The Effects of Water Quality on Macroinvertebrates

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Abstract

This experiment focused on the question; to what extent does nitrate, pH, temperature, and dissolved oxygen affect the biodiversity of macroinvertebrates? The hypothesis states that if over time, dissolved oxygen levels are high, nitrate is less than 1 ppm, pH levels are neutral, and water temperature is between 14 degrees Celsius and 20 degrees Celsius, then the population of macroinvertebrates will be more diverse because these conditions promote more food sources and varied habitats for the most macroinvertebrate species. The independent variable is time. The dependent variables measured are nitrate in parts per million, pH, water temperature in Celsius, dissolved oxygen in parts per million, and the biodiversity of macroinvertebrates. The controls for this experiment are the time of observation, the study site, the testing equipment, and the GLOBE protocols. This experiment was conducted by using LaMotte Dissolved Oxygen and Nitrate kits, a Jellas pH meter, and an alcohol filled thermometer to measure water quality parameters and collecting macroinvertebrates according to GLOBE Protocols. The data partially supported the hypothesis. This is because the data showed moderately high dissolved oxygen, pH close to neutral, and water temperature closer to 20 degrees Celsius promoted more biodiversity. It seemed these levels were more optimal for the most amount of classifications of macroinvertebrates, including pollution sensitive, moderately sensitive, and pollution tolerant. If this experiment would be continued, there would be more data collected after precipitation events.

Keywords: water quality, nitrate, dissolved oxygen, pH, water temperature, macroinvertebrates, biodiversity

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Research Question and Hypothesis

This experiment is designed to find how nitrate, pH, water temperature, and dissolved oxygen contribute to the biodiversity of macroinvertebrates. The research question is; to what extent does nitrate, pH, temperature, and dissolved oxygen affect the biodiversity of macroinvertebrates? The independent variable is time. The dependent variables measured are nitrate in parts per million, pH, water temperature in Celsius, dissolved oxygen in parts per million, and the biodiversity of macroinvertebrates. The controls for this experiment are the time of observation, the study site, the testing equipment, and the GLOBE protocols. The hypothesis states if over time, dissolved oxygen levels are high, nitrate is less than 1 ppm, pH levels are neutral, and temperature is between 14 degrees Celsius and 20 degrees Celsius then the population of macroinvertebrates will be more diverse because these conditions promote more food sources and varied habitats for the most macroinvertebrate species.

Introduction

Freshwater biomes, including lakes, rivers, and streams, are low in salt and can be seasonal or permanent bodies of water. The mineral composition and water chemistry can depend on the surrounding environment and soils the water has passed through. The upper parts of a river are usually fast-flowing and narrow. This is also where riffles and runs are common. Riffles are fast-flowing, shallow areas with tumultuous waters running over rocks. Runs are similar, but are deeper. A glide is similar to a run, but has no surface turbulence. The middle is wide and slower. The lower end is the slowest flowing part of the river. The cloudiness, the

strength of the current, oxygen levels, nutrient content, and temperature depend on the volume of water and the land cover the river runs through (Morgan, 1995). Toms Creek, the study site for this experiment, is classified as a HQ-CWF, MF stream and then it transitions into a CWF, MF classified stream when it reaches Carroll Valley Park. HQ means that the water has a high water quality. CWF means that it supports cold water fish. MF means that it also supports migratory fishes (Hallinan, 2018).

Macroinvertebrates are water dwelling animals that have no backbone. Different types of macroinvertebrates are sensitive to different water quality levels. Pollution sensitive macroinvertebrates include the stonefly, caddisfly, mayfly, and the water penny. Moderately sensitive macroinvertebrates include the cranefly, crayfish, damselfly, and dragonfly. A non-pollution sensitive macroinvertebrate is the midge. Macroinvertebrates are indicators of water quality conditions over long periods of time (Arlington County, 2018). Many species spend their premature years in water and then leave and live on land. At the immature stages of macroinvertebrates, increased dissolved oxygen is necessary for them to survive. Depending on the season, different macroinvertebrates will be present depending on their food supply. The macroinvertebrates who eat algae will be more abundant in the summer because algae is more copious in the summer. The amount of sediment and the substrate on the bottom will also change the species of macroinvertebrates present. Species who eat small food particles will prefer muddier substrates. Runoff and industrial pollutants can lower pH and this can debilitate the macroinvertebrates' shells and exoskeletons (Utah State University, 2018).

Varied macroinvertebrates have different life cycles. There is complete metamorphosis, which has four stages of life including egg, larva, pupa, and winged adult. The second life cycle

is incomplete metamorphosis, which has three stages of life including egg, larva, and flying adult (Hamilton Harbor, 2014). Macroinvertebrates adapt to the environment they live in. The species that live in the rifles of a stream will stick to surrounding rocks. Those that live in the glides most likely have a flat shape to avoid being swept downstream. Macroinvertebrates who live in slow moving water will burrow in the substrate or develop shells to protect themselves from predators. Some species have gills to absorb dissolved oxygen while others go to the surface to breathe oxygen from the atmosphere (Hamilton Harbor, 2014).

The diet of each species also determines where its habitat is located. Collectors include the caddisfly and mayfly who gather food or construct nets to catch food. Collectors eat dissolved organics, algae, bacteria, feces, and plants. They live on the bottom of the stream. Shredders include the caddisfly, mayfly, and stonefly who use their mouth attachments to shred, bore, cut, and bite to find and consume food. They eat leaves and vegetation that have fallen into the water. Shredders live in areas with extended tree canopy. Scrapers include the caddisfly and the mayfly who use razor like mouth attachments to scrape surfaces for algae. They live in lighter areas of the stream where algae grows. Predators include stoneflies, beetles, dragonflies, and alderflies whose bodies are designed to catch, kill, and eat live organisms. They will live in any habitat (Hamilton Harbor, 2014). Narrower streams with denser riparian vegetation are more likely to have shredders and collectors because of the lack of light and habitat. Other types of macroinvertebrates will be present, but not in such copious amounts. The wider and sunnier the stream environment, the more scrapers and collectors there will be (National Park Service, 2018).

Macroinvertebrates can be indicators of pollution and the effectiveness of pollution control endeavors. They can also show trends in water quality over extended periods of time as well as specify areas of concern in streams and rivers (Enviroscience, 2018). Macroinvertebrates indicate long term water quality while the more prevalent chemical water testing gives only a quick snapshot of the water quality and may not indicate a problem if the chemical testing is done before or long after a problematic event (CBF, 2018). Macroinvertebrates are essential to the ecosystem as some consume algae and organic matter and decrease nutrients in the water (Hepp, Milesi, Biasi and Restello, 2018). Water temperature appears to be a major component in discerning how species of macroinvertebrates develop, affecting the length of egg incubation and hatching in species of mayflies and stoneflies, as well as growth through the larval stages (National Park Service, 2018).

Human habitation has large impacts on macroinvertebrates. When vegetation in and around bodies of water is damaged or cleared, this removes necessary food sources, breeding grounds, and shade. This will lead to a rise in water temperature altering the environment for macroinvertebrates who are sensitive to this change. Dams and irrigation reduce water flow which causes a buildup of sediment, modifying the substrate (Sayre, 1996). Excess nutrients from runoff from farms and businesses cause a change in the plants around and present in the water because of the alteration of water chemistry. If the different water chemistry is not compatible to the vegetation, the plants may die. When this occurs, the decomposition process of the plants soak up extra dissolved oxygen and the process of eutrophication will start. Eutrophication is when an excess of nutrients causes algae to grow and decrease the light coming into the water, which kills the plants in the water, depletes the dissolved oxygen levels, and can

lead to death of macroinvertebrates. Eutrophication causes a lack of habitat, food, and oxygen production. This is a common problem in the Chesapeake Bay, the watershed in which Toms Creek is located. Spills and run-off from businesses and residential areas also change the water chemistry. Water chemistry can change quickly, and reflect a spill or weather event, but unless the measurements are taken right after the occurrence, it may be difficult to detect the change.

Macroinvertebrates are good indicators of water quality because they live in one aquatic habitat. Macroinvertebrates are valid bioindicators of stream health because they spend up to one year or more in a stream, have little maneuverability, are an abundant, primary food source for many fish, and are good indicators of local conditions. The more diverse the population of macroinvertebrates, the healthier the stream. A healthy stream can support diversity in macroinvertebrates because more food sources will be present, the habitats will be more varied, and more macroinvertebrates can live in fair and good conditions rather than poor conditions. If macroinvertebrates are monitored consistently, it will be easy to detect a change in the water quality, reflected by the number, type, and diversity of the macroinvertebrates collected (Science Learning Hub, 2018).

Orders of macroinvertebrates include the Ephemeroptera (Mayfly), Plecoptera (Stonefly), Trichoptera (Caddisfly), Megaloptera (Dobsonfly / Hellgrammite), Coleoptera (Aquatic Beetles), Diptera (True Flies), Odonata (Dragonfly and Damselfly), Pelecypoda (Clams), Gastropoda (Snails), and Hemiptera (True Bugs) (World Book Inc., 2011). Trichoptera have very short antennae, three pairs of legs each with one tarsal claw, and one pair of prolegs. They make cases to protect themselves using wood, sand grains, gravel, and other organic particles. Megaloptera have large mandibles, can be 2-10 cm long, and have 7-8 lateral filaments. Ephemeroptera have

gills on about all seven abdominal segments and three tails. Plecoptera have two tarsal claws, can be 1-3 cm in length, and two long filamentous tails. Coleoptera have shell like wings and chewing mouthparts. Odonata have distinctive antennae, large eyes, 1-5 cm length, extendible lower jaw, short filamentous antennae, large compound eyes, and an elongated hinged mouth. Diptera can be 5-8 cm in length. Pelecypoda have bivalve shells. Hemiptera have wings hardened near the base and membranous everywhere else and tube-like sucking mouthparts (Western U.P. Center for Science, Mathematics, and Environmental Education, 2018). The macroinvertebrate population has been in decline because of human habitation and climate change (Hepp, et. al., 2018).

Water temperature is vital to the health of streams and the aquatic life that lives in these bodies of water. Water temperature is important to biodiversity because different organisms thrive in different temperatures. Temperature affects other aspects of water chemistry, so it is referred to as the master variable. Higher temperatures can reduce the dissolved oxygen in water, which can damage biodiversity in aquatic species. Consistently warm temperatures can cause an excess of nutrients. It can also negatively affect circulation of the water which can alter nutrient and salinity levels. Temperatures naturally change at different points in the length of a stream depending on the source of water, geographical location, and other factors. Some specific factors include melted snow, a recent rainstorm, groundwater, the amount of water in the stream, air temperature, and vegetation. The Chesapeake Bay is the largest estuary in the United States and an important habitat for innumerable aquatic species. Rising air temperatures are causing stream temperatures to rise. Warmer stream water coming into the Bay can damage plants and animals as well as intensify the effects of nutrient pollution the Bay is currently struggling with.

Since 1960, the water temperature overall has increased within the Chesapeake Bay region (EPA, 2016).

pH is the measure of acidity or basicness of water. pH has a range of 0-14 with 7 indicating a neutral pH. A pH lower than 7 is acidic and above 7 is basic. pH measures the amount of hydrogen and hydroxyl ions present. Water containing more hydrogen ions is considered acidic. Water containing more hydroxyl ions present is considered basic. pH is measured in logarithmic units. Every number denotes a 10 fold change in the acidity or basicness in the water. The pH of water dictates the solubility and biological availability of chemical components such as nutrients and heavy metals. Normal rainfall has a pH of about 5.6 which is moderately acidic in part to carbon dioxide gas from the atmosphere. Pollution can change water's pH, which in turn can harm animals and vegetation existing in the water (USGS, 2018).

Excess nitrate can cause negative effects on these plants and animals (EPA, 2016). Nitrates (NO_3) are an essential source of nitrogen (N) for plants. When nitrogen fertilizers are used to enrich soils, nitrates may be carried by rain, irrigation and other surface waters through the soil into groundwater. When it flows into the nearest body of water, it can decrease the amount of dissolved oxygen in the water due to eventual eutrophication. Eutrophication is the process in which nutrients are excessive, usually from runoff, resulting in too much algae. The layer of algae on top of the water blocks sunlight from getting to the bottom of the body of water, which can harm some bottom dwelling plants and animals. Nitrate is one of the key nutrients involved in this process. Most of the nutrients come from runoff and fertilizers are caught up in the runoff (EPA, 2016).

Dissolved oxygen is the amount of gaseous oxygen in water. Dissolved oxygen (DO) may vary from 0 ppm to 18 ppm. Readings above 18 ppm are physically impossible. Readings lower than 5 ppm are concerning. Readings at or below 3 ppm create an environment that most aquatic organisms cannot survive in. Low dissolved oxygen readings indicate pollution or the environment is stressed (GLOBE, 2015). A reason for decreased DO may be fertilizer runoff from farm fields and lawns. The fertilizer which was meant to make land plants grow better now makes the aquatic plants do the same. If the weather becomes cloudy for many days, the plants will consume much of the DO. When the increased numbers of aquatic plants eventually die, they support tremendous amounts of bacteria which also consume large amounts of DO as the bacteria decomposes the dead plants (EPA, 2016). Temperature has an inverse effect on dissolved oxygen. Warmer temperatures cause lower dissolved oxygen levels which could be harmful to aquatic life. Cold water can sustain more dissolved oxygen. Oxygen can enter water through groundwater and the atmosphere (USGS, 2018).

The percent EPT and biotic index are ways to monitor for pollution sensitivity relating to macroinvertebrates and are quantitative ways to monitor stream health. The percent EPT is short for the number of Ephemeroptera (mayflies), Plecoptera (stoneflies), and Trichoptera (caddisflies). These taxa are very sensitive to pollution so they are preferable indicators for quality. The more percent EPT taxa indicates better water quality. The biotic index is a comparison of the abundance of taxa and their tolerance to environmental stress that can indicate organic and nutrient pollution. The lower the biotic index, the better the water quality (Leaf Pack Network, 2017). EASI is a set of procedures from the Senior Environmental Corps. EASI utilizes another way to determine water quality through the number of macroinvertebrates called the

water quality score. The number of macroinvertebrates in a group of pollution sensitivity is multiplied by the weighing factor for the number group of macroinvertebrates. This will provide a number to add to the rest of the pollution groups after the previous calculations have been done for each group (Belt, 2018)

This project is important to environmental groups, hydrologists, and environmental scientists because the outcomes of this experiment because it will affect their research. The Borough of Carroll Valley will care because the testing site is within the Carroll Valley Park so the outcomes will help them determine if they need to intervene in the local water quality. Local businesses such as Ski Liberty and several nearby golf courses because they are both outdoor environments that are involved with Toms Creek, possibly having an impact on the quality of Toms Creek. The study of macroinvertebrates has become increasingly important within the past few years because of increased commercialization of land in the area as well as in the whole country. This is important because bodies of water in the local area are part of the Chesapeake Bay watershed. If problems are solved in smaller regions, it will help increase the health of the Chesapeake Bay. This is also important because scientists need to know how chemical parameters affect the biological aspects of a body of water to create ideal environments and properly address and solve problems. This experiment will also help scientists distinguish what macroinvertebrates are more likely to live in different chemical conditions.

Previous research related to this experiment includes studies performed by NOAA on the population of macroinvertebrates in the Great Lakes. They found that substantial population changes were occuring as a result of decreased nutrient pollution and the introduction of invasive species (NOAA, 2018). NOAA has also conducted surveys on several regions on different

categories of macroinvertebrates including pollution tolerant, moderately sensitive, and sensitive and how they can carry contaminants through a river (NOAA, 2018). This data will benefit the local community as a whole because most homes in the area are supplied by well water. The health of streams will affect the groundwater that supplies these homes. The macroinvertebrates in local bodies of water are natural indicators of water chemistry. If one species of macroinvertebrates is prominent over a long period of time, after this research and continued studies, it is possible to pinpoint what the specific cause of this response is. This project applies to the real world because the data from this experiment will help the community of residents who use well water and is incredibly important to anglers and the ecotourism industry so economically important to this area. It will also supply information that applies to streams and bodies of water all over the world. This experiment more specifically will help scientists understand factors that can be targeted in the Chesapeake Bay depending on the health of its watershed.

Materials and Methods

- 1 0.254 mm mesh sifting tray
- 1 kick net
- Lamotte Dissolved Oxygen Kit
    - 30 mL of Manganous Sulfate Solution
    - 30 mL of Alkaline Potassium Iodide Azide
    - 30 mL of Sulfuric Acid
    - 60 mL of Sodium Thiosulfate
    - 30 mL of Starch Indicator Solution

    - 1 direct reading titrator
    - 1 25 mL test tube
    - 1 60 mL glass water sampling bottle
- Lamotte Nitrate Kit
    - 250 mL of Mixed Acid Reagent
    - 10g of Nitrate Reducing Reagent
    - 1 dispenser cap
    - 1 0.1g spoon
    - 2 5 mL test tubes
    - 2 10 mL test tubes
    - 1 60 mL water sampling bottle
    - 1 0.5 mL pipet
    - 1 Axial Reader
    - 1 distilled water ampoule
    - 1 Nitrate- Nitrogen Comparator 0-10 ppm
- Jellas pH Meter
- Alcohol filled thermometer
- 1 pair of goggles
- 1 box of gloves

Mapping the Study Site

As stated from GLOBE Protocols:

1. Select a section of the bank at least 50 meters long as the study area, if possible. The area should contain the sampling site where the water measurements are collected as well as a variety of habitats.
2. Use the measuring tape to measure a straight transect, at least 50 meters long, parallel to the shoreline, and within 10 meters of the bank. The transect will be varying distances from the water if the bank is not straight.
3. Place flags at the two ends and at every 2 meters along the transect.
4. Start drawing the map using the flags to help keep it to scale. Mark the transect and flag positions on the map.
5. Draw the waterline or bank by measuring from each flag directly to the water, placing a small dot on the map to show the waterline, then connect the dots with a dotted line to indicate the bank.
6. Put in the opposite bank or indicate the approximate distance to the opposite bank if known.
7. Use an arrow to indicate the direction of water flow or the inlet and outlet of the water body.
8. Create a key with symbols for special features found at the site. Use these symbols to indicate where special features are located on the map. Suggested features to include:
    a. Within the sampling area: riffle areas, pools, vegetated areas, logs, rocky areas, gravel bars, sand bars, bridges, docks, jetties, dams, etc.

Documenting the Study Site

As stated from GLOBE Protocols:

1. Fill in the information on the top of Site Definition Sheet.
2. Locate the Hydrosphere Study Site following the GPS Protocol Field Guide.
3. Record the name of the water body being sampled, using the name commonly used in maps. Record whether the water is saltwater or freshwater.
4. If the water site is moving water, record whether it is a stream, river, or other and its approximate width in meters.
5. Record whether the sample location is an outlet, bank, bridge, boat, inlet or pier.
6. Record whether the bottom is clear.
7. Record the material from which the bank or channel is made.
8. Record the type of bedrock, if known.
9. Record the manufacturer and model number for each chemical test kit being used if any.

Macroinvertebrates

As stated from GLOBE Protocols:

1. Locate the areas where the five samples will be collected on the map and in the water.
2. If collecting water chemistry measurements, do before collecting macroinvertebrates. Be careful not to disturb the areas where will be macroinvertebrates will be collected.
3. Fill a bucket with water from the site.
4. While holding the sieve over a second bucket, pour water through the sieve. Use the sieved water to fill (and refill as needed) the plastic squirt or spray bottles. Keep sieved water in the shade.
5. Rinse sieve downstream of the sampling sites.

6. Begin sampling in the area farthest downstream. Place the 1 x 1 meter quadrat on the bottom of the stream so that two sides are perpendicular to the water flow.
7. Hold the Kick-net vertically in the water column, perpendicular to the water flow. Press the Kick-net firmly against the bottom of the streambed lined up with the quadrat and one meter downstream of the quadrat. Water must not flow above or under the net.
8. Start working in the part of the quadrat farthest away from the net. Overturn and scrape the undersides of rocks and wood found in the quadrat. The rocks and wood may be placed outside the quadrat until the sample is collected. Place large crustaceans and mollusks directly in the bucket. If large organisms escape outside the quadrat, mentally note their identity and numbers to record on the Freshwater Macroinvertebrate Identification Data Sheet later.
9. After scraping rocks and wood, use the feet, hands, or a stick to disturb the stream bottom within the quadrat for exactly 3 minutes.
10. Lift the Kick-net from the water by moving the bottom of the frame forward in a scooping motion so that nothing escapes from the net.
11. Return to shore with net.
12. Place the net over the square of white fabric.
13. Carefully remove large organisms and large debris with hands or forceps and put them in a tray half filled with the sieved water from the site.
14. Lift the net and squirt water on the net to concentrate all organisms and small debris in one corner of the net.

15. Place the corner of the net with the sample into a bucket. Tip the net and squirt water to move all of the contents into the bucket.
16. Rinse the square of white fabric into the bucket to make sure that all the macroinvertebrates are in the sample.
17. Place the bucket in the shade until ready to sort, identify, and count organisms.
18. Repeat steps 6 -17 for the other two samples.
19. Use the Sorting, Identifying, and Counting Freshwater Macroinvertebrate Protocol Lab Guide to sort, identify and count the macroinvertebrates collected.

Sorting, Identifying, and Counting Freshwater Macroinvertebrates

As stated from GLOBE Protocols:

1. Fill out the top portion of the Freshwater Macroinvertebrates Identification Data Sheet.
2. Put on gloves.
3. Use a basting syringe or forceps to pick out large organisms from buckets. Put these organisms in a tray.
4. If there is rocks in the sample, take them out of the bucket and use the spray bottle to rinse the rocks over the sample bucket before discarding the rocks.
5. If the water in the buckets is clear, free of debris, and rather a small amount, pour sample on tray to sort. Go to step 13.
6. If there is a lot of water, sediments or debris, pour the samples through the sieves. Place the sieve with the finer mesh size below the other sieve. Hold the sieves inside the top of a clean bucket.

7. Gently and slowly pour the water from the bucket containing the organisms into the sieves. If a sieve is clogged, gently tap the bottom of the clogged sieve to allow water to escape.
8. Every so often, transfer and rinse the contents of the sieves into trays using a squirt bottle.
9. Rinse twigs over the sieves.
10. Put twigs in a tray with water. Examine twigs for macroinvertebrates.
11. Rinse the bucket several times with spray bottles and pour the water down the sieves.
12. Invert each sieve over a tray and squirt water on the back of the sieve to remove contents.
13. Use identification keys to identify individuals to the most detailed level possible (Phylum, Class, or Order required and Family, Genus, or Species if possible). Keep in mind that appendages like legs and antennae may be missing because they may have broken in the net or the sieves.
14. Use the vials to sort organisms into different taxa. If the taxon of an organism is not known, place in a separate vial to examine later under a dissecting scope or with the help of an expert.
15. If organisms are large and clinging to debris, use forceps to gently pull them free. If they are floating or swimming, use a basting syringe or an eyedropper to catch them.
16. To count the number of individuals in each taxon, isolate organisms a few at a time using forceps, an eye dropper, or a basting syringe and transfer them into another jar. Keep a tally on paper.
17. Count macroinvertebrates in each taxon up to 100 individuals. If there is more than 100 individuals in a taxon, three things are possible: 1. report >100, 2. continue counting, 3.

use the Freshwater Macroinvertebrate Subsampling Field Guide to estimate the total number of organisms of this taxon.

18. As the macroinvertebrates are counted, look closely at the individuals to make sure that there are no mistakes in identification. Report the total number of organisms found for each taxon on the Macroinvertebrates Identification Data Sheet. Include organisms that were counted at the site but could not be collected because they escaped.
19. Enter data into the GLOBE data entry site.

Dissolved Oxygen

As stated from GLOBE Protocols:

1. Fill in the top of the Hydrosphere Investigation Data Sheet.
2. Put on the gloves and goggles.
3. Rinse the sample bottle and hands with sample water three times.
4. Place the cap on the empty sample bottle.
5. Submerge the sample bottle in the sample water.
6. Remove the cap and let the bottle fill with water. Move the bottle gently or tap it to get rid of air bubbles.
7. Put the cap on the bottle while it is still under the water.
8. Remove the sample bottle from the water. Turn the bottle upside down to check for air bubbles. If there is air bubbles, discard this sample. Collect another sample.
9. Follow the directions in the dissolved oxygen kit to test the water sample.

    Instructions for LaMotte Dissolved Oxygen Test Kit

    a. Remove cap from the bottle.

b. Immediately add 8 drops of Manganous Sulfate Solution and add 8 drops of Alkaline Potassium Iodide Azide.
c. Cap the bottle and mix by inverting several times. A precipitate will form.
d. Allow the precipitate to settle below the shoulder of the bottle.
e. Add 8 drops of Sulfuric Acid.
f. Cap and gently invert the bottle to mix the contents until the precipitate and the reagent have totally dissolved. The solution will be clear yellow to orange if the sample contains dissolved oxygen.
g. Fill the titration tube to the 20 mL line with the fixed sample. Cap the tube.
h. Depress plunger of the Titrator.
i. Insert the Titrator into the plug in the top of the Sodium Thiosulfate titrating solution.
j. . Invert the bottle and slowly withdraw the plunger until the large ring on the plunger is opposite the zero line on the scale.
k. Turn the bottle upright and remove the Titrator.
l. Insert the tip of the Titrator into the opening of the titration tube cap.
m. Slowly depress the plunger to dispense the titrating solution until the yellow-brown color changes to a very pale yellow. Gently swirl the tube during the titration to mix the contents.
n. Carefully remove the Titrator and cap. Do not to disturb the Titrator plunger.
o. Add 8 drops of Starch Indicator Solution. The sample should turn blue.

p. Cap the titration tube. Insert the tip of the Titrator into the opening of the titration tube cap.

q. Continue titrating until the blue color disappears and the solution becomes colorless.

r. Read the test result directly from the scale where the large ring on the Titrator meets the Titrator barrel. Record as ppm Dissolved Oxygen. Each minor division on the Titrator scale equals 0.2 ppm.

10. Record the dissolved oxygen in water sample on the Data Sheet.
11. Calculate the average of the three measurements.
12. Each of the three measurements should be within 1 mg/L of the average. If one of the measurements is not within 1 mg/L of the average, find the average of the other two measurements. If both of these measurements are within 1 mg/L of the new average, record this average.
13. Discard all used chemicals into the waste container. Clean dissolved oxygen kit with distilled water.
14. Enter data into the GLOBE data entry site.

Nitrate

As stated from GLOBE Protocols:

1. Fill out the top portion of the Hydrosphere Investigation Data Sheet. In the Nitrate section fill in the kit manufacturer and model.
2. Put on gloves and goggles.

3. Follow the instructions in the kit to measure the nitrate nitrogen. Use the Low Range Test (0 – 1 mg/L) unless previous results indicate that the site typically has greater than 1 mg/L nitrate nitrogen. If using powdered reagents, use the surgical mask when opening these products. Use clock or watch to measure the time if the kit requires to shaking the sample.

    Instructions for LaMotte Nitrate Test Kit

    a. Fill the water sampling bottle with sample water.
    b. Fill one test tube to the lower line (5 mL) with sample water.
    c. Dilute to second line with Mixed Acid Reagent. Cap and mix.
    d. Wait 2 minutes.
    e. Use the 0.1 g spoon to add one level measure (avoid any excess)
    f. of Nitrate Reducing Reagent.
    g. The mixing procedure is extremely important. Cap tube. Invert tube slowly and completely 30 times in 1 minute to insure complete mixing.
    h. Wait 10 minutes.
    i. Insert test tube into Axial Reader (2071). Fill two test tubes to the 10 mL line with sample water. Place in Axial Reader. Match sample color to a color standard. Record as ppm Nitrate-Nitrogen.
    j. To convert to nitrate, multiply by 4.4. Record as ppm Nitrate.

4. Match the color of the treated sample water with a color in the test kit. Record the value as ppm nitrate-nitrogen for the matching color. Record all three nitrate-nitrogen values on the Data Sheet.

5. Calculate the average of the three measurements.
6. Check to see if each of the three measurements is within 0.1 ppm of the average (or within 1.0 ppm of the average if using the high range test). If they are, record the average on the Data Sheet. If they are not, read the color measurements again. Calculate a new average. If the measurements are still not within range discuss possible problems with the teacher.
7. Enter data in the GLOBE data entry site.

Water Temperature

As stated from GLOBE Protocols:

1. Fill out the top portion of the Hydrosphere Investigation Data Sheet.
2. Put on the gloves.
3. Slip the rubber band around the wrist so that the thermometer is not accidentally lost or dropped into the water.
4. Check the alcohol column on the thermometer to make sure there are no air bubbles trapped in the liquid. If the liquid line is separated, notify the teacher.
5. Put the bulb end of the thermometer into the sample water to a depth of 10 cm.
6. Leave the thermometer in the water for three minutes.
7. Read the temperature without removing the bulb of the thermometer from the water.
8. Let the thermometer stay in the water sample for one more minute.
9. Read the temperature again. If the temperature has not changed, go to Step 10. If the temperature has changed since the last reading, repeat Step 8 until the temperature stays the same.

10. Record the temperature on the Hydrosphere Investigation Data Sheet.
11. Calculate the average of the three measurements.
12. All temperatures should be within 1.0˚ C of the average. If they are not, repeat the measurement.
13. Enter data into GLOBE data entry site.

pH

As stated from GLOBE Protocols

1. Fill in the top portion of the Hydrosphere Investigation Data Sheet. Check pH meter as the instrument.
2. Put on the gloves.
3. Remove the cap from the meter that covers the electrode.
4. Rinse the electrode on the meter and the area around it with distilled water in the wash bottle. Blot the meter dry with a clean paper towel. Do not rub the electrode or touch it with the fingers.
5. Rinse the electrode with distilled water and blot dry again.
6. Calibrate the pH meter according to the manufacturer's directions.
7. Rinse a 100-mL beaker three times with sample water.
8. Pour 50 mL of sample water into the 100-mL beaker.
9. Put the electrode part of the meter into the water.
10. Stir once with meter. Do not let the meter touch the bottom or sides of the beaker. Wait for one minute. If the pH meter is still changing numbers, wait another minute.
11. Enter data into GLOBE data entry site.

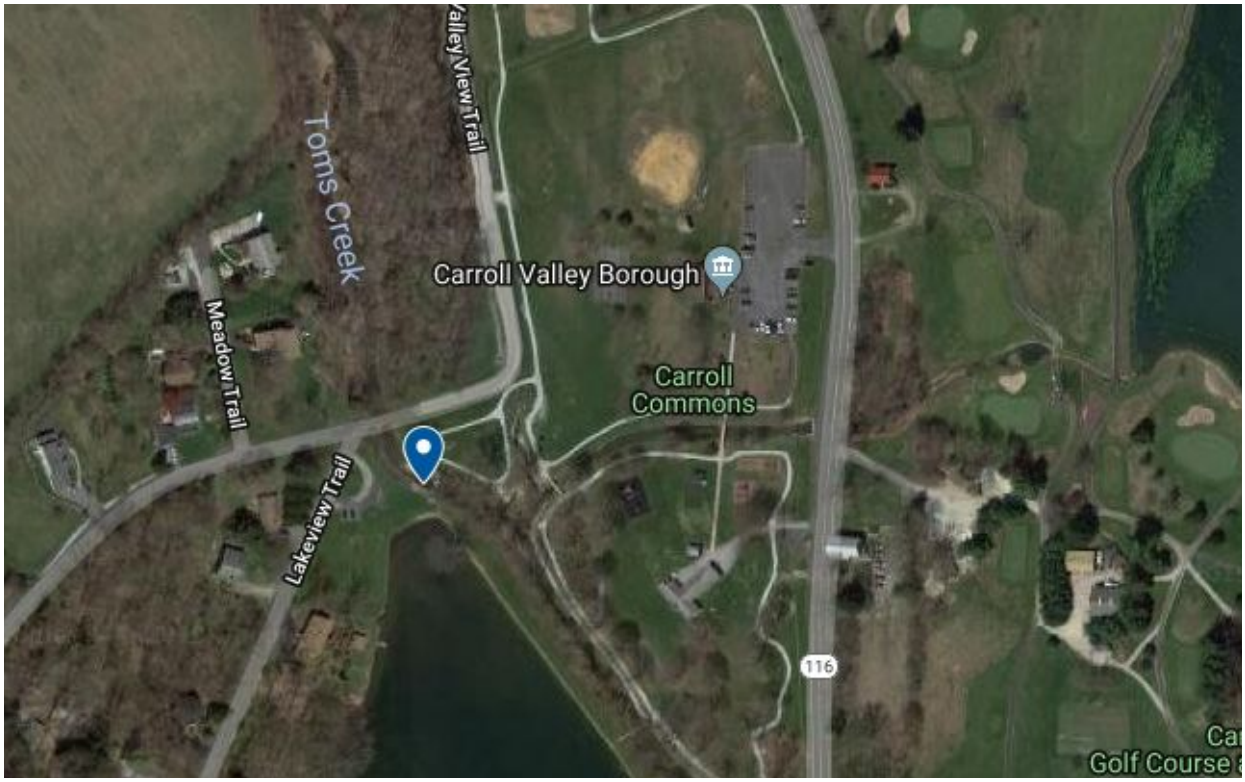
Take water chemistry measurements for three days a month with macroinvertebrate collection on the second day.

Calculating Percent EPT

1. Add the total number of mayflies, stoneflies, and other caddisflies.
2. Divide the number of EPT individuals by the total number of individuals in the samples.
3. Convert to percentage.

Calculating Biotic Index

1. Multiply the Total # of each taxon and the Pollution Tolerance Value which will equal the Total Tolerance Value.
2. Sum the Total Tolerance Value column.
3. Divide Total Tolerance Value by Total # of Individuals.
4. Enter in Leaf Pack Network calculator.



Map 1: This depicts a satellite image of the study site in Carroll Valley, PA, Tom's Creek.

Data Summary

Date	Dissolved Oxygen Averages (ppm)	pH Averages (logarithmic units)	Nitrate Averages (ppm)	Water Temperature Averages (Celsius)
9/22/18	5	5.2	0	19.5
9/23/18	5	5.3	0	19.3
9/24/18	5	5.3	0	19.4
10/13/18	7	7.7	0	18
10/14/18	11	8.1	0	13
10/15/18	9	7.9	0	15.0
11/25/18	10	4.4	0	9
11/26/18	10	4.2	0	9

11/27/18	10	4.4	0	10
12/19/18	8	3.4	0	5
12/20/18	9	3.3	0	5
12/21/18	9	3	0	5.0

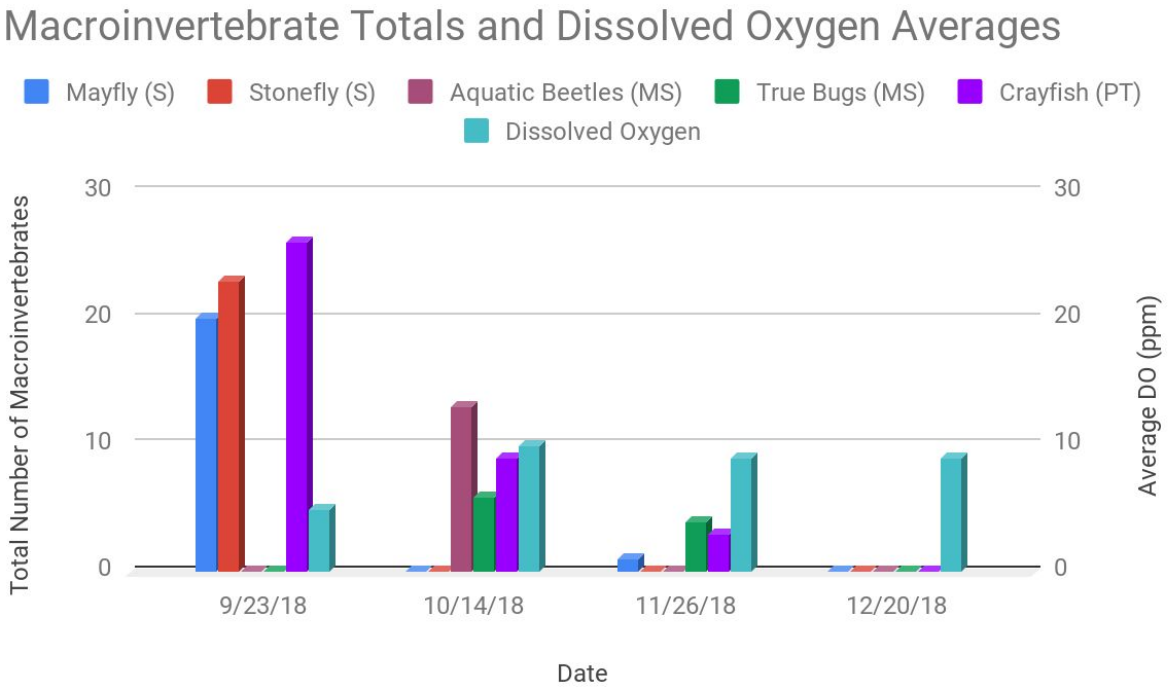
Table 1: This table shows the averages for the water chemistry data collected from September to December. Many precipitation events happened in the later months of 2018. A fewer of these interrupted testing but the more prominent events were in November, from 11/17-11/19, and again in December, from 12/14-12/16.

Date	Trial	Mayfly (S)	Stonefly (S)	Caddisfly (S)	Dobsonfly / Hellgramite (S)	Aquatic Beetles (MS)	True Bugs (MS)	Dragonfly and Damselfly (MS)	Crayfish (PT)
9/23/18	Total	20	23	0	0	0	0	0	26
10/14/18	Total	0	0	0	0	13	6	0	9
11/26/18	Total	1	0	0	0	0	4	0	3
12/20/18	Total	0	0	0	0	0	0	0	0

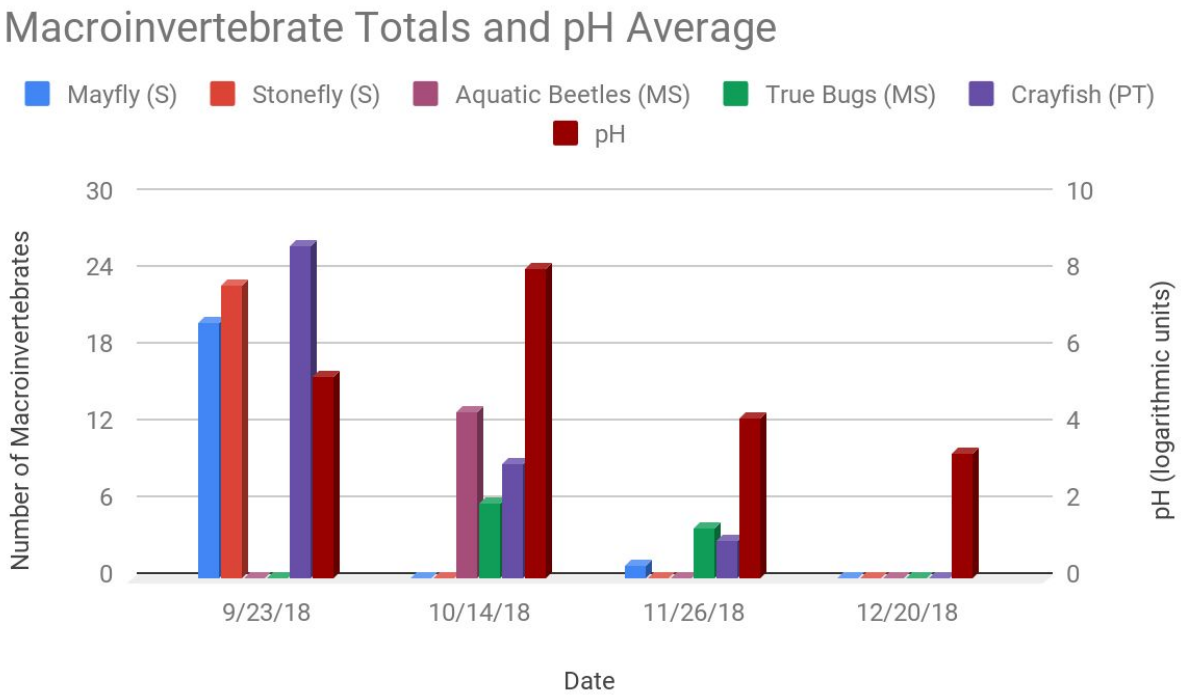
Table 2: This table shows all the macroinvertebrate data collected in September through December on the second day of each three day testing period.

Time of Year	Percent EPT	Biotic Index
Fall	62.3	3.26
Winter	14.3	4.8

Table 3: This table shows the drastic difference in the biotic index and percent EPT from fall to winter. This difference is not necessarily good or bad, because water quality parameters are expected to change drastically between these months and many species of macroinvertebrates cannot survive in such different conditions or their life cycle affects this as well.

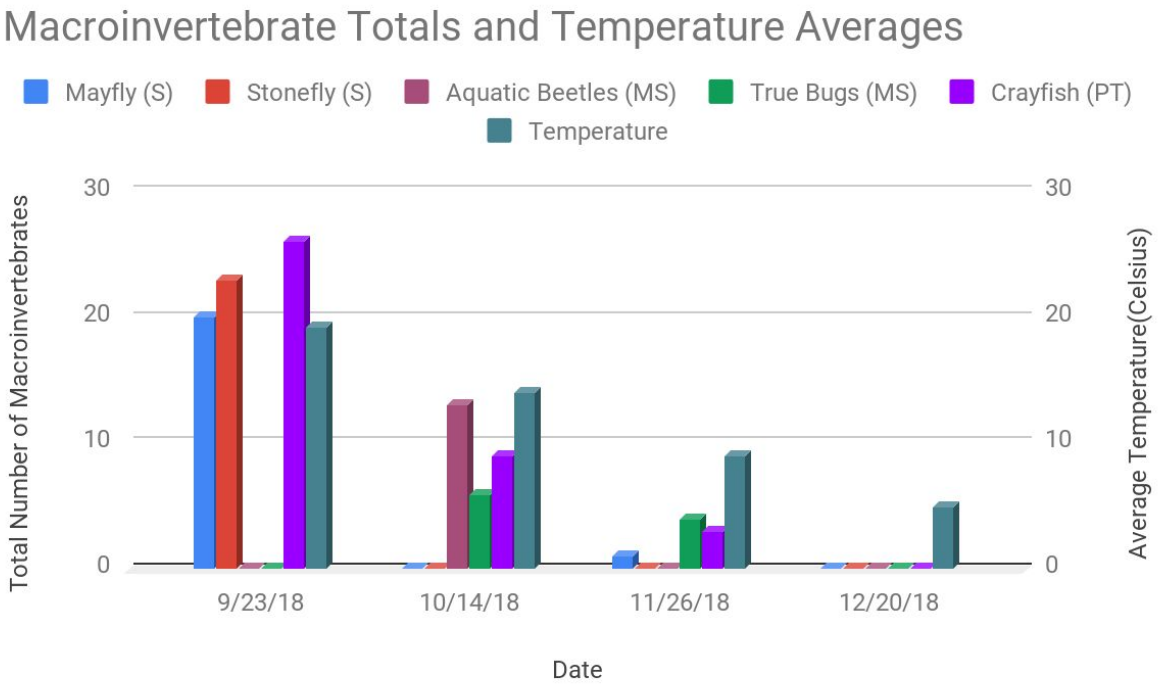


Graph 1: This graph shows the correlation between dissolved oxygen and the biodiversity of macroinvertebrates. This shows that the number of macroinvertebrates decreased as dissolved oxygen increased, as well as the taxa of macroinvertebrates changed. The cause of this could also be a result from decreased temperature as the seasons changed from fall to winter. The macroinvertebrates are categorized by the amount of pollution tolerance, (S) being sensitive, (MS) being moderately sensitive, and (PT) being pollution sensitive.

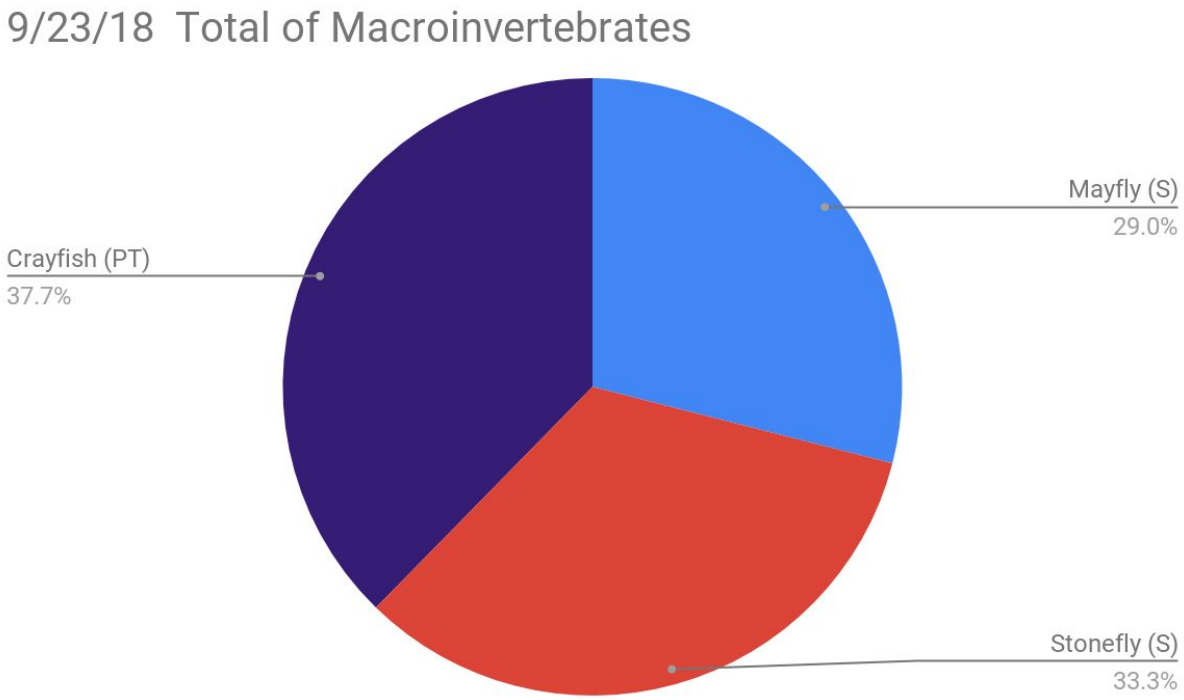


Graph 2: This graph shows the correlation between pH and the biodiversity of macroinvertebrates.

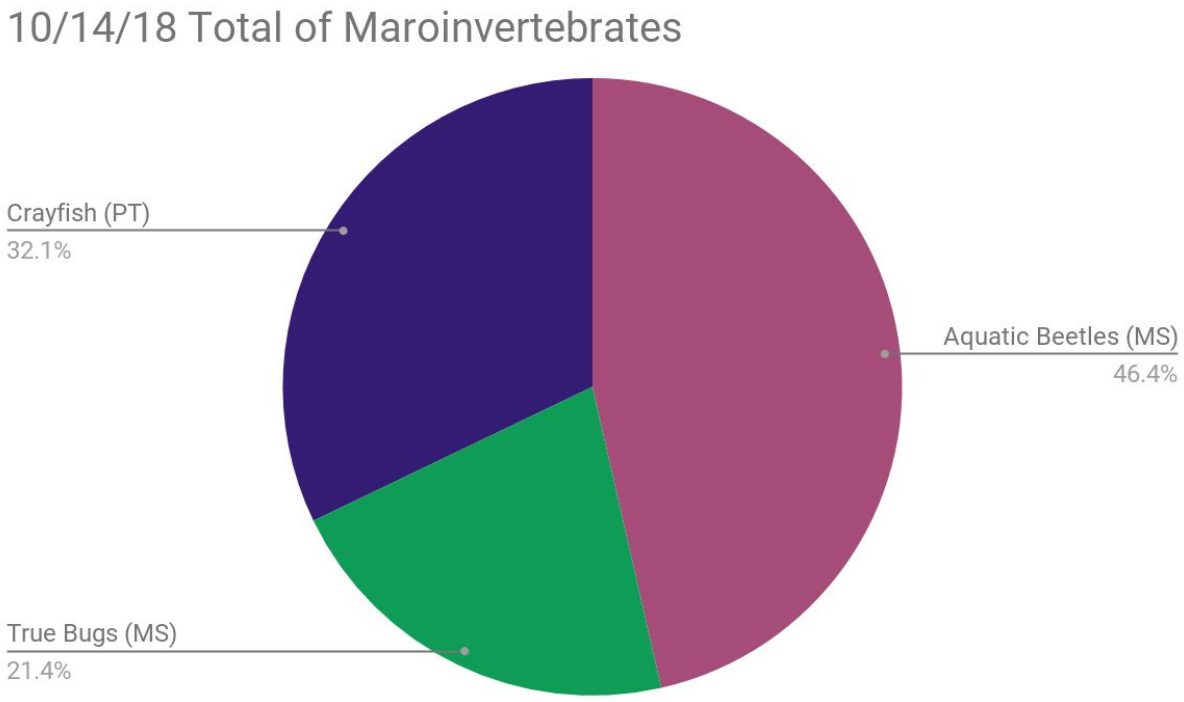
When the pH was closer to neutral, more macroinvertebrates were present. When the pH decreased, the taxa were mainly tolerant species or no species were present.



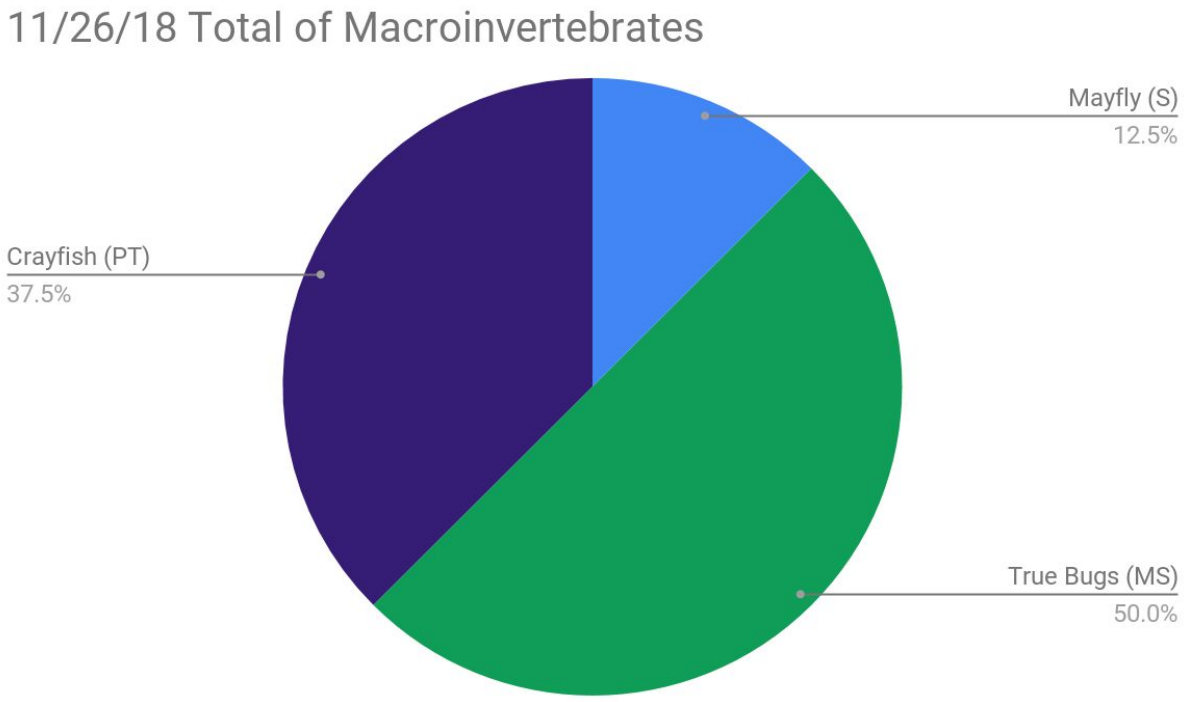
Graph 3: This graph shows the relationship between water temperature and the biodiversity of macroinvertebrates. As the temperature lessened, the number of macroinvertebrates lessened until no macroinvertebrates were found.



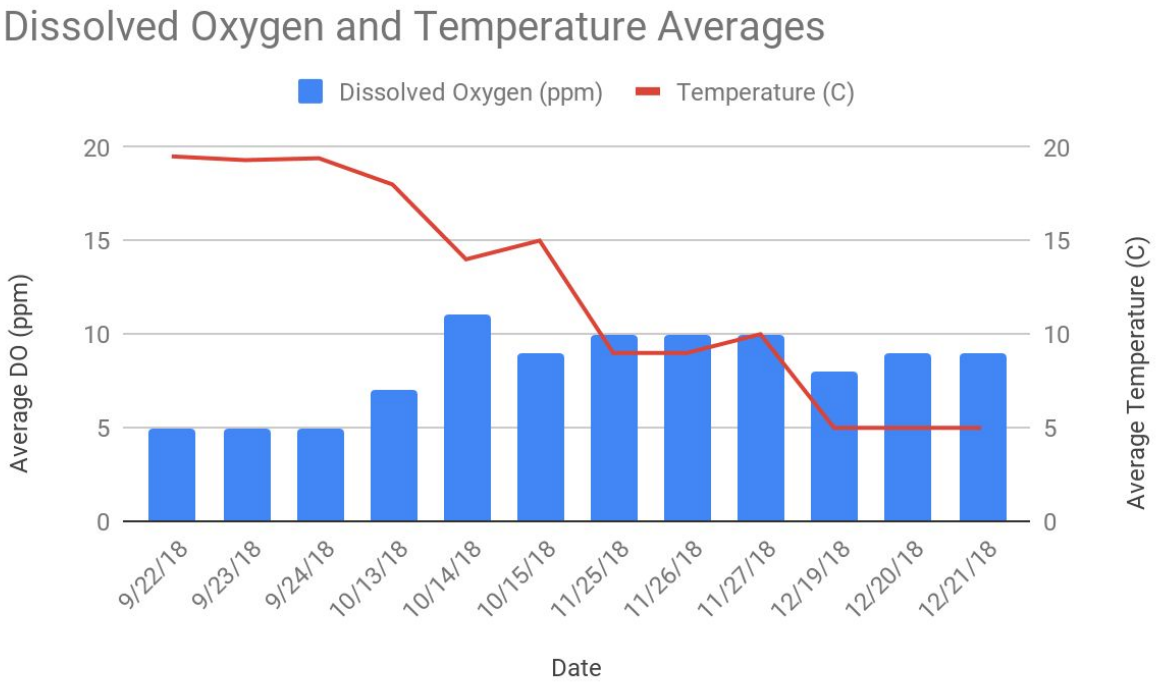
Graph 4: This pie chart shows the percentage of each taxa of macroinvertebrate found on September 23, 2018. The predominant type is crayfish.



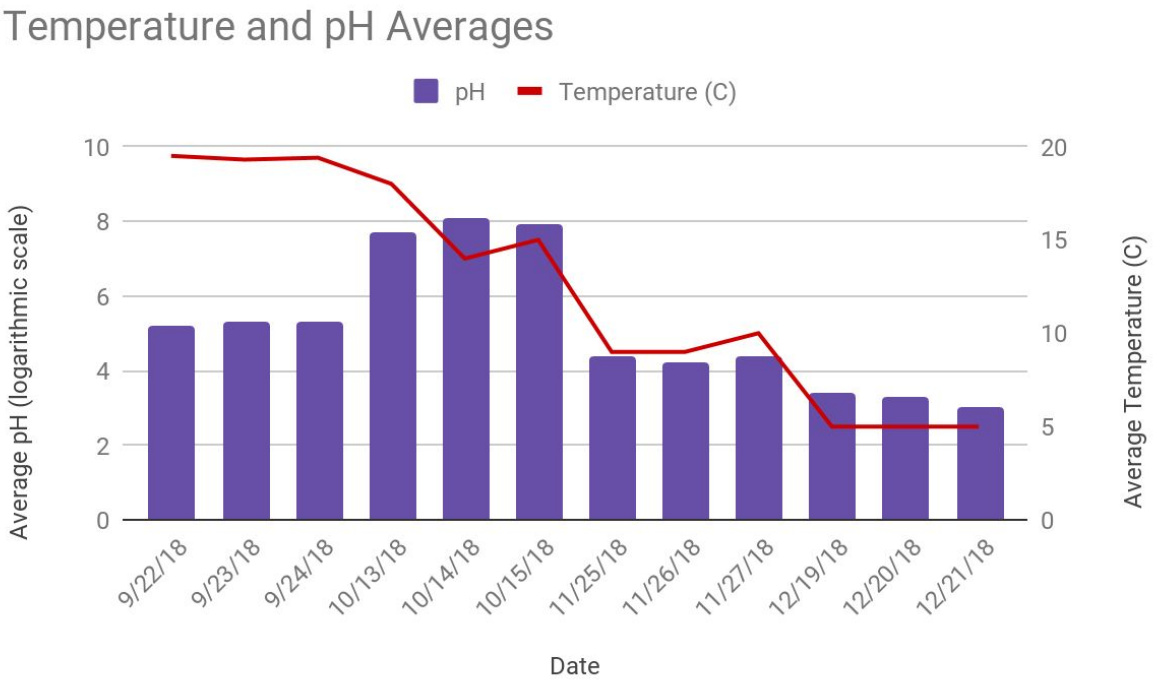
Graph 5: This pie chart shows the percentage of macroinvertebrates found on October 14, 2018. The more predominant taxa of macroinvertebrates was aquatic beetles.



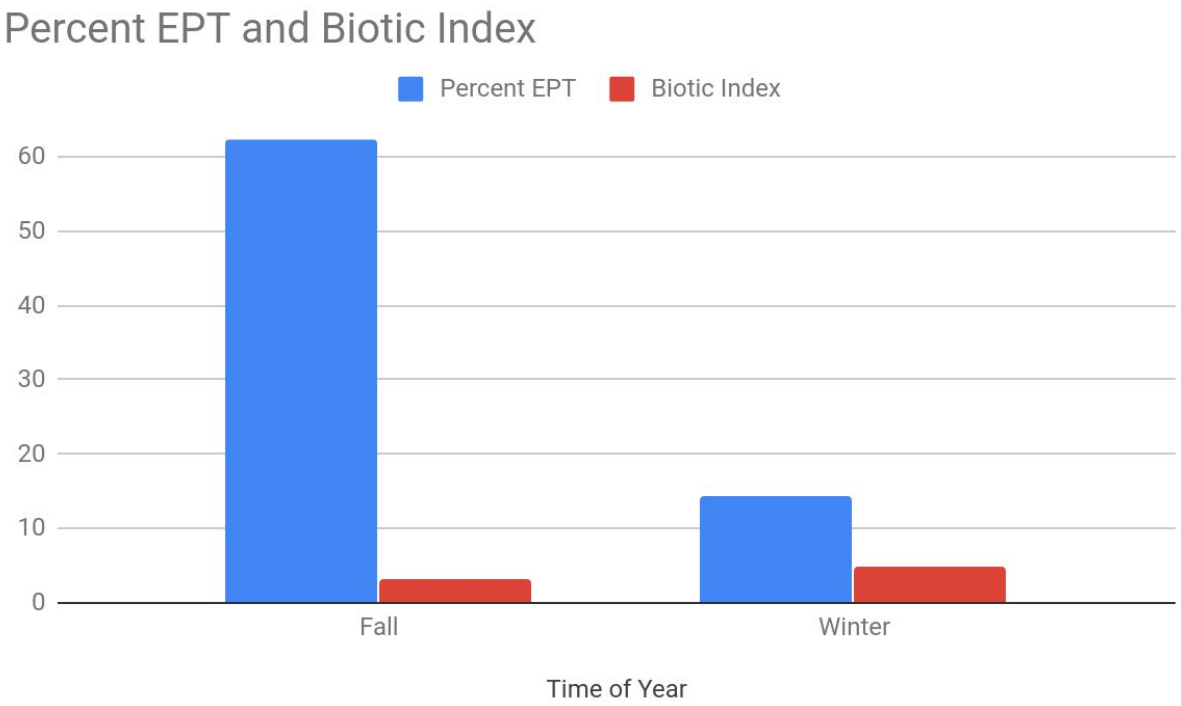
Graph 6:This pie chart shows the percentage of macroinvertebrates found on November 26, 2018. The more predominant taxa of macroinvertebrates was true bugs.



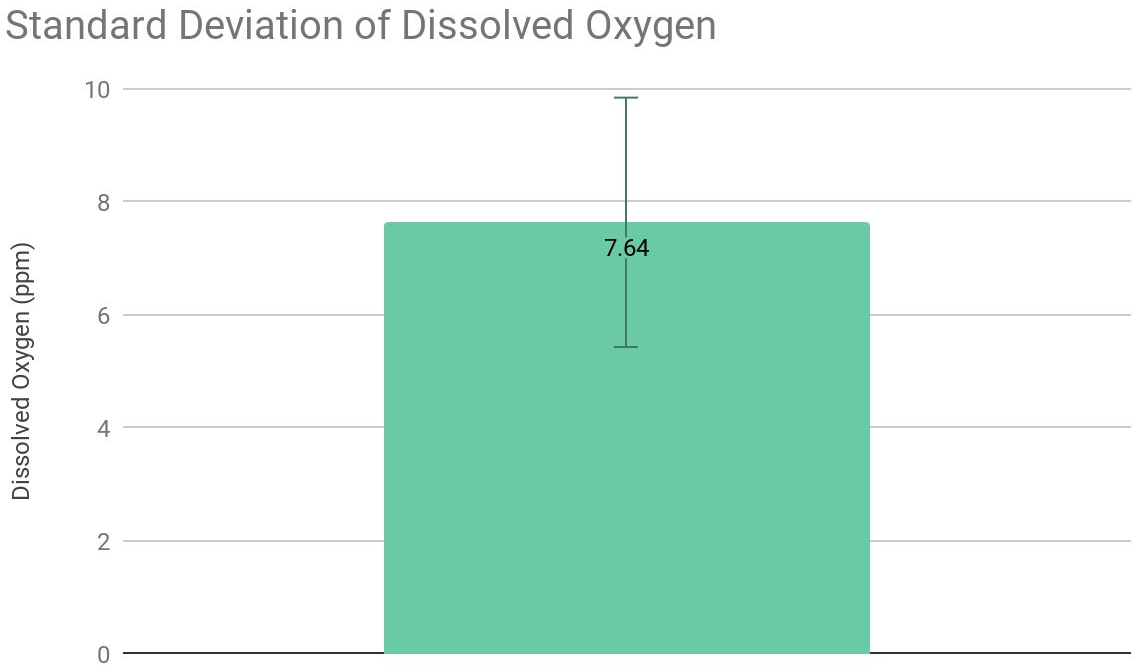
Graph 7: This graph shows the correlation between the dissolved oxygen and water temperature. Water temperature and dissolved oxygen have an inverse correlation, so as temperature increases, DO decreases.



Graph 8: This graph shows the correlation between pH and water temperature. pH decreases as the water temperature increases.

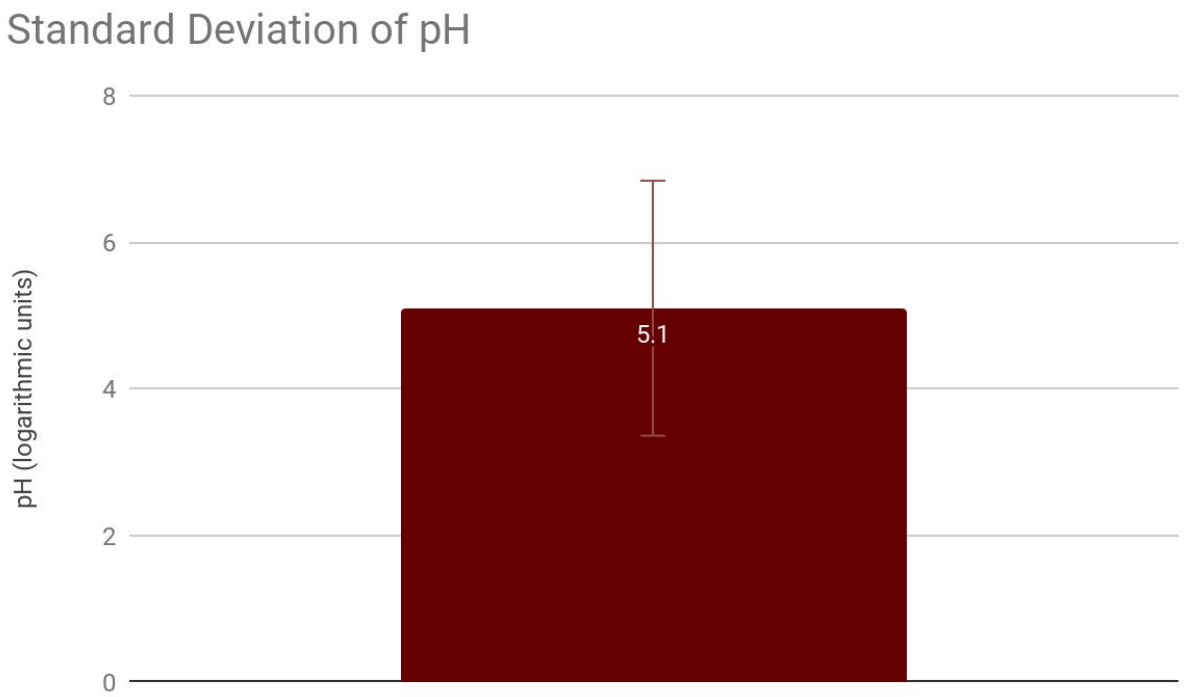


Graph 9: This graph shows the difference in percent EPT and biotic index from the fall to winter. The percent EPT was much higher in the fall than the winter because the amount of Ephemeroptera, Plecoptera, and Trichoptera drastically decreased. This is also what attributes to the higher biotic index. More tolerant taxa were present in the winter so the tolerance value is higher, which means that the quality of the water is lower than in the fall.

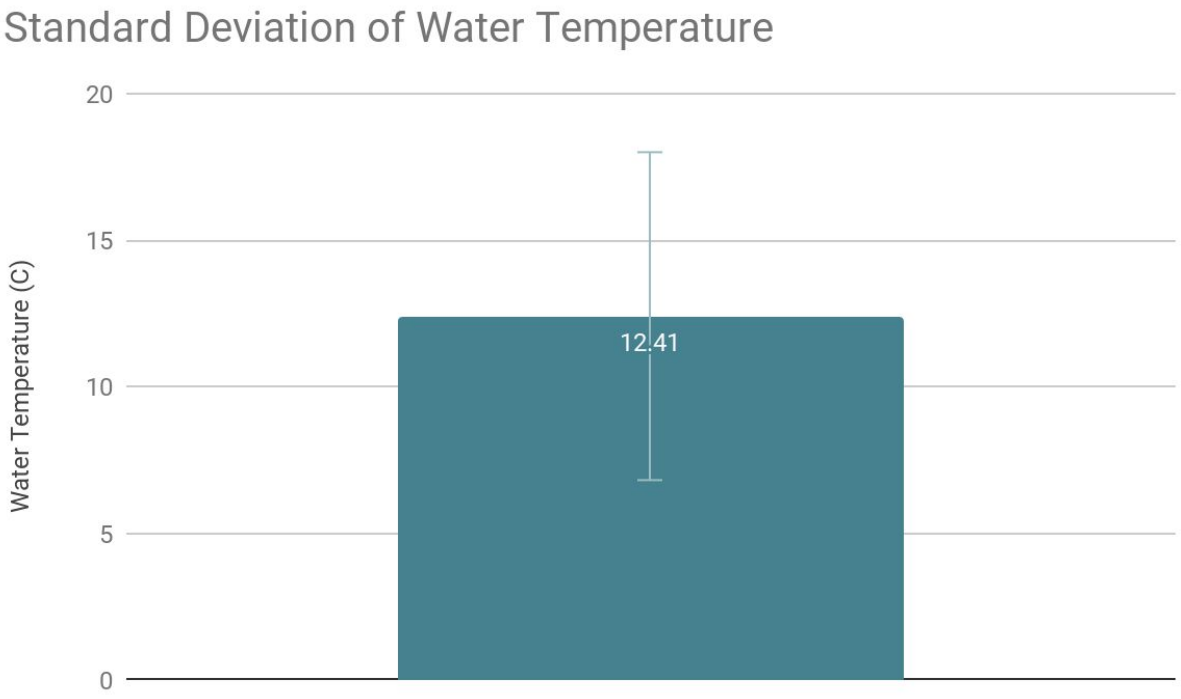


Graph 10: This graph shows the standard deviation of the data taken all four months. The standard deviation was 2.2. This means that the dissolved oxygen data could vary 2.20 units away from the average, meaning it could be 5.44 or 9.84. The recommended for dissolved

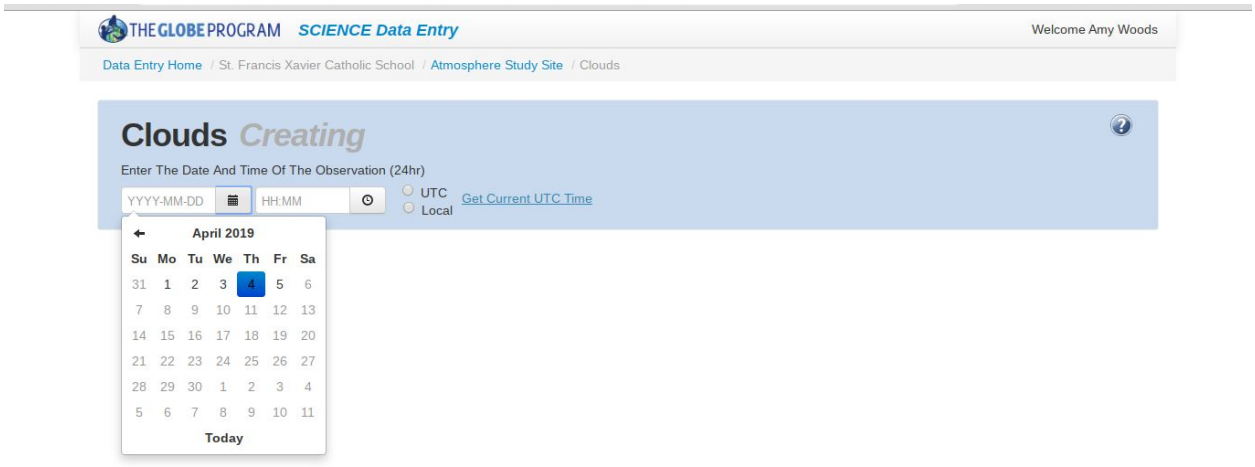
oxygen is around 5 ppm. Most of the data falls within this range except the higher deviation.



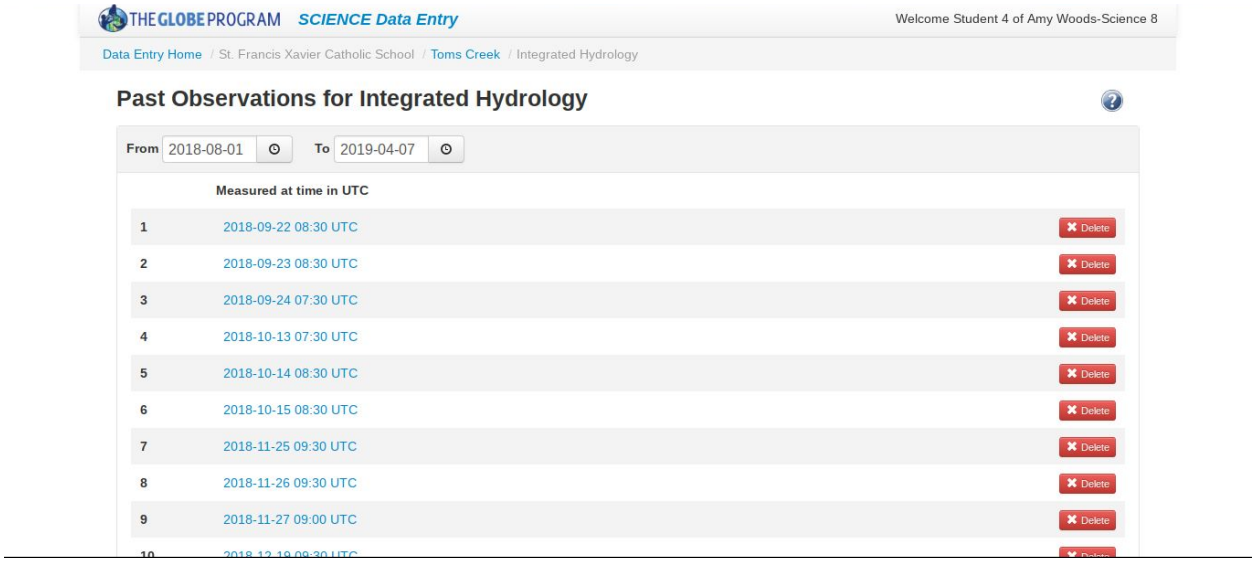
Graph 11: This graph shows the standard deviation of the pH data. The standard deviation was 1.75. This means that the data could be 1.75 units lower or higher than the average, 3.35 or 6.85. The recommended range for most aquatic animals is 6-8 units. The averages and the higher possible deviation are within the range.



Graph 12: This graph shows the standard deviation of the water temperature. The standard deviation was 5.59. This means that the data could be 5.59 units lower or higher than the average, 6.82 or 18. The range for most macroinvertebrates 14 degrees Celsius and 20 degrees Celsius. The average temperatures are mostly in the range accept the lower deviation.



Picture 1: This image shows data being entered into the GLOBE website. Water quality data and macroinvertebrate data was entered for each of the days observations were made.



Picture 2: This image show the history of observations made and entered into GLOBE.

Analysis and Results

The results of this experiment show that the biodiversity of macroinvertebrates was greater when the dissolved oxygen was moderately high and decreased when the levels increased. The amount of macroinvertebrates decreased as the levels were consistently between 8 ppm and 10 ppm. The correlation between nitrate and the biodiversity of macroinvertebrates was inconclusive because nitrate remained zero consistently through the experiment. The biodiversity of macroinvertebrates was higher when the pH was closer to neutral levels. When the pH rose above or became more acidic, the biodiversity decreased, which was apparent in the change in the number of organisms from October to December. The water temperature slowly decreased over the four months of data collection. As the temperature decreased, so did the number of macroinvertebrates.

Discussion

The data collected from September to December showed that the number of macroinvertebrates decreased as the temperature decreased, pH became more acidic, and dissolved oxygen increased. The data from September and October showed that the same number of species of macroinvertebrates were present, but macroinvertebrates with all varied classifications of pollution and water quality toleration were present when the dissolved oxygen was moderately high, temperature was closer to twenty degrees Celsius, and pH was close to neutrality. Pollution and quality sensitive (S) means that those macroinvertebrates are sensitive to organic pollution and drastic changes in water quality. Moderately sensitive (MS) means that those macroinvertebrates are sensitive to organic pollution and water quality changes, but are less sensitive than the previous classification. Pollution and quality tolerant (PT) means that

these macroinvertebrates can be sustained in organic polluted water or water with poor water quality. From November to December, dissolved oxygen, nitrate, and pH stayed consistent. Temperature decreased though, until no macroinvertebrates were found. Although, the correlation between nitrate and macroinvertebrates is unclear with this amount of data because nitrate has been consistently zero.

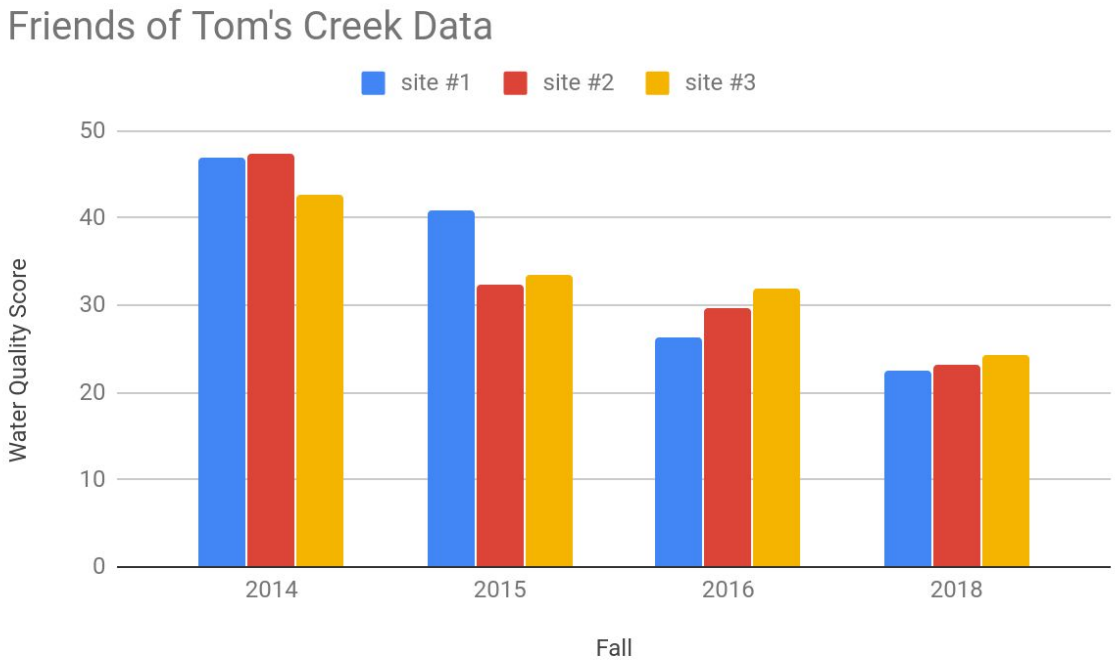
As shown in Graph 1, when the dissolved oxygen was consistently around five in September, the biodiversity of the macroinvertebrates had a wider range of taxa that were pollution and water quality tolerant and pollution and quality sensitive. The progression of the data showed that when the dissolved oxygen increased in October and continued to increase, is the point where the biodiversity started to decrease. However, this could be due to the decrease in temperature because temperature and dissolved oxygen have an inverse relationship, changing of seasons, and macroinvertebrate life cycle.

Graph 3 shows that when the temperature decreased is when the population was concentrated with tolerant taxa. The number of macroinvertebrates seemed to consistently decrease with the temperature. The water temperature seems to have a large impact on the biodiversity and number of macroinvertebrates.

Graph 2 displays that when the pH was closer to neutrality the taxa had a higher variety of pollution and quality tolerance, rather than when the pH became really acidic and the biodiversity decreased.

The data shows that nitrate stayed consistently at zero so no conclusion could be made on how nitrate affected the biodiversity of macroinvertebrates.

Overall, the conditions in September and October seemed more optimal for the most amount of taxa. The macroinvertebrate data led to the conclusion that the percent EPT of Toms Creek in the fall was 62.3. This means that it has a fair amount of EPT taxa in the fall, which is a good indicator that Toms Creek is in good health. The percent EPT in the winter was 14.3. This means that the number of EPT taxa drastically decreases from fall to winter. The biotic index of Toms Creek in the fall is 3.26. This indicates that Toms Creek has excellent water quality and organic pollution is unlikely. The biotic index in the winter is 4.8. This means that the quality of the water decreased slightly from excellent to good. As an added comparison, additional macroinvertebrate data was provided by the Friends of Toms Creek, an organization that monitors the health of the stream. The data provided was in the format for an organization called The Senior Environment Corps. It was contributed by Wayne Belt.



Graph 13: This graph shows the water quality scores collected by The Friends of Toms Creek from 2014 to 2018, except 2017, when data was not available.

Fall	site #1	site #2	site #3	Average
2014	47	47.4	42.7	45.7
2015	40.8	32.3	33.4	35.5
2016	26.4	29.6	32	29.3
2018	22.4	23.2	24.4	23.3
Average	34.15	33.13	33.13	

Table 4: This table shows the water quality scores from The Friends of Toms Creek. Data was collected every year, except 2017, when data was not available. The table shows the scores for each site every year, then the average of all the sites for that year on the side, and the average of the site for all the years.

Since 2014, the data collected by The Friends of Toms Creek has shown a steady decrease in water quality scores from good to fair. Future collaboration with The Friends of

Toms Creek will provide more data for the winter months and the data collection will take place in March.

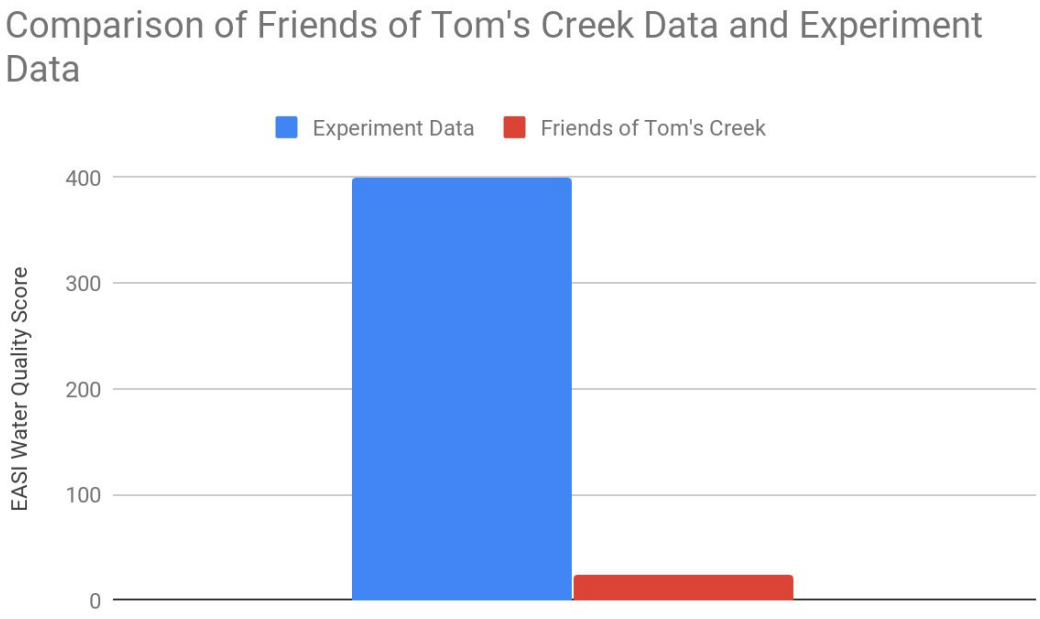


Figure 14: This graph shows the difference between the water quality score calculated by the Friends of Toms Creek and the data from this experiment. The water quality score of the experiment data was much higher. The water quality score for the experiment data was 399. The water quality score from the Friends of Toms Creek was 23.3. This might be because of the difference in time that the data was collected or a weather event may have occurred.

Time of Year	Experiment Data	Friends of Tom's Creek
Fall	399	23.3

Table 5: This table shows the water quality scores from this experiment and The Friends of Toms Creek from the fall months.

Conclusion

This experiment focused on determining how nitrate, pH, water temperature, and dissolved oxygen affect the biodiversity of macroinvertebrates. The hypothesis states that if over time, dissolved oxygen levels are high, nitrate is less than 1 ppm, pH levels are neutral, and water temperature is between 14 degrees Celsius and 20 degrees Celsius then the population of macroinvertebrates will be more diverse because these conditions promote more food sources and varied habitats for the most macroinvertebrate species. The data showed that biodiversity was highest when dissolved oxygen was moderately high, pH was close to neutral, and temperature was about 20 degrees Celsius. Water temperature seems to play a large part in the variety of macroinvertebrates. The trends in the data of the temperature and macroinvertebrates were consistent and very apparent. The diversity lessened as temperature steadily decreased. The diversity seemed to be higher when the dissolved oxygen was moderate because as the dissolved oxygen increased, the diversity and number of macroinvertebrates decreased. The pH data showed that the biodiversity was greater when the levels were less acidic. As the pH became more acidic, the diversity decreased. The role of nitrate in the biodiversity of macroinvertebrates is undetermined because nitrate was consistently at zero. A rain event and general timing of the season in the macroinvertebrate life cycles may be another change in the macroinvertebrate taxa and population. Further research on role of the life cycles of macroinvertebrates will be conducted in the near future and will be compared to the results from this experiment.

Based on the data from this experiment, the revised hypothesis states that if over time, dissolved oxygen levels are moderately high, nitrate is less than 1 ppm, pH levels are close to neutrality, and temperature is about 20 degrees Celsius then the population of macroinvertebrates

will be more diverse because these conditions promote more food sources and varied habitats for the most macroinvertebrate species. The water quality score from The Friends of Toms Creek was very different from the water quality score from the experiment data as seen in figures 14 and 15. The causes of this might be because the experiment data is from more than one month and the other data from only one date, if there was a weather event that caused changes in the stream, and many other possibilities. The standard deviation showed that the data deviated somewhat from the average. For all of the standard deviations, the higher possibility was in acceptable ranges for most aquatic species. The highest deviation was 5.59 for temperature and the lowest was 0 for nitrate because the data stayed consistent throughout the experiment.

During testing, possible areas of error in measuring the data include increased amounts of precipitation, error in reading the data, and malfunction of the equipment. Problems encountered in this experiment include flooding of the study site, learning how to use new materials, data being unavailable because of the government shutdown, and managing time for this project. This has been solved by collecting more data, testing the equipment before logging down data, and planning ahead of testing. If this experiment was repeated, there would be an attempt to gather more data to see if the role of nitrate could be determined. Further investigation on this experiment in the future will include taking more measurements to discern what effects the life cycles and seasons have on the biodiversity of macroinvertebrates

One of the most prominent obstacles encountered in this experiment has been gathering data from different locations. Data from the Chesapeake Bay will also continue to be pursued. The reason data needs to be collected throughout the watershed is to try to see firsthand how the quality of upstream waters affect the entire watershed. With the Chesapeake Bay being such a

"National Treasure," and laws being passed in New York and Pennsylvania, it is important for people not in direct contact with the Bay to understand how their actions influence the Bay. It is certainly not "Out of sight, out of mind," but that is what seems to have been happening these past decades. Contact has been made with several different organizations over many months in regards to macroinvertebrate data from the Chesapeake Bay. Despite the best efforts, only limited data has been available for use in this experiment. Data that has been recently published in reports is outdated in relation to this experiment. Many organizations have made commitments to provide data and unfortunately have not followed through at this time. Water quality data has been gathered, but is not as useful to this experiment without macroinvertebrate data. This is an objective to be pursued in the future.

Data will still continue to be sought out to further support the findings presented in this experiment. Data will continue to be taken over the course of the school year to see how the trends change over the different seasons. This project applies to the real world because more people are becoming citizen scientists and the more studies there are on individual environments, the better scientists can understand hydrology and other sciences as a whole. This project is valuable and important to the community because many homes in south central Pennsylvania are supplied water through wells, which is groundwater that is affected by the water quality on the surface. Based on the data collections, these findings will also be taken and presented at the Northeastern GLOBE Regional Symposium in Boston, Massachusetts in the spring of 2019.

Acknowledgements

I would like to thank my parents for helping take data and supplying materials, Mr. Todd Toth at NASA Goddard for providing the LaMotte Dissolved Oxygen and Nitrate kits, Mr. Joe

Hallinan for providing information on the background of Toms Creek, Mr. Belt, for providing more data from Toms Creek, the Chesapeake Bay Foundation, Mr. Floess, for advice on fluency and graphing, and Mrs. Woods for providing assistance through the entire process. I would not have been able to perform this experiment without the help of these people.

GLOBE Student Research Badges

Data Scientist

Many sets of data were compiled, analyzed and compared in this experiment. Data from The Friends of Toms Creek has been analyzed and compared to the data from this experiment. There was collaboration with the Chesapeake Bay Foundation on ideas for methods and data collection. Water quality data from the NOAA Chesapeake Bay buoys was gathered, and will possibly be added in the future months of this experiment.

Community Impact

This project came about after research concerning increasing commercialization in Pennsylvania as well as worldwide. This project will help the local community because many residents in the Carroll Valley area are supplied by well water, which is affected by water quality on the surface. The angling and ecotourism industries are crucial to the economy of south central Pennsylvania. Monitoring macroinvertebrates and water quality will help keep these industries thriving in this area. This experiment can help scientists understand factors that can be targeted in the Chesapeake Bay depending on the health of its watershed. The data from this experiment will also supply information that applies to streams and bodies of water all over the world.

STEM Professional

Collaboration with the Chesapeake Bay Foundation, Mr. Wayne Belt, Mr. Hallinan, and Mr. Todd Toth has improved this project tremendously. Mr. Hallinan provided research that put the interpretations of the data into context. The Chesapeake Bay Foundation provided individual advice to improve the experiment. Mr. Belt collaborated on this experiment to compare data from The Friends of Toms Creek to the data from this experiment to further look at the different natural processes that affect macroinvertebrates. Mr. Todd Toth made this project possible by providing equipment suited to the project.

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