achnanthes sp.*	*Gomphonema sp.*	*Navicula radiosa*	*Nav sp.*	*Unid. benthic.*
St. Croix	1	0	1	1	3	0	2	2	2
Miss.	0	1	0	0	0	0	0	0	0
Lab	4	2	2	4	3	2	0	0	5
None	6	1	1	0	0	0	0	1	1

Diatoms were found in 48% of the juveniles dissected. Table 1 shows all benthic diatoms found through gut analysis of juveniles collected. A total of 45 benthic diatoms were found. Table 2 shows all planktonic diatoms found through gut analysis of juveniles collected. A total of four planktonic diatoms were found. There was no statistically significant difference between percent benthic diatoms and water type (p = 0.7824). There was also no statistically significant difference between percentage benthic and sediment type (p = 0.4036). However, there was a statistically significant difference between the number of benthic diatoms and habitat (p = 0.04130.

Table 2: Planktonic diatoms found through gut analysis

Water Type	*Stephanodiscus sp.*	*Cyclotella menaghiniana*	*Aulacoseira ambigua*
St. Croix	1	2	0
Mississippi	0	0	1

Conclusion

The goals of this project were to determine the best sediment/water combination for growth and survivorship of *Quadrula quadrula* mussels, as well as to determine possible food sources for juveniles. No past studies had been performed on *Q. fragosa* or on species closely related to *Q. fragosa* for a basis to compare results.

As seen in Figures 1 and 4, the overall best combination for both growth and survivorship of *Q. quadrula* juveniles appeared to be St. Croix River sediment with Mississippi River water. However, this was not statistically shown.

It can be statistically shown that juveniles had larger growth sizes at the end of four weeks in River sediment treatments as opposed to lab and no sediment treatments (p = 0.0003). Growth sizes in different water types showed no statistically significant difference (p = 0.1762).

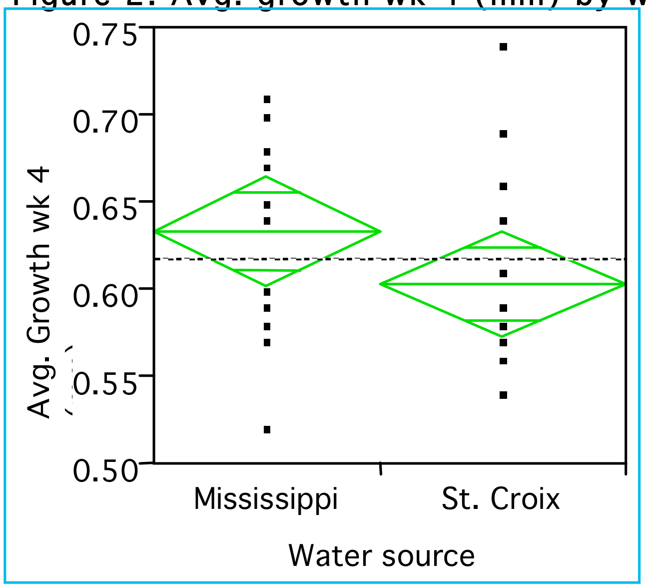
Juveniles had higher survivorship at the end of four weeks in river sediment treatments as opposed to lab and no sediment treatments (p = 0.0257). Water type appeared to have an effect on survivorship as well. Juveniles had a significantly higher survivorship at the end of four weeks in Mississippi River water as opposed to St. Croix River water (p = 0.0116).

Data showed the best food source for juveniles was *Achnanthes sp*. diatoms, which is a benthic algae, showing a frequency of 30 as seen in Table 1. *Achnanthes sp*. constituted 66.7% of all diatoms found in diatom guts. Diatom species found in the potentially best sediment, the St. Croix sediment, included: *Achnanthes exigua, Achnanthes lanceolata, Achnanthes minutissima, Achnanthes sp., Navicula radiosa*, *Navicula sp*., and unidentified sp. Of the diatoms found, 92% were benthic while the other 8% were planktonic, as seen in Tables 1 and 2. This indicates that after two weeks *Q. quadrula* juveniles were pedal feeding and had not switched to filter feeding. This is statistically supported (p = 0.0413). There was no statistical difference between sediment types as well as between water types in the percentage of benthic diatoms found (p = 0.4036 and 0.7824 respectively); this indicates that both sediment and water type had no effect on the types of diatoms found. Overall, it would be recommended that a river sediment with Mississippi River water treatment be used to culture *Quadrula fragosa*.

For further study, water chemistry tests could be performed on the Mississippi River and St. Croix River water to determine if factors such as pH, ammonia, and other pollutants affect juvenile growth. In addition, juveniles could be cultured for a longer period, allowing for more substantial growth differences. Gut contents could be analyzed on a weekly basis to provide a more accurate range of algae preference.

Appendix A

Figure 2: Avg. growth wk 4 (mm) by water type



Oneway Anova

Summary of

RSquare	0.066694
RSquare Adj	0.032127
Root Mean Square	0.05858
Mean of	0.617931
Observations (or Sum	29

t-Test

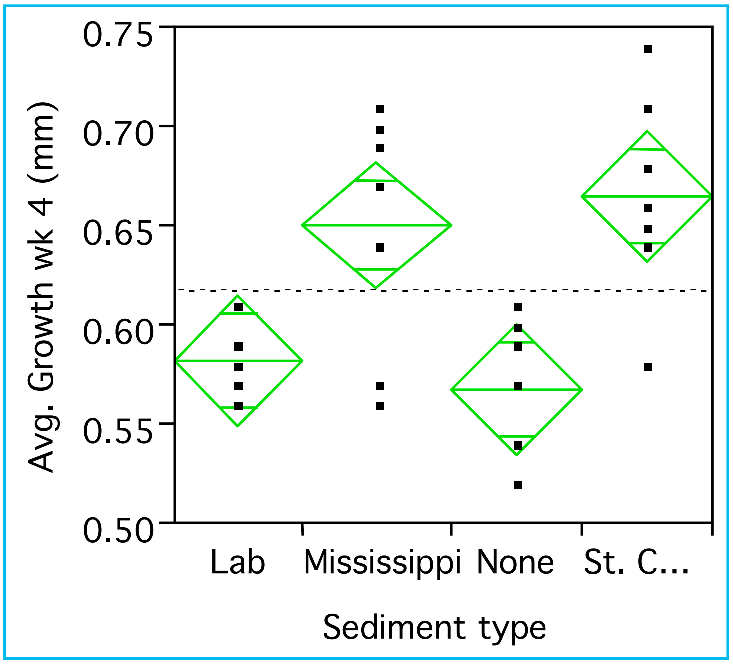
	Difference	t-Test	DF	Prob>|t|
Estimate	0.030238	1.389	27	0.1762
Std Error	0.021769			
Lower 95%	-0.01443			
Upper 95%	0.074904			

Assuming equal

Analysis of

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	1	0.00662110	0.006621	1.9294
Error	27	0.09265476	0.003432	Prob>F
C Total	28	0.09927586	0.003546	0.1762

Figure 3: Avg. growth wk 4 (mm) by sediment type



Oneway Anova

Summary of Fit

RSquare	0.519071
RSquare Adj	0.461359
Root Mean Square Error	0.043701
Mean of Response	0.617931
Observations (or Sum Wgts)	29

Analysis of Variance

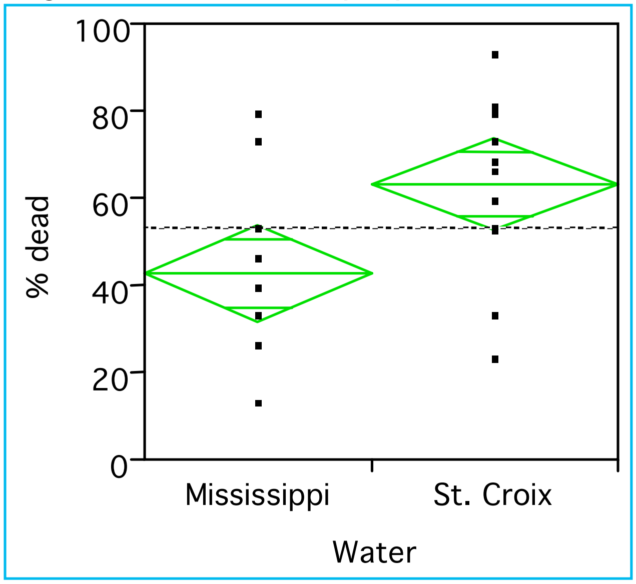
Source	DF	Sum of Squares	Mean Square	F Ratio
Model	3	0.05153122	0.017177	8.9942
Error	25	0.04774464	0.001910	Prob>F
C Total	28	0.09927586	0.003546	0.0003

Means for Oneway Anova

Level	Number	Mean	Std Error
Lab	7	0.582857	0.01652
Mississippi	8	0.651250	0.01545
None	7	0.567143	0.01652
St. Croix	7	0.665714	0.01652

Std Error uses a pooled estimate of error variance

Figure 5: % mortality by water



Oneway Anova

Summary of

RSquare	0.213809
RSquare Adj	0.184691
Root Mean Square	20.53998
Mean of	53.54828
Observations (or Sum	29

t-Test

	Difference	t-Test	DF	Prob>|t|
Estimate	-20.6833	-2.710	27	0.0116
Std Error	7.6329			
Lower 95%	-36.3446			
Upper 95%	-5.0220			

Assuming equal

Analysis of

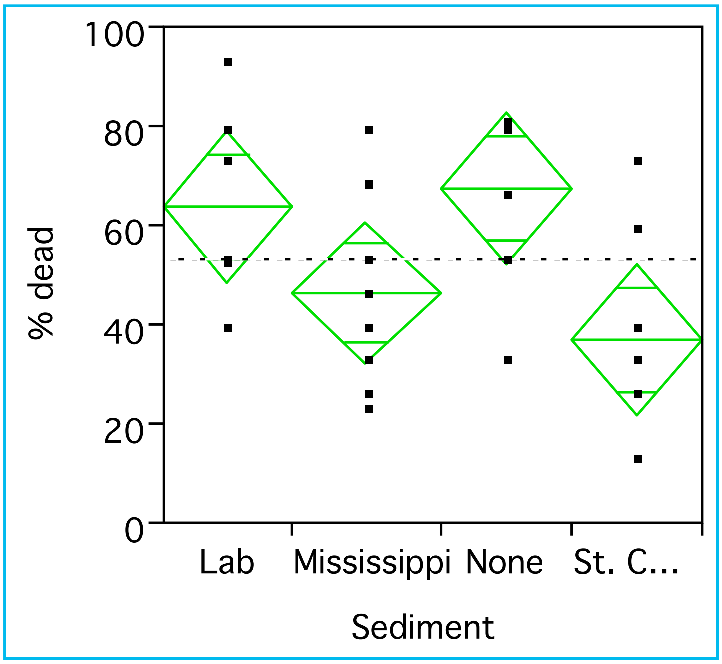
Source	DF	Sum of Squares	Mean Square	F Ratio
Model	1	3097.864	3097.86	7.3428
Error	27	11391.048	421.89	Prob>F
C Total	28	14488.912	517.46	0.0116

Means for Oneway

Level	Number	Mean	Std Error
Mississippi	14	42.8500	5.4895
St. Croix	15	63.5333	5.3034

Std Error uses a pooled estimate of error

Figure 6: % mortality by sediment



Oneway Anova

Summary of Fit

RSquare	0.305593
RSquare Adj	0.222264
Root Mean Square Error	20.06111
Mean of Response	53.54828
Observations (or Sum Wgts)	29

Analysis of Variance

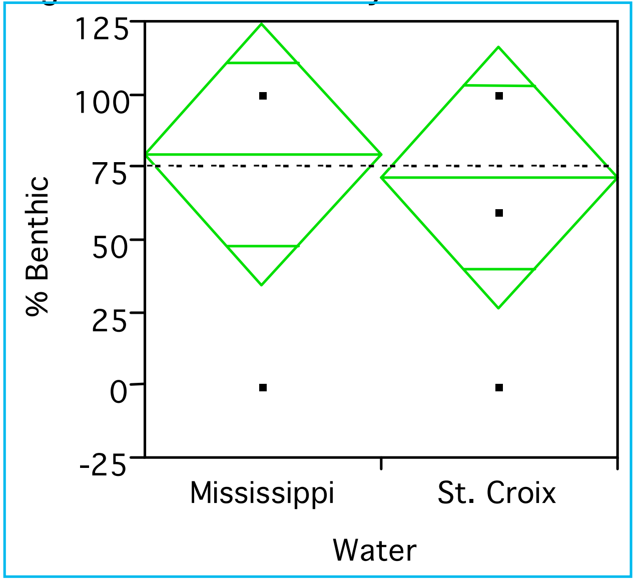
Source	DF	Sum of Squares	Mean Square	F Ratio
Model	3	4427.705	1475.90	3.6673
Error	25	10061.207	402.45	Prob>F
C Total	28	14488.912	517.46	0.0257

Means for Oneway Anova

Level	Number	Mean	Std Error
Lab	7	63.7286	7.5824
Mississippi	8	46.5375	7.0927
None	7	67.8000	7.5824
St. Croix	7	37.1286	7.5824

Std Error uses a pooled estimate of error variance

Figure 7: % Benthic by water



Oneway Anova

Summary of Fit

RSquare	0.010101
RSquare Adj	-0.11364
Root Mean Square	44.27189
Mean of	76
Observations (or Sum	10

t-Test

	Difference	t-Test	DF	Prob>|t|
Estimate	8.0000	0.286	8	0.7824
Std Error	28.0000			
Lower 95%	-56.5687			
Upper 95%	72.5687			

Assuming equal

Analysis of

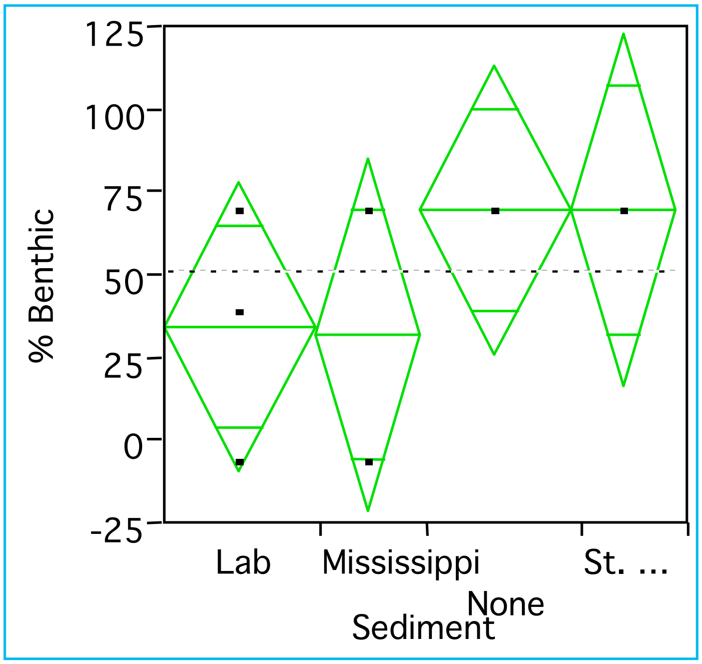
Source	DF	Sum of Squares	Mean Square	F Ratio
Model	1	160.000	160.00	0.0816
Error	8	15680.000	1960.00	Prob>F
C Total	9	15840.000	1760.00	0.7824

Means for Oneway

Level	Number	Mean	Std Error
Mississippi	5	80.0000	19.799
St. Croix	5	72.0000	19.799

Std Error uses a pooled estimate of error

Figure 8: % Benthic by sediment



Oneway Anova

Summary of Fit

RSquare	0.364478
RSquare Adj	0.046717
Root Mean Square	40.96069
Mean of Response	76
Observations (or Sum Wgts)	10

Analysis of Variance

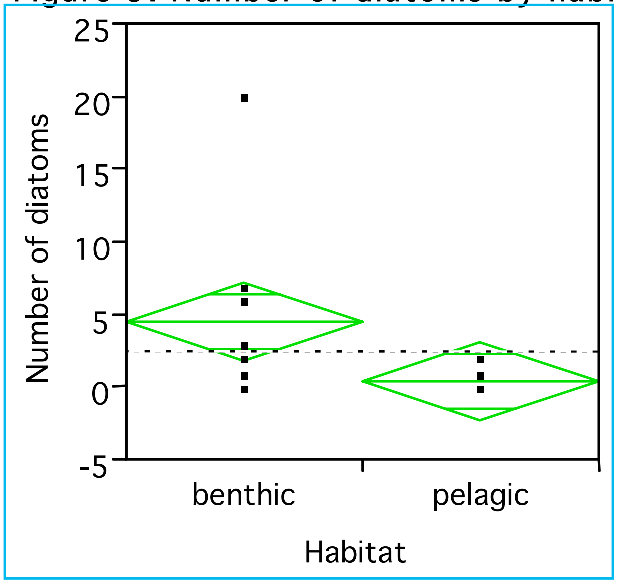
Source	DF	Sum of Squares	Mean Square	F Ratio
Model	3	5773.333	1924.44	1.1470
Error	6	10066.667	1677.78	Prob>F
C Total	9	15840.000	1760.00	0.4036

Means for Oneway Anova

Level	Number	Mean	Std Error
Lab	3	53.333	23.649
Mississippi	2	50.000	28.964
None	3	100.000	23.649
St. Croix	2	100.000	28.964

Std Error uses a pooled estimate of error variance

Figure 9: Number of diatoms by habitat



Oneway Anova

Summary of Fit

RSquare	0.211511
RSquare Adj	0.167706
Root Mean Square Error	4.273952
Mean of Response	2.5
Observations (or Sum Wgts)	20

t-Test

	Difference	t-Test	DF	Prob>|t|
Estimate	4.20000	2.197	18	0.0413
Std Error	1.91137			
Lower 95%	0.18439			
Upper 95%	8.21561			

Assuming equal variances

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	1	88.20000	88.2000	4.8285
Error	18	328.80000	18.2667	Prob>F
C Total	19	417.00000	21.9474	0.0413

Means for Oneway Anova

Level	Number	Mean	Std Error
benthic	10	4.60000	1.3515
pelagic	10	0.40000	1.3515

Std Error uses a pooled estimate of error variance

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