INFLUENCE OF NIRATES AND PHOSPHATES TO FRESWATER MACROINVERTEBRATES BIODIVERSITY

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ABSTRACT

Nitrates are families of chemical compound that composes of nitrogen and oxygen and are one of the main sources in which plant obtains nitrogen wherein Phosphates are occurs in bodies of water containing an atom of phosphorus and oxygen. Phosphorus is essential to the development od biological organisms both in their photosynthetic and metabolic processes. On the other hand, Freshwater macroinvertebrates are any species or animals small enough to be categorized in macro that doesn't posses a backbone. These organisms are often called exothermic or cold-blooded species. Benthic macroinvertebrates are small aquatic organisms whose habitats are under the rocks. Benthic macroinvertebrates are used to indicate water quality. In this study, we experimented whether the presence of Nitrates and Phosphates has a direct affect on these fresh water macroinvertebrates in three (3) selected sites in Wawa Dam. It turns out that not only nitrates and Phosphates can affect the biodiversity of macroinvertebrates but also the habitat they are one. The type of habitat greatly contributed to the different variety of species of macroinvertebrates in each site. In doing so, we also used the macroinvertebrates to test the water quality by using the Biotic Index Value wherein we measure the biotic value of each species.

INTRODUCTION

Background of the Study

Macroinvertebrates are any animal that doesn’t possess backbones and can be seen through the naked eye. These organisms are often called exothermic or cold-blooded species. Benthic macroinvertebrates are small aquatic organisms whose habitats are under the rocks. Benthic macroinvertebrates are used to indicate water quality. They respond to quality disturbance of water due to pollution, and that includes their diversity. Nitrates and Phosphates are great contributors to the environment. They are essential for the growth of plants and animals but they are greatly affected and deteriorated by human activities which cause various problems in the environment.

Statement of the Problem

Nitrate and phosphate post a significant threat to freshwater macroinvertebrates when delivered without treatment at levels past suggested limits in oceanic climate. While resistance levels of a wide scope of taxa have been set up along slopes of natural contamination, little is thought concerning how freshwater macroinvertebrates in tropical African locales react to raised degrees of nitrate and phosphate. Presence of raised nitrate and phosphate levels in freshwater biological systems can drive touchy macroinvertebrate gatherings to elimination. This has been an issue not only to the locals but also to the farmers and fishermen whose livelihood depends on bodies of water. Thus, it is a must to determine whether the presence of nitrates and phosphates affects the diversity of macroinvertebrates.

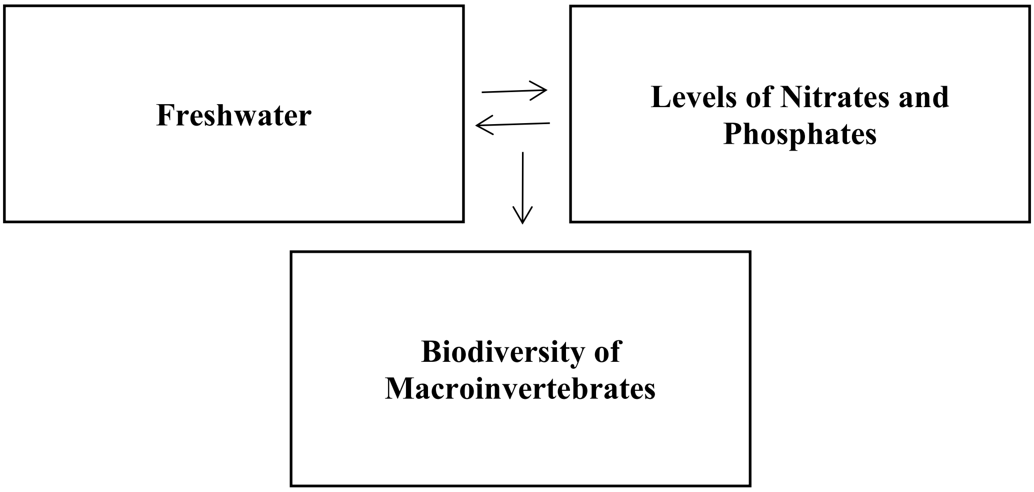
This study aims to answer the following questions;

1. What type of relationship is there between the factors to the biodiversity of macroinvertebrates?

2. How are the levels of phosphate and nitrates, and the quality of water influential to the biodiversity of macroinvertebrates?

3. If there is such a relationship, what is the greatest factor to the biodiversity of macroinvertebrates?

Conceptual Framework



Significance of the Study

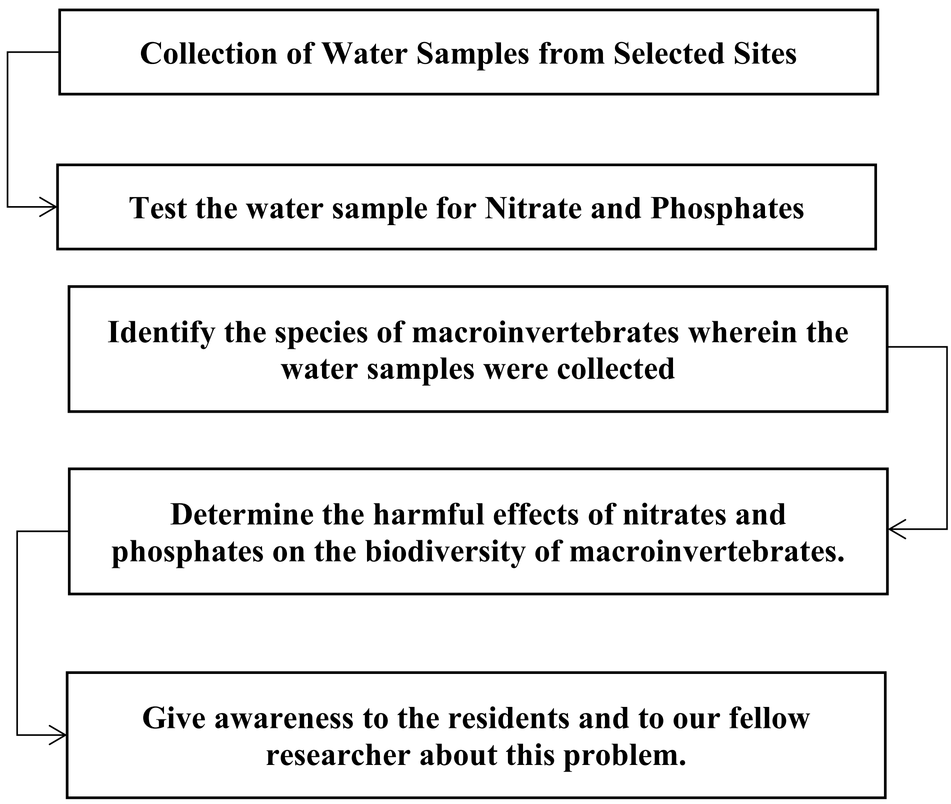
With the increase of urbanization and commercialization of agricultural products being such are nitrates and phosphates: main components of fertilizers which are widely used and as a part of standardization of agriculture. And with growing concern of harmful algal blooms through the process of eutrophication amongst which is encouraged by the said organic compounds. It is very necessary to monitor such concerns as these may lead to public health and environmental concerns affecting many communities and lives who depend on the bodies of water they reside. Inclusive also to the hydrosphere are its inhabitants such as the freshwater macroinvertebrate.

By observing the aquatic flora of our water bodies not only it ensures balance and stability but also ensures the future of our planet.

Scope and Limitation

This study, The Influence of the accumulated nitrates and phosphates to Freshwater Macroinvertebrates Biodiversity only focal point is to finding the correlation or the relationship between the accumulated nitrates and phosphates to the biodiversity of freshwater macroinvertebrates and will not answer nor interpolate other subject unrelated. This will include concepts of biodiversity, water quality, and water chemistry and will not investigate any further beyond these concepts.

Theoretical Framework



Hypothesis

H_a: The levels of phosphates and nitrates detected has any relationship or relativity on the diversity of macroinvertebrates and the quality of water directly or inversely.

H_o: The levels of phosphates and nitrates detected have no relationship or any subsequent effects on the diversity of macroinvertebrates and the quality of water.

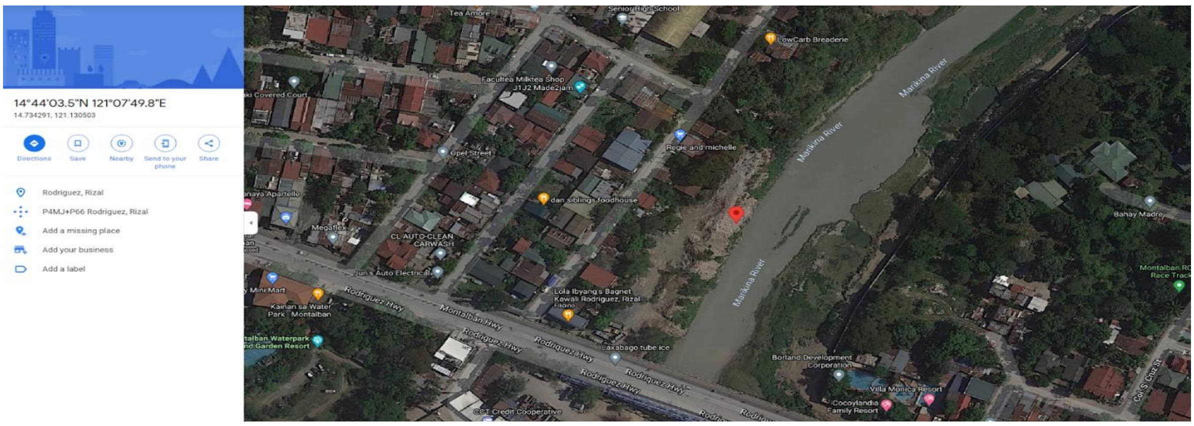
METHODOLOGY

Globe Protocol

This study will include and follow protocols from the Globe program, an organization sanctioned and organized by the National Aeronautical and Space Administration (NASA) under the US government. These protocols being such; pH protocol, Nitrates ($NO^{-}{}_{3}$) protocol, and Freshwater Macroinvertebrate protocol. These will be strictly and rigorously followed for precision and accuracy of data, and the credibility of the research.

Selection of Possible Testing Site

Selecting a fine and quality site has a significant impact on the success of conduct of in-situ testing in finding the total nitrate/phosphate level and analysis of macroinvertebrates. A 50 x 50 meters section of the stream, river, or lake. Access to safety and efficiency to sampling is highly preferred. Locations from Rodriguez, Rizal was chosen by the researcher as it had a connection and is a tributary to one of the major river systems in Metro Manila, the Pasig-Marikina River. Before the selection of the site, preliminary ocular visitations had been made to ensure not only the safety, stability, and access to safety, but also to observe proper protocols such as the Site selection protocol.



Mapping of Hydrosphere Study Site

Preparation of Testing Kits and Equipment

Calibration of pH Meter

Before using the pH meter, it is required to calibrate it for the assurance of precise and accurate data. According to the Globe Protocols in order to calibrate the pH Meter, you will need to Rinse a 100-mL beaker three times with sample water and pour 50 mL of sample water into the 100-mL beaker. Put the electrode part of the meter into the water and stir it once. Do not let the meter touch the bottom or sides of the beaker and wait for one minute. If the pH meter is still changing numbers, wait another minute. Using a pH

Meter (Electrical Conductivity Greater than 200 mS/cm) Field Guide GLOBE® 2014 pH

Protocol - 11 Hydrosphere. Record pH value on the data sheet under observer 1 and repeat it the steps twice using the newly collected water samples. Record conductivity and pH values under Observer 2 and Observer 3. Calculate the average on the Data Sheet. Rinse the electrode with distilled water then turn off the meter.

Preparation of Nitrate and Phosphate Kit

In preparation of the Nitrate and Phosphate kit, the protocols provided by the GLOBE were followed. The first step is to put on latex gloves to avoid exposure, and follow the guides and instructions that is included in the kit to measure nitrates and Phosphates. Use a clock or a timer for a precise and accurate result then match the treated sample water with a color in the test kit.

Preparation of Macroinvertebrate sampling

For the preparation of macroinvertebrate sampling, lay a quadrat and place the net downstream. Someone will hold the net while the other one will use a shovel or a trowel to lift the substrate and place it on the net. Move the bottom of the net back and forth to wash out the finer sediments. Lift up the net out of the water and make sure the water flows out of the net and no organisms escape by climbing out. Use the sieved water to concentrate all organisms and debris at the bottom of the net. Place the collected samples on the net on a bucket until you are ready to sort and organize them out. Repeat the process for all site.

Collection of Data

Nitrate test

Fill a clean test tube with 5 ml of water sample collected in each site to be tested. Add 10 drops of Nitrate solution #1, holding the vertically to assure the number of drops. Cap the test tube in repeatedly invert the test tube to mix the solution. Before dropping the Nitrate solution test #2, make sure to vigorously shake the bottle for at least 30 seconds to ensure the accuracy of each test. Now add 10 drops of the Nitrate solution test #2 on the test tube containing the solution of the collected water sample mixed with Nitrate solution test #1. Cap the test tube and shake vigorously for 1 minute. Use a clock or a timer for a more precise result. Wait 5 mins for the color to develop.

Compare the color of the solution to the appropriate color card provided in the kit.

Phosphate test

For the Phosphate test, fill a clean test tube with 5 ml of the water sample collected in each site to be tested. While holding the test tube vertically, add 6 drops from Phosphate test solution #1. Cap and shake the test tube vigorously for 5 seconds. Add another 6 drops of Phosphate test solution #2 on the test tube then cap and shake it vigorously for 5 seconds. Wait 3 mins for the color to develop. Compare the color of the solution to the appropriate color card provided in the kit.

Macroinvertebrate sampling

In collecting the samples of Macroinvertebrate, a bast syringe or forceps are needed to pick out a large organism from your bucket or container and put these in a tray or any flat surfaces where you can easily sort and organize them. If there are rocks or other sediments that was mixed in the container, take them out and rinse the rocks over the sample bucket before discarding the rocks. If the water in your buckets is clear, free of debris, and rather a small amount, pour sample on tray to sort. Strain the sample filled water using a sieve to remove the water and to contain the rocks, sediments and macroinvertebrates caught in the sieve. Place the sieve with the finer mesh size below the other sieve. Gently and slowly pour the water from the bucket containing the organisms into the sieves. Transfer and rinse the sample on the sieves into the tray using a squirt bottle. Make sure to rinse the bucket every so often with your spray bottle and strain the water through the sieve. Report the total number of organisms found for each taxon on Macroinvertebrates Identification Data Sheet.

RESULTS

A. Hydrology Investigation Data Sheet

This sheet provided by GLOBE Program to help assess and record the data collected. Nitrates and Phosphates are the parameters used in this study wherein the pH of the water is our controlled variable. We collected 3 samples with 3 replicates on 3 different sites specifically in Wawa Dam located in Rodriguez, Rizal. As shown in the table below.

Phosphates			
	Site 1	Site 2	Site 3
Rep 1	1 ppm	0.5 ppm	2 ppm
Rep 2	2 ppm	1 ppm	2 ppm
Rep 3	1 ppm	1 ppm	2 ppm

Table 1: Phosphate levels in 3 different sites in Wawa Dam

As seen in Table 1, the levels of Phosphates in Site 1 have an average of 1 ppm, while the site 2 has a relatively lower Phosphate level of an average of 0.075 ppm and lastly for site 3 which has a higher level of Phosphates with an average of 2 ppm.

Nitrates			
	Site 1	Site 2	Site 3
Rep 1	0 ppm	0 ppm	0 ppm
Rep 2	0 ppm	0 ppm	0 ppm
Rep 3	0 ppm	0 ppm	0 ppm

Table 2: Nitrate levels in 3 different sites in Wawa Dam

As seen in Table 2, all sites have shown no presence of nitrates in the selected location. According to archive.epa.gov, this is because Nitrates dissolves quickly in water and especially in flowing water. [1]

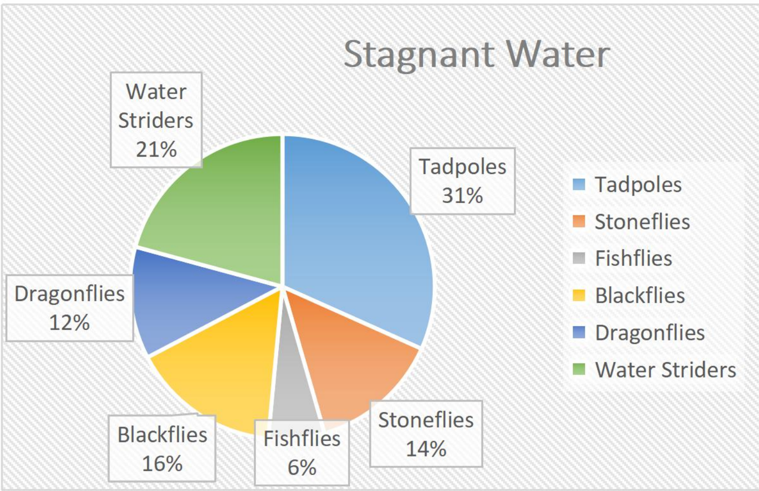


Chart 1: Site 1 Stagnant Water

1 Environmental Protection Agency. (2012, March 6). *5.7 nitrates*. EPA. Retrieved March 9, 2022, from https://archive.epa.gov/water/archive/web/html/vms57.html

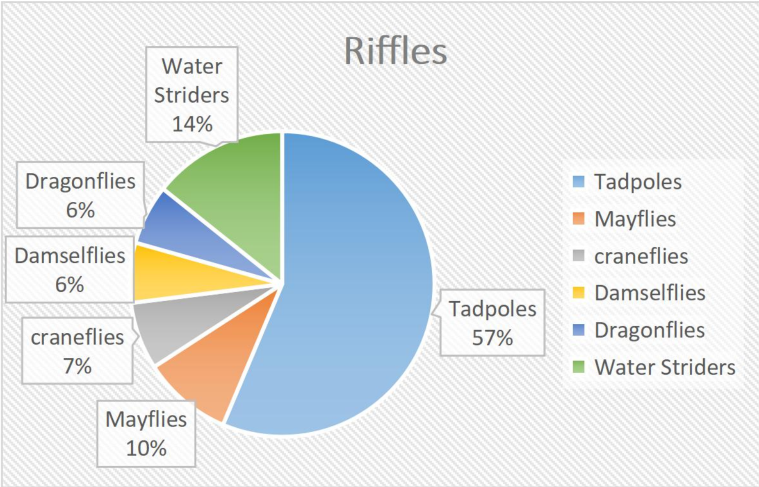


Chart 2: Site 2 Riffles

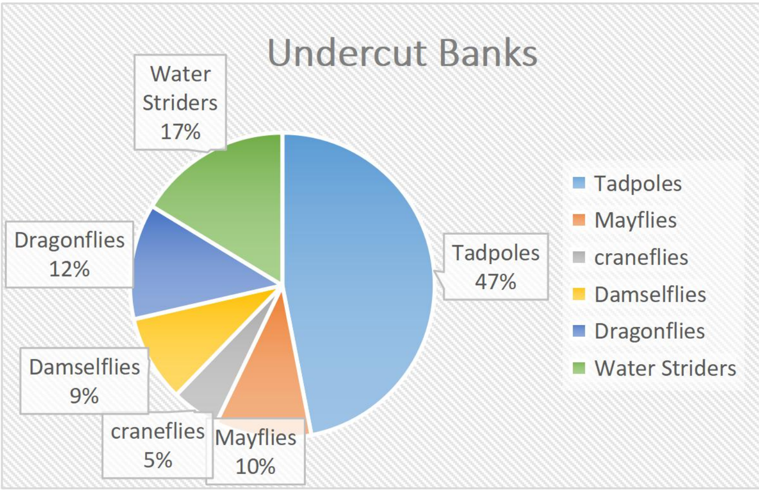


Chart 3: Site 2 Undercut Banks

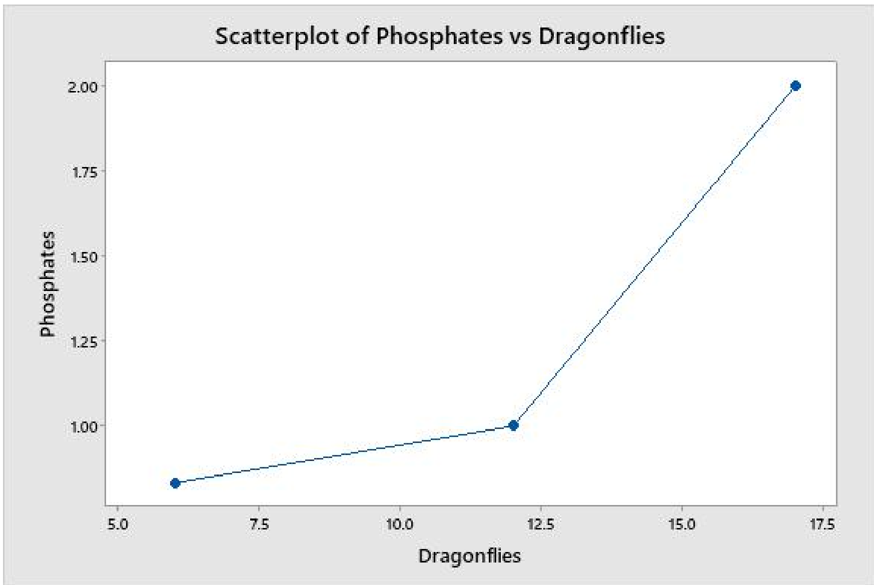


Chart 4: Scatterplot of Phosphates vs. Water Strider

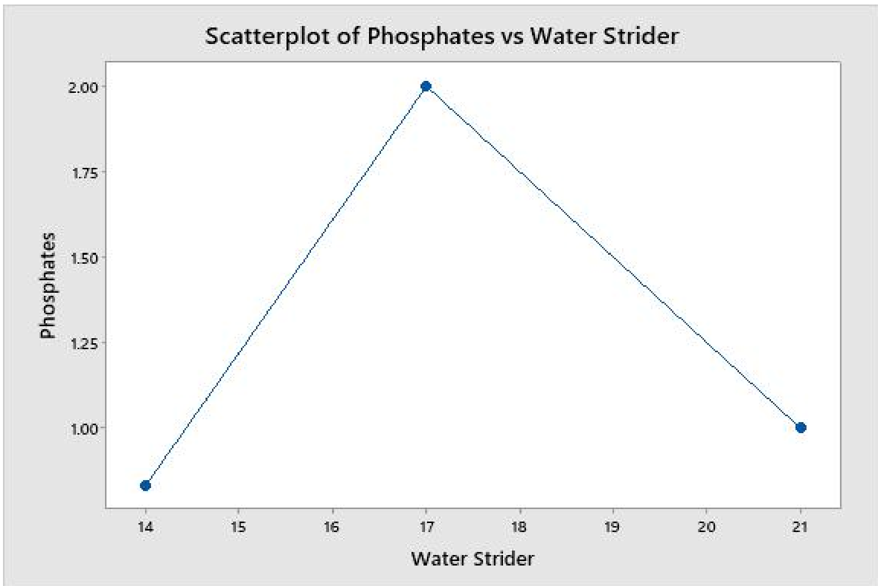


Chart 5: Scatterplot of Phosphate vs. Dragonflies

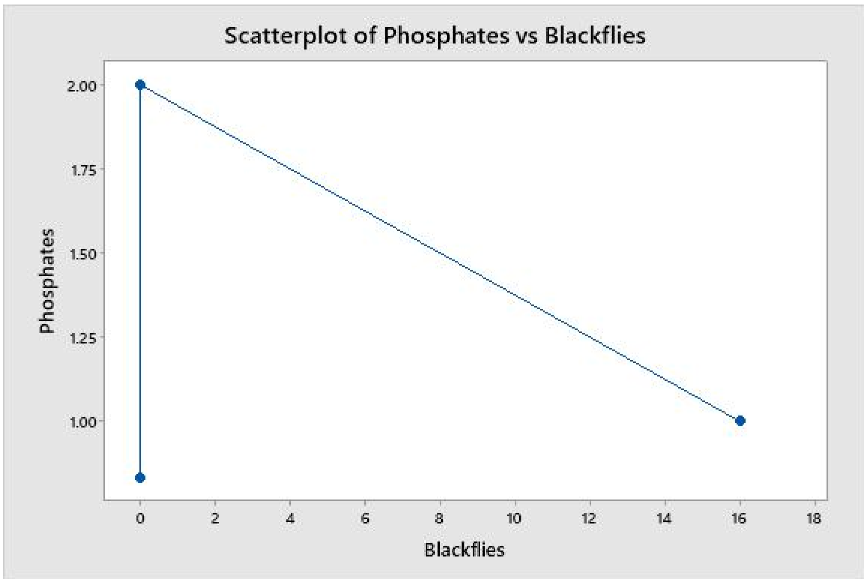


Chart 6: Scatterplot of Phosphate vs. Blackflies

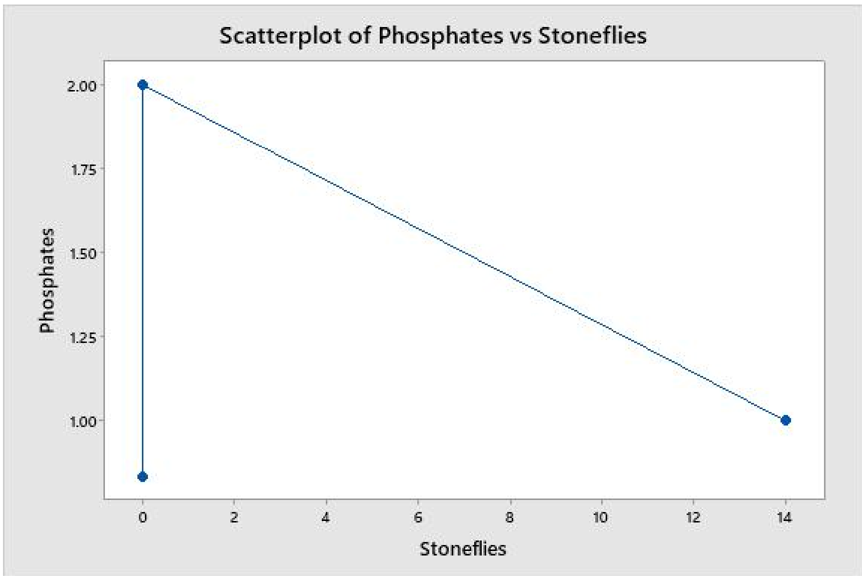


Chart 7: Scatterplot of Phosphate vs. Stoneflies

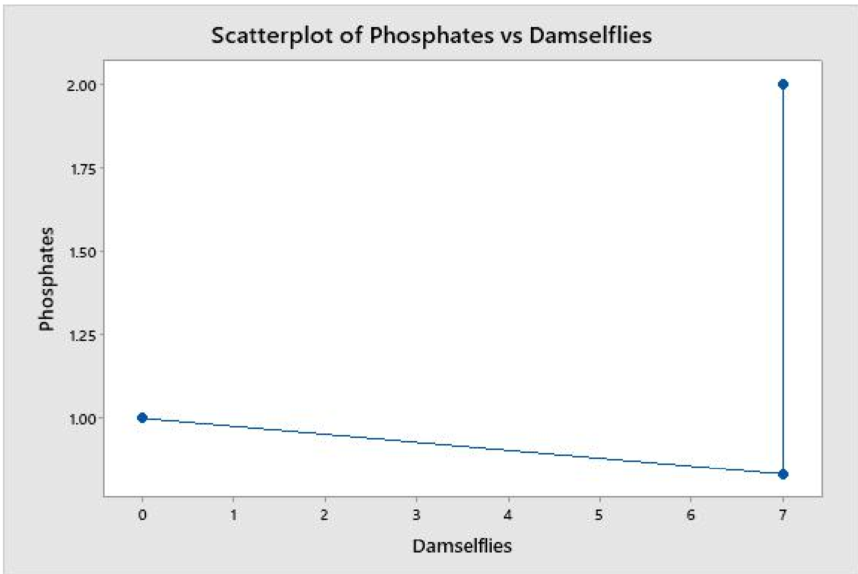


Chart 8: Scatterplot of Phosphate vs. Damselflies

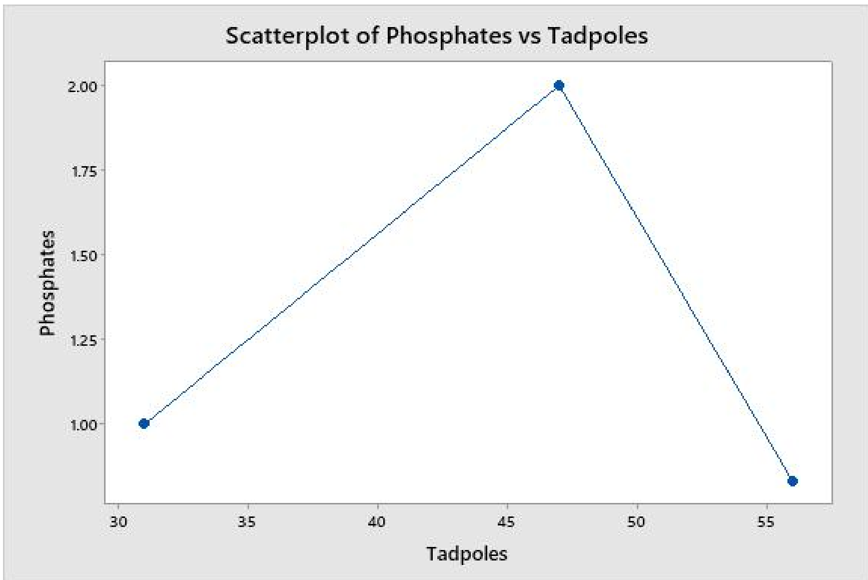


Chart 9: Scatterplot of Phosphate vs. Tadpoles

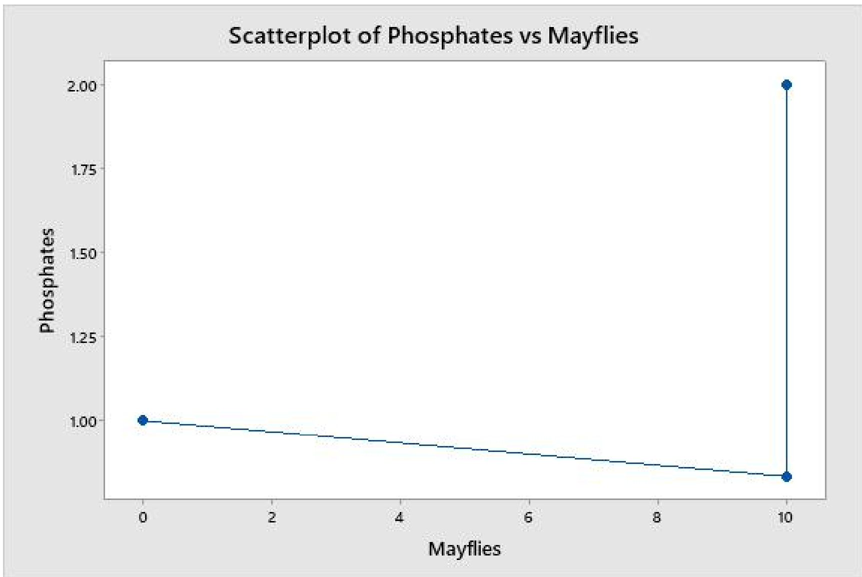


Chart 10: Scatterplot of Phosphate vs. Mayflies

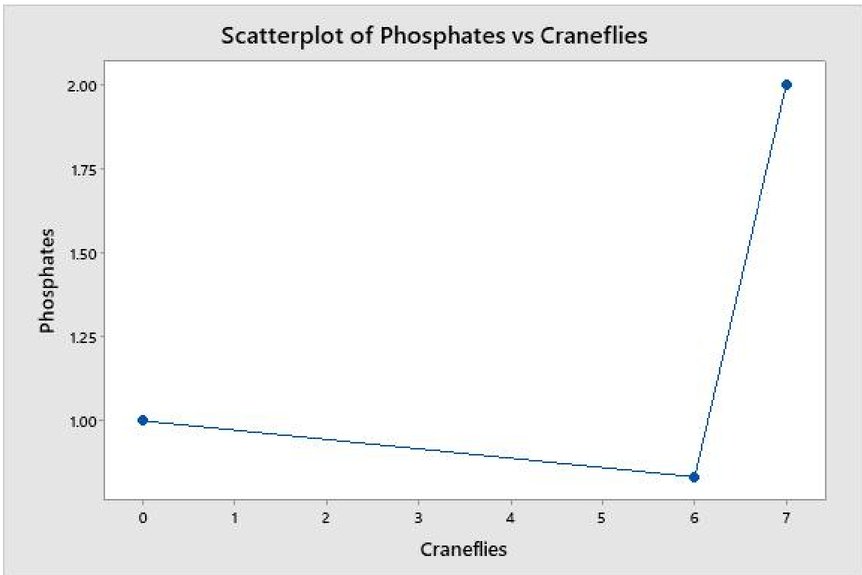


Chart 11: Scatterplot of Phosphate vs. Craneflies

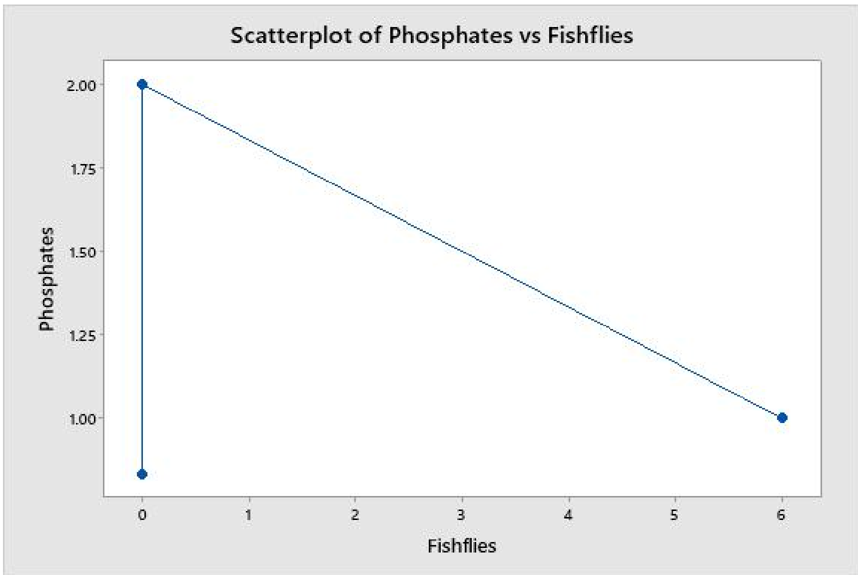


Chart 12: Scatterplot of Phosphate vs. Fishflies

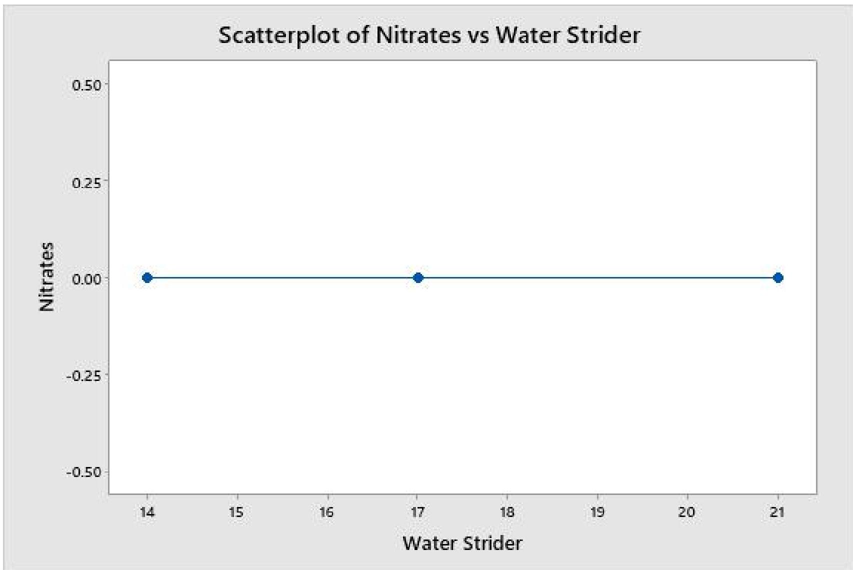


Chart 13: Scatterplot of Nitrates vs. Water Strider

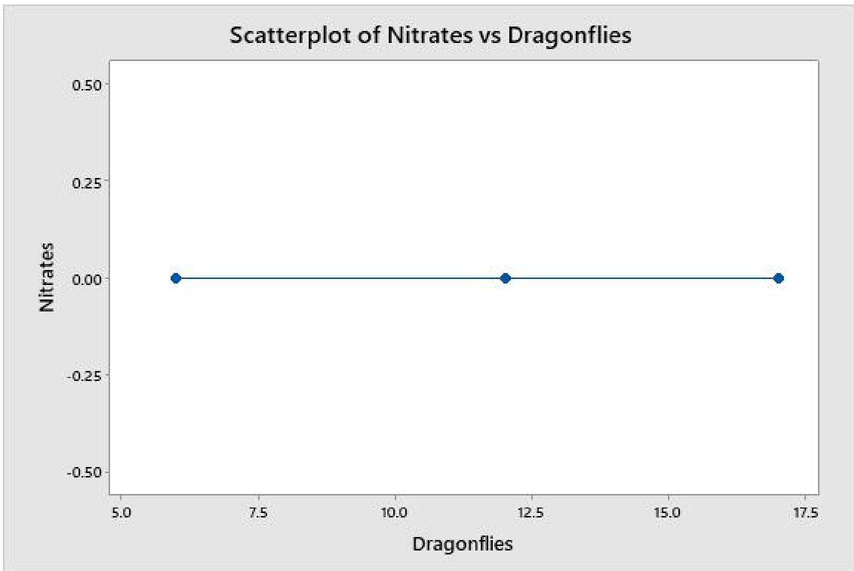


Chart 14: Scatterplot of Nitrates vs. Dragonflies

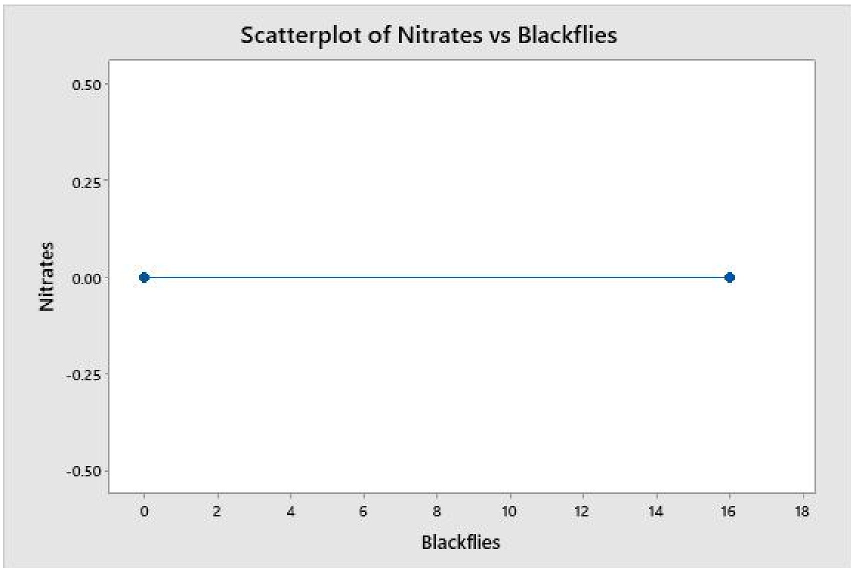


Chart 15: Scatterplot of Nitrates vs. Blackflies

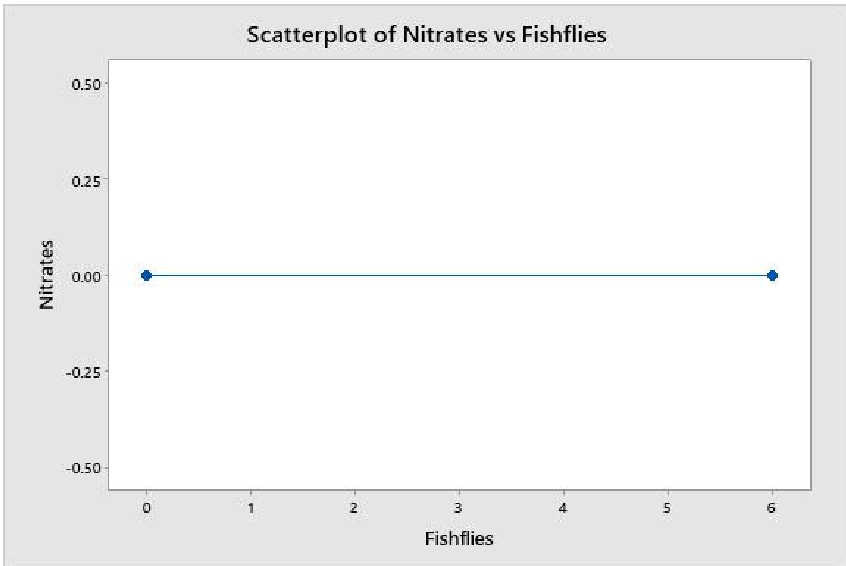


Chart 16: Scatterplot of Nitrates vs. Fishflies

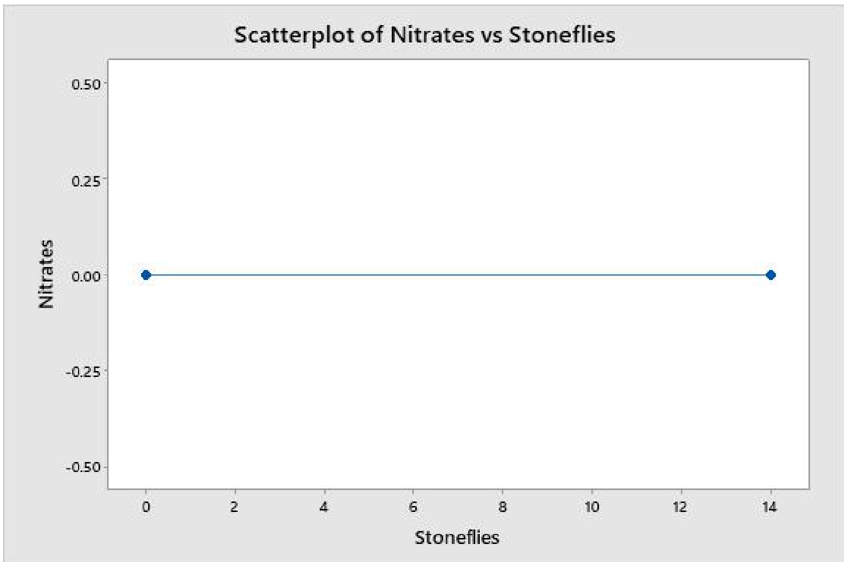


Chart 17: Scatterplot of Nitrates vs. Stoneflies

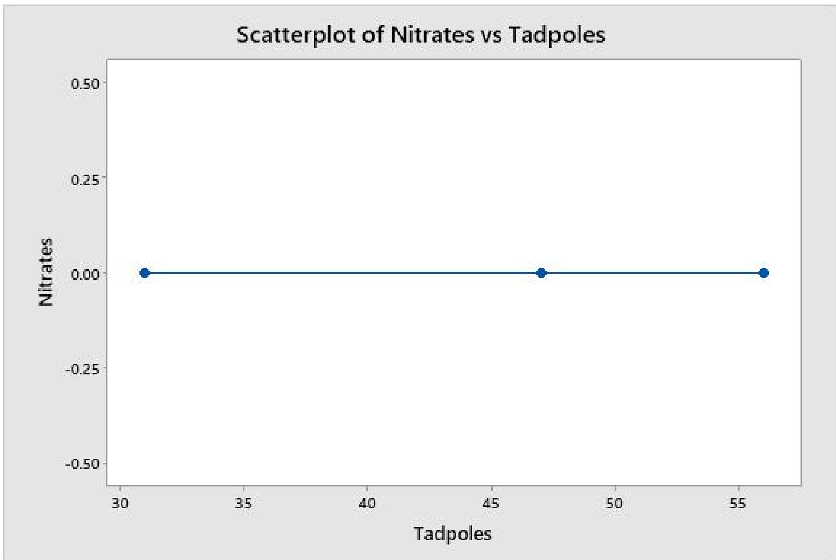


Chart 18: Scatterplot of Nitrates vs. Tadpoles

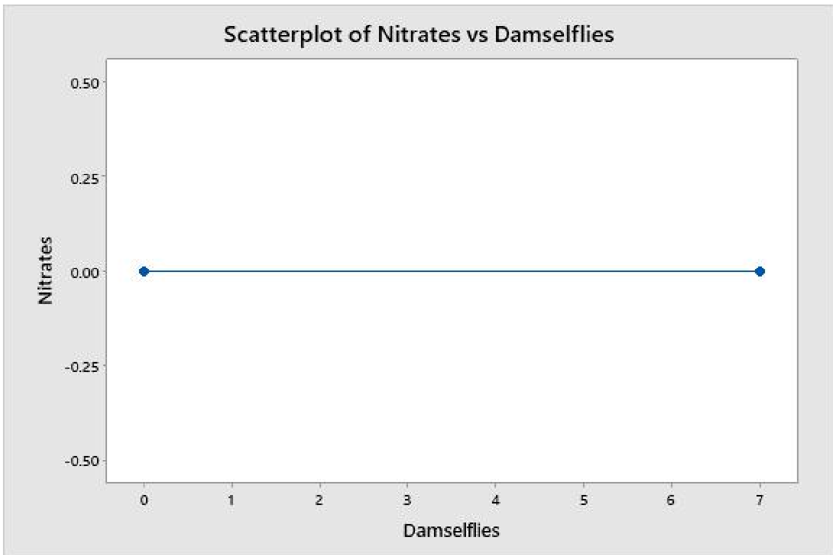


Chart 19: Scatterplot of Nitrates vs. Damselflies

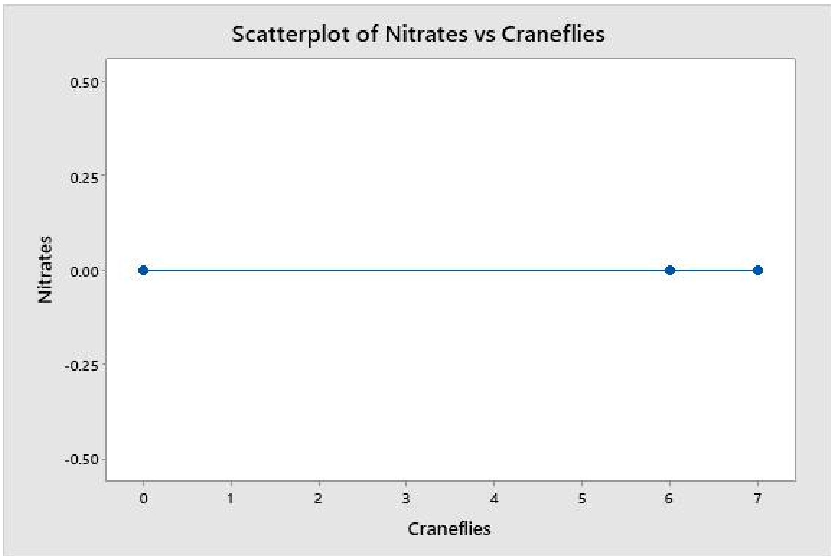


Chart 20: Scatterplot of Nitrates vs. Craneflies

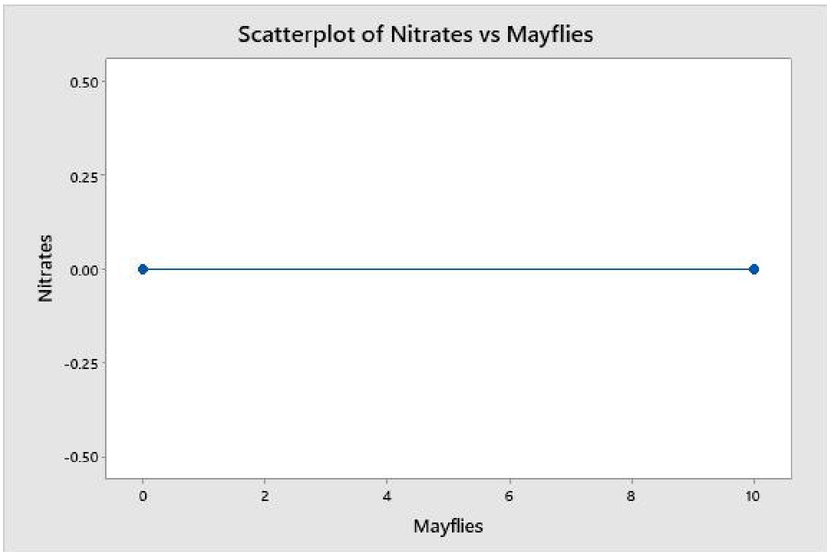


Chart 21: Scatterplot of Nitrates vs. Mayflies

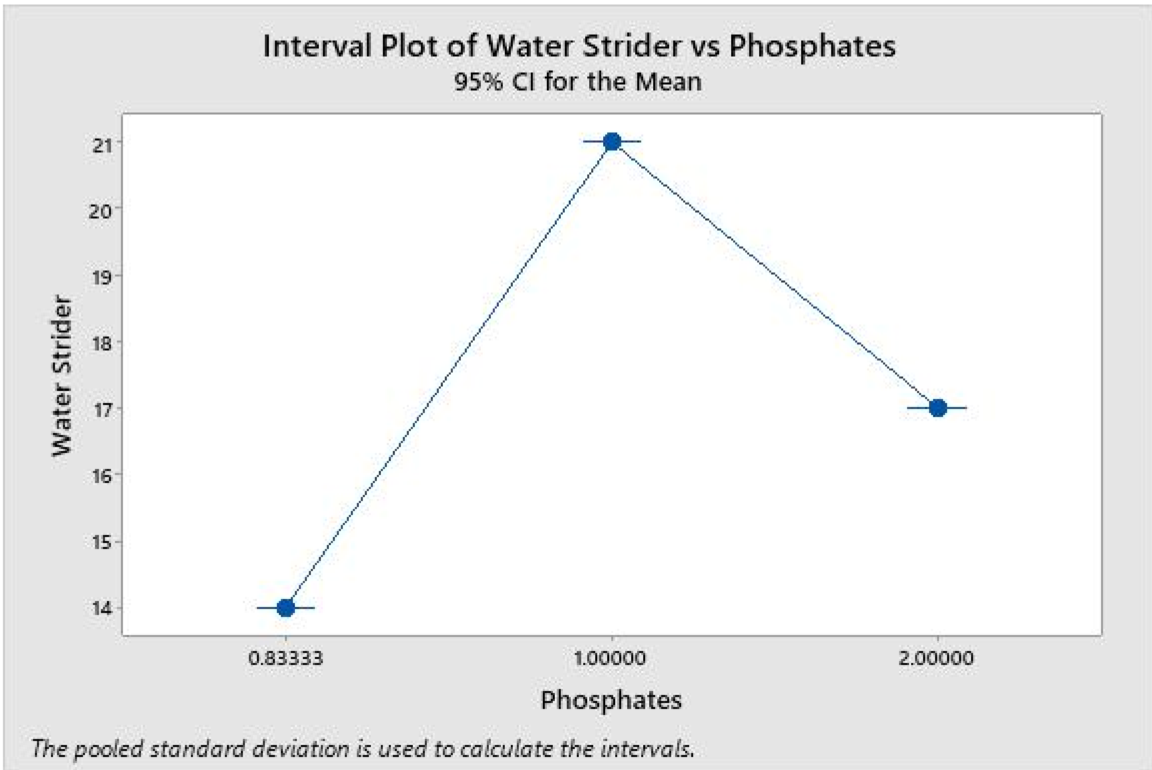


Chart 22: One-way ANOVA: Water Strider versus Phosphates

Factor Information

Factor	Levels	Values
Phosphates	3	0.83333, 1.00000, 2.00000

Analysis of Variance

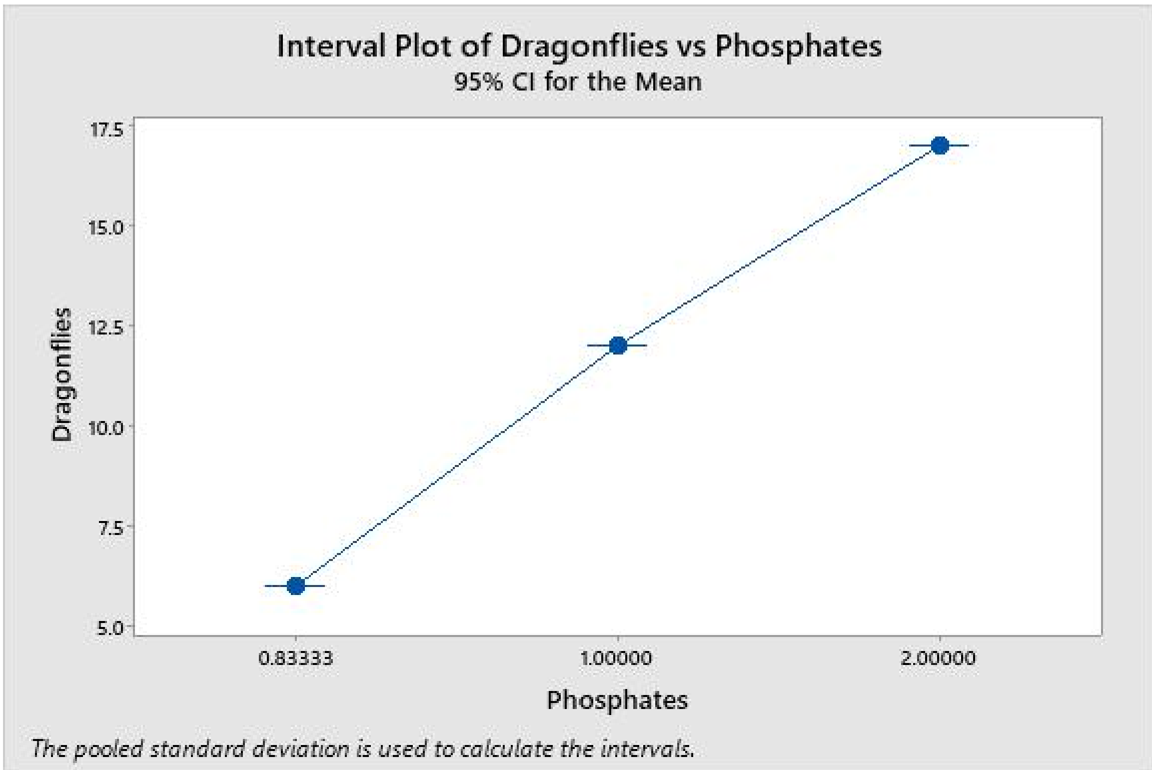
Source	DF	Adj SS	Adj MS	F-Value	P-Value
Phosphates	2	24.67	12.33	*	*
Error	0	*	*		
Total	2	24.67			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
*	100.00%	*	*

Means

Phosphates	N	Mean	StDev	95% CI
0.83333	1	14.00	*	(*, *)
1.00000	1	21.00	*	(*, *)
2.00000	1	17.00	*	(*, *)



*Pooled StDev = **

Chart 23: One-way ANOVA: Dragonflies versus Phosphates

Factor Information

Factor	Levels	Values
Phosphates	3	0.83333, 1.00000, 2.00000

Analysis of Variance

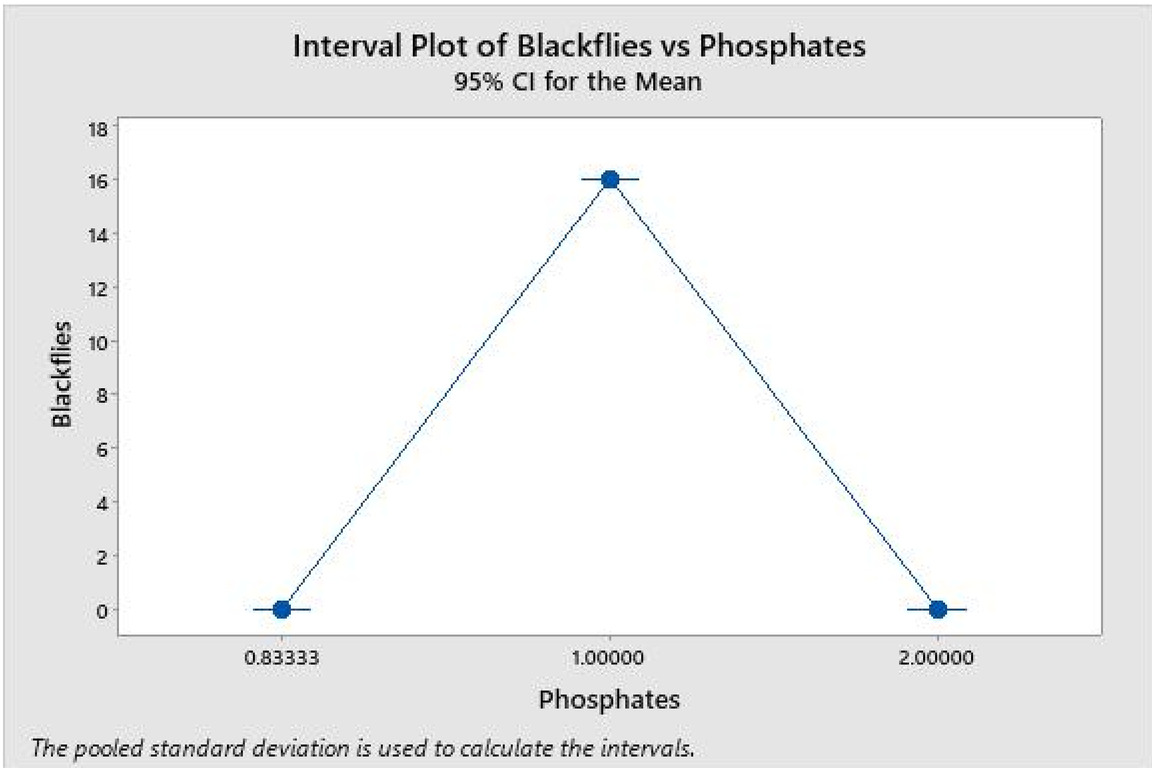
Source	DF	Adj SS	Adj MS	F-Value	P-Value
Phosphates	2	60.67	30.33	*	*
Error	0	*	*		
Total	2	60.67			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
*	100.00%	*	*

Means

Phosphates	N	Mean	StDev	95% CI
0.83333	1	6.000	*	(*, *)
1.00000	1	12.00	*	(*, *)
2.00000	1	17.00	*	(*, *)



*Pooled StDev = **

Chart 24: One-way ANOVA: Blackflies versus Phosphates

Factor Information

Factor	Levels	Values
Phosphates	3	0.83333, 1.00000, 2.00000

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Phosphates	2	170.7	85.33	*	*
Error	0	*	*		
Total	2	170.7			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
*	100.00%	*	*

Means

Phosphates	N	Mean	StDev	95% CI
0.83333	1	0.000000	*	(*, *)
1.00000	1	16.00	*	(*, *)
2.00000	1	0.000000	*	(*, *)

*Pooled StDev = **

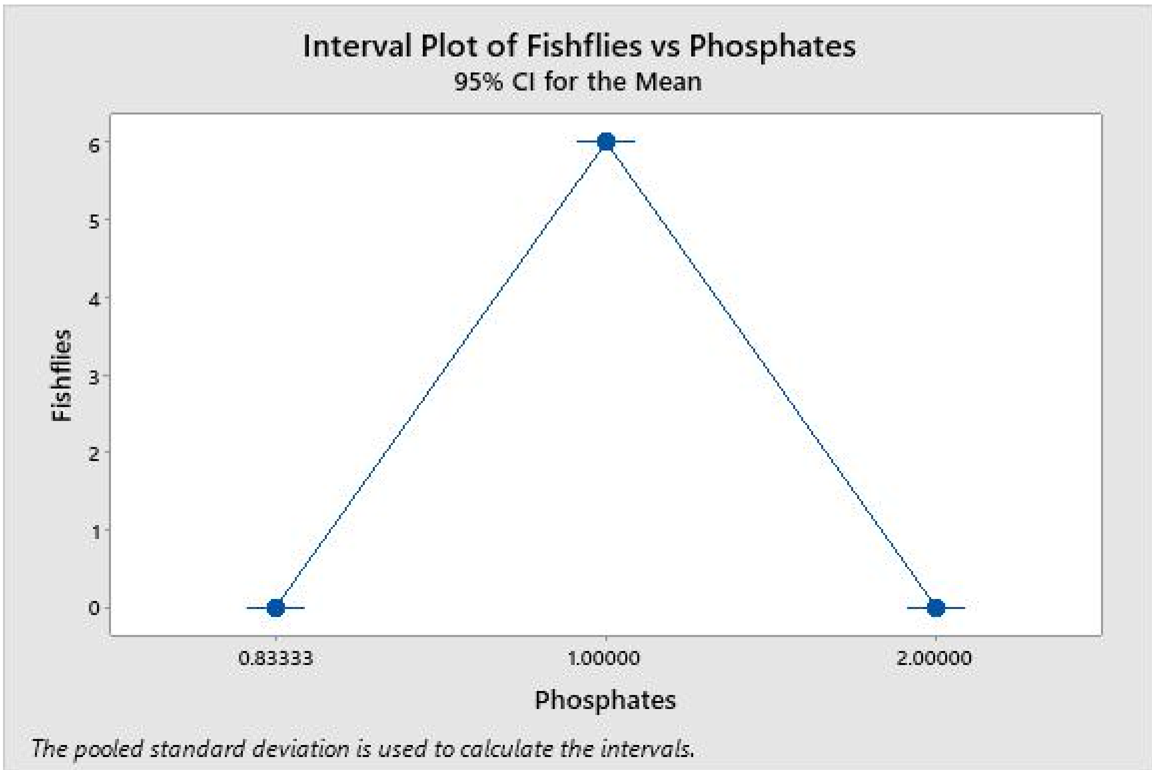


Chart 25: One-way ANOVA: Fishflies versus Phosphates

Factor Information

Factor	Levels	Values
Phosphates	3	0.83333, 1.00000, 2.00000

Analysis of Variance

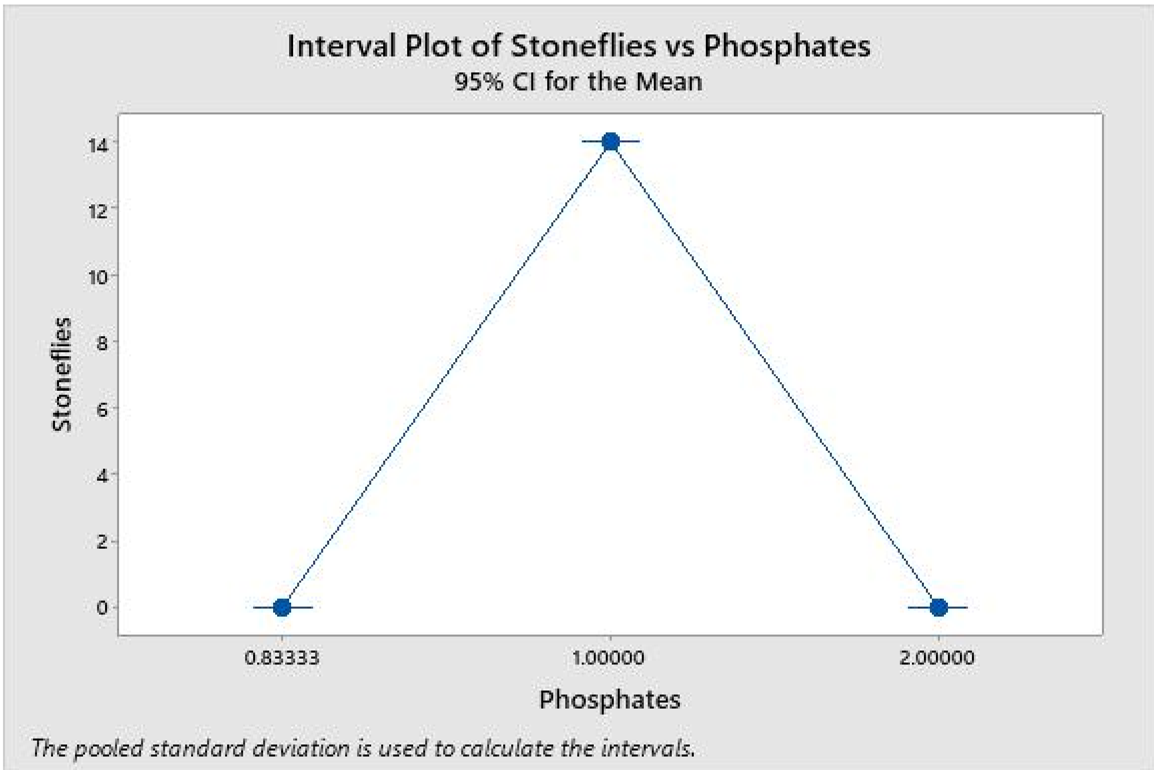
Source	DF	Adj SS	Adj MS	F-Value	P-Value
Phosphates	2	24.00	12.00	*	*
Error	0	*	*		
Total	2	24.00			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
*	100.00%	*	*

Means

Phosphates	N	Mean	StDev	95% CI
0.83333	1	0.000000	*	(*, *)
1.00000	1	6.000	*	(*, *)
2.00000	1	0.000000	*	(*, *)



*Pooled StDev = **

Chart. 26: One-way ANOVA: Stoneflies versus Phosphates

Factor Information

Factor	Levels	Values
Phosphates	3	0.83333, 1.00000, 2.00000

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Phosphates	2	130.7	65.33	*	*

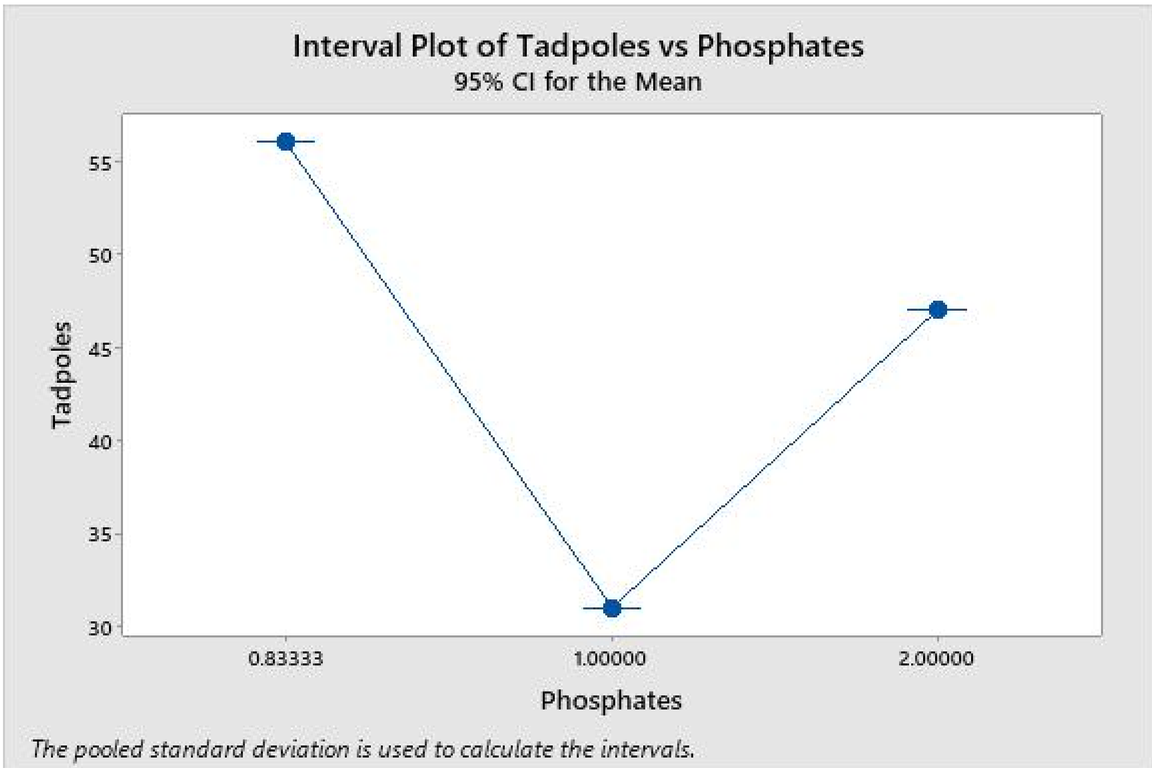
Error	0	*	*
Total	2	130.7	

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
*	100.00%	*	*

Means

Phosphates	N	Mean	StDev	95% CI
0.83333	1	0.000000	*	(*, *)
1.00000	1	14.00	*	(*, *)
2.00000	1	0.000000	*	(*, *)



*Pooled StDev = **

Chart 27: One-way ANOVA: Tadpoles versus Phosphates

Factor Information

Factor	Levels	Values
Phosphates	3	0.83333, 1.00000, 2.00000

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Phosphates	2	320.7	160.3	*	*
Error	0	*	*		
Total	2	320.7			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
*	100.00%	*	*

Means

Phosphates	N	Mean	StDev	95% CI
0.83333	1	56.00	*	(*, *)
1.00000	1	31.00	*	(*, *)
2.00000	1	47.00	*	(*, *)

*Pooled StDev = **

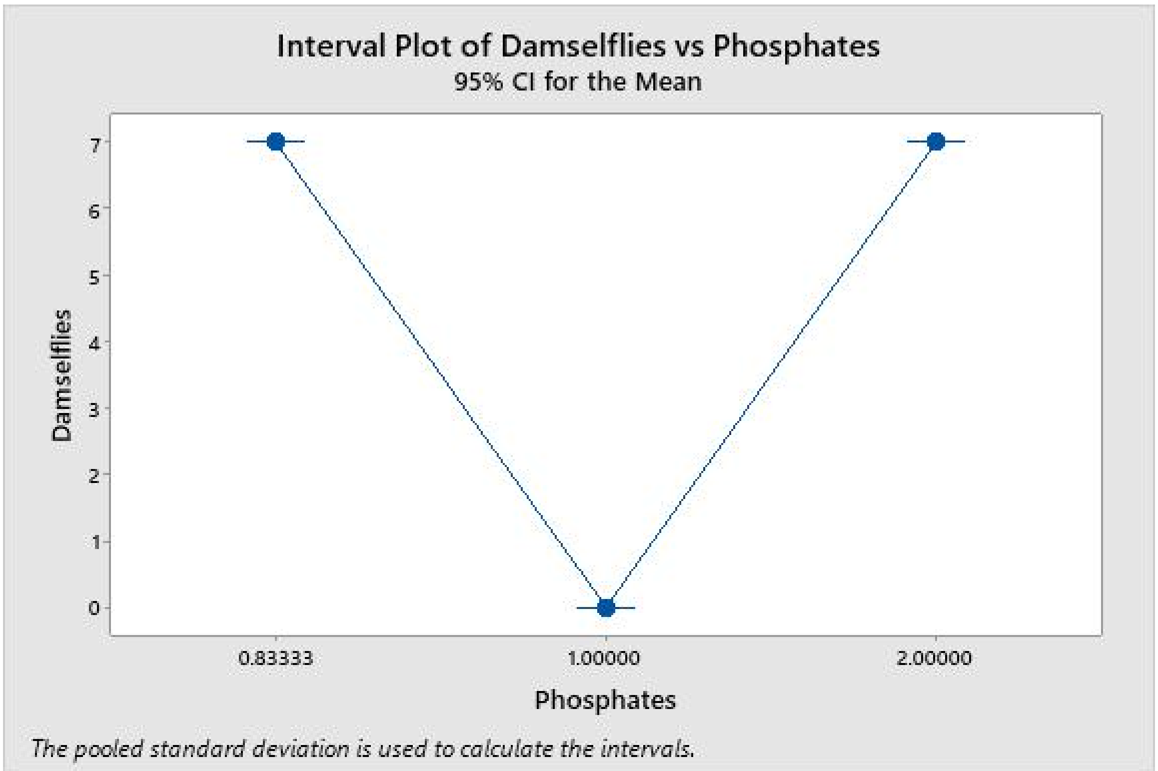


Chart 28: One-way ANOVA: Damselflies versus Phosphates

Factor Information

Factor	Levels	Values
Phosphates	3	0.83333, 1.00000, 2.00000

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Phosphates	2	32.67	16.33	*	*
Error	0	*	*		
Total	2	32.67			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
*	100.00%	*	*

Means

Phosphates	N	Mean	StDev	95% CI
0.83333	1	7.000	*	(*, *)
1.00000	1	0.000000	*	(*, *)
2.00000	1	7.000	*	(*, *)

*Pooled StDev = **

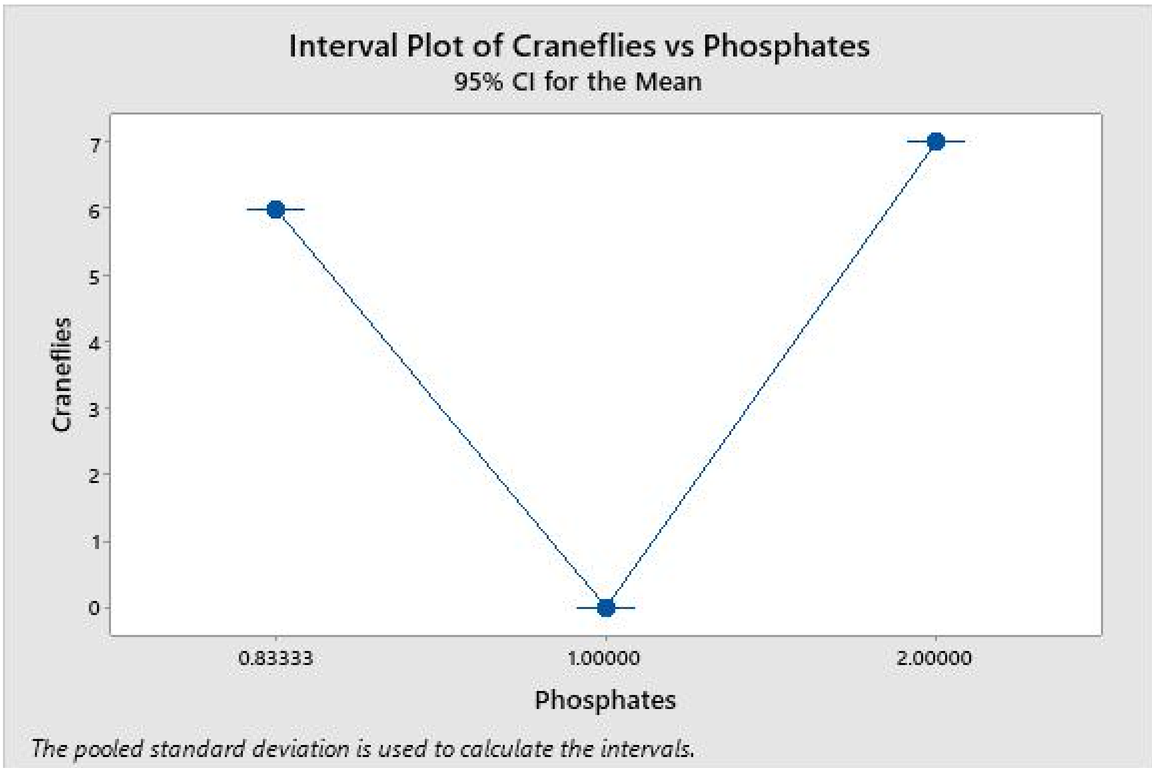


Chart 29: One-way ANOVA: Craneflies versus Phosphates

Factor Information

Factor	Levels	Values
Phosphates	3	0.83333, 1.00000, 2.00000

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Phosphates	2	28.67	14.33	*	*
Error	0	*	*		

Total	2	28.67

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
*	100.00%	*	*

Means

Phosphates	N	Mean	StDev	95% CI
0.83333	1	6.000	*	(*, *)
1.00000	1	0.000000	*	(*, *)
2.00000	1	7.000	*	(*, *)

*Pooled StDev = **

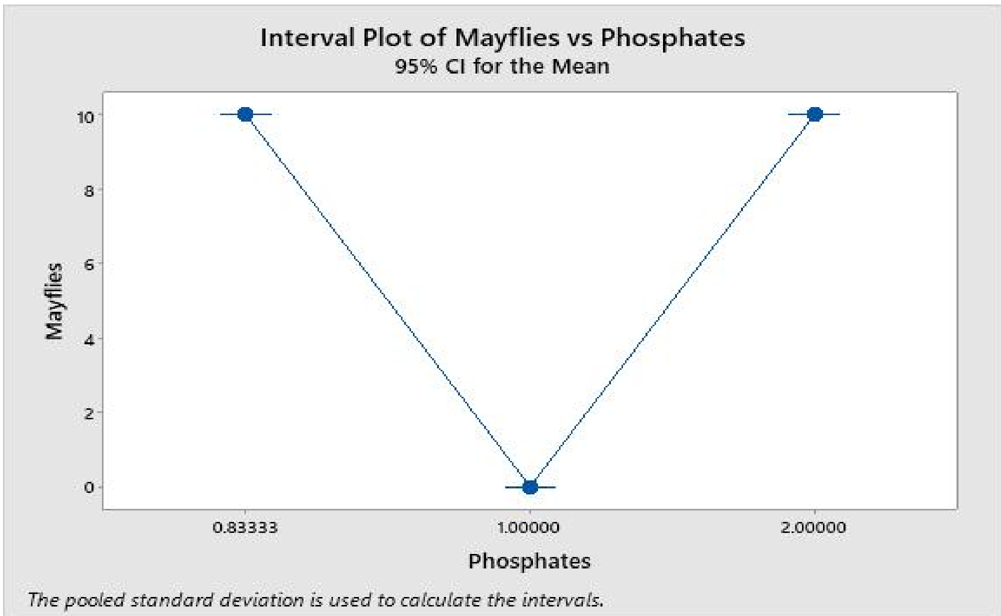


Chart 30: One-way ANOVA: Mayflies versus Phosphates

Factor Information

Factor	Levels	Values
Phosphates	3	0.83333, 1.00000, 2.00000

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Phosphates	2	66.67	33.33	*	*
Error	0	*	*		
Total	2	66.67			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
*	100.00%	*	*

Means

Phosphates	N	Mean	StDev	95% CI
0.83333	1	10.00	*	(*, *)
1.00000	1	0.000000	*	(*, *)
2.00000	1	10.00	*	(*, *)

*Pooled StDev = **

DISCUSSION

The average Biotic Index value on site 1 is 69.5 which shows that the water quality is good while on site 2 has an average of 98.2 and has an excellent water quality. On site 3, the biotic index has an average of 73, which indicates it has a good water quality on Undercut Banks. The water qualities in each site are on the higher scale of the Hilsenhoff spectrum. The biotic index values were significantly different because the macroinvertebrates have different tolerances to pollution and nutrient levels, and different dissolved oxygen needs. It was found out that different species were found in each site i.e.; in stagnant waters, macroinvertebrates such as stoneflies, blackflies, Dragonflies, fishflies, frogs and water striders because it is easier for them to live in a habitat that there's only a little movement on the water unlike in riffle where there are more frogs, mayflies and craneflies present because of their gills and sucker like characteristic which help them cling on to rocks in rough waters.

There are some unexpected results that we didn't anticipate such as recording and collecting data from water sample and getting no ppm for nitrates it is maybe due to a fact that the site is a river and is constantly moving downstream. The purpose of this study is to analyze whether there is a direct effect of Nitrates and Phosphates on freshwater macroinvertebrates, in doing so, we also found out the water quality using the biotic factors that are living in that area. The biotic index scale ranges from one to ten; 1 to 3.75 indicates an excellent water quality; 3.76 to 5 is a good water quality; 5.1 to 6.5 is a fair water quality; and 6.6 to 10 is a poor water quality.

CONCLUSION

Our Null Hypothesis is that the levels oh Phosphates and Nitrates detected and has no relationship or any subsequent effects on the diversity of macroinvertebrates and the quality of water while our Alternative Hypothesis is that the levels of Phosphates and Nitrates detected has any relationship or relativity on the diversity of macroinvertebrates and the quality of water directly or inversely. The team rejected our Null Hypothesis for as shown in the results above, although no there are 0 parts per million of Nitrates in each site, but the Phosphates collected varies depending on the site, does have an effect on the diversity of the freshwater macroinvertebrates. The alternative hypothesis was accepted because the results shown there is a dependent relationship on the biodiversity of macroinvertebrates to the levels of Phosphates in each site.

In doing this study, one must also take note of Photosynthesis wherein it is one way for dissolved oxygen to enter the water. The dissolved Oxygen may have change between the course of time and depending on the temperature too. Having a high temperature will most likely to increase the amount of dissolved oxygen on the water.

The suggestions we can give to further improve the study is to make sure to write or take down notes on the different macroinvertebrate or even better, you could also take a pic and identify and after collecting the samples. Not only will it save you time but it will also be much easier tracking the macroinvertebrates you found, including the ones flying for you can't really catch them easily. Another suggestion is to bring 3 buckets or container and collect 3 samples in one go. In that way, you don't have to go back and forth every after the testing for Nitrates and Phosphates.

Different questions formulated as we were conducting the experiment and collecting data such as, why are there no nitrates found in each site? Does weather affect the amount of dissolved oxygen on the water affecting the amount of phosphates and nitrates on the water? Is it possible for the location to have another species if its not for the amount of phosphates of the water? Would we have collected different species of Macroinvertebrate if we had collected the data at night or in a different season? Would the levels of Nitrates and Phosphates be different if we conducted the study at night? Those questions piqued my interests to further continue this study and get different data in doing so. In conclusion, the lower the levels of Phosphate, the more macroinvertebrates present in that area. The type of habitat also greatly contributed on the type of macroinvertebrates that lives in that area and clearly, this study on freshwater macroinvertebrate will serve to be helpful to researchers and scientist in this field of study.