The Effect of Riparian Vegetation and Abiotic Factors on Freshwater Ecosystems

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Abstract

This experiment was conducted to observe the impact of riparian vegetation and abiotic factors on freshwater ecosystems and the macroinvertebrates that inhabit them. Two leaf packs were placed in the toop property pond on campus, one with exclusively oak leaves and one with mixed vegetation. While the leaf packs remained in the water, abiotic testing was done using a water quality test kit to assess various water characteristics including but not limited to dissolved oxygen, pH, and nitrate level. The data was then used to calculate the biotic index and EPT index of the sample, and an overall conclusion about the water quality was made. Macroinvertibrates showed preference to riparian vegetation type of one leaf pack. The abundance and richness of the macroinvertebrates also seemed to be affected by abiotic factors of the water.

Introduction

The following experiment was conducted to observe and determine the effects of abiotic and biotic conditions on the macroinvertebrates in the freshwater ecosystem of the toop pond located near the Brookdale campus. It is assumed that various factors such as the vegetation found in the riparian zone, pollution levels and overall water quality will impact the organisms of the ecosystem in either a negative or positive way. In a freshwater community, the riparian vegetation plays a crucial role in the characteristics and quality of the freshwater environment. Riparian vegetation may act as a buffer, preventing pollutants from entering the water, as well as providing organic matter in the form of debris which may fall into the water, creating habitat and providing nutrients for freshwater macroinvertebrates (Dunkerley). The habitats that the riparian vegetation create can be found in the form of leaf packs, which are composed of detritus (decomposing particles of matter comprised of leaves). These leaf packs can be found on the edge of a freshwater system and they house a diverse group of organisms such as stoneflies and caddisflies which utilize the leaf packs for food and shelter (Merrit and Wallace).

Freshwater macroinvertebrates, though small, play a very important role in both the functioning of the ecosystem they live in and in understanding the complexities of freshwater environments. Aside from being a food source for many predators, macroinvertebrates provide a freshwater ecosystem with most of its species diversity and richness. They are also considered biological indicators and often studied to determine the water quality of an environment (Meixler and Bain). For example, a significant amount of caddisflies (*Trichoptera*) can be very sensitive to pollutants and are unable to live in areas with moderate to significant water pollution. There are many more Caddisflies present in water with little or no pollution. Due to their sensitivity to pollutants, they are often used as one of the more prominent biological indicators and in the EPT index (Morse).

Macroinvertebrates can be classified as any of the following functional groups: shredders, grazers, collectors, or predators. The functional group depends on how the organism obtains its food, not necessarily by what the organism consumes (Wallace). Collectors (also known as gatherers), for example, tend to feed on the already small particles of food. Shredders feed by scraping and shredding larger leaves in the water and can even assist in the decomposition of wood, while grazers feed by grazing on algal growth. Predatory macroinvertebrates feed on the other macroinvertebrates and help to control the keep prey populations under control (Wallace). The manner in which these organisms live and interact with their environment is crucial to the functioning of a freshwater ecosystem. Along with bacteria and fungi, macroinvertebrates comprise the foundation of a unique and complex environment home to many organisms.

Macroinvertebrates are strongly influenced by the quality of the water which surrounds them. Water quality is a broad concept which can refer to a multitude of characteristics, including but not limited to whether or not it is safe to drink, if it can support life and if it's contaminated with any pollutants (water quality). In order to determine a waterbody's quality, testing can be done to observe the abiotic factors of the environment and water in question. Nonliving aspects of the environment such as water pH, temperature, dissolved oxygen, biochemical oxygen demand, turbidity, phosphate and nitrate are all factors that impact a freshwater ecosystem. The abiotic factors of an environment can vary with temperature, presence of pollutants and waste, and growth of vegetation.

One of the calculations performed in this experiment was the water's EPT index, which assesses water quality through the presence of three significant groups of macroinvertebrates. The presence of the macroinvertebrates Ephemeroptera (mayflies), Plecoptera (stoneflies) and Trichoptera (caddisflies) are all taken into account and used to assess water quality and anthropogenic effects on the ecosystem being observed (Stewart). As previously mentioned, caddisflies are extremely sensitive to water pollutants, which makes them important biological indicators. Likewise, stoneflies also require a generally clean water habitat to survive, and play a vital role in the food web in a majority of freshwater ecosystems (Stewart). Mayflies comprise a large amount of the macroinvertebrates of these ecosystems, are an important link in the food chain, and are particularly susceptible to pH fluctuations (Brittain and Sartori).

Additionally, the water's biotic index was determined using data of the amount and diversity of the species found in the experiment combined with the organisms' ability to tolerate pollution. An ecosystem's biotic index can be utilized to assess the water quality of an ecosystem and to determine if any stressors are harming an ecosystem and the organisms that inhabit it. In general, species diversity dissipates as the level of environmental stress caused by pollutants increases. The most sensitive species will disappear first because they are more vulnerable to pollution, while species with a higher pollutant tolerance will be able to withstand harsh conditions (biotic indices).

The goal of the experiment was to determine the effects of abiotic factors and riparian vegetation on freshwater ecosystems. In order to conduct the experiment, artificial leaf packs were created to observe the impact that the type of riparian vegetation may have on macroinvertebrate diversity. One leaf pack had exclusively oak leaves, while the other leaf pack contained a mixture of various leaves found. Both leaf packs were placed in the same pond for the same amount of time, and it is assumed that leaf pack composition will impact macroinvertebrates differently despite being in the same pond. Creating leaf packs is a beneficial way to observe the impact of riparian vegetation on a freshwater system because it is simple to set up, repeatable, affordable and reliable. A similar experiment was conducted by Isis Sanpera-Calbet, Antoine Lecerf and Eric Chauvet on the influence of vegetation diversity on the impact of shredders in the Ruisseau de Peyreblanque, a river in France. Sanpera-Calbet, Lecerf and Chauvet studied leaf packs made of one species (beech, hazel or ash) in comparison with leaf packs which had all of the leaves combined in different amounts. The leaf packs in this experiment were then assessed for species diversity and preference to leaf type (Sanpera-Calbet, Lecerf and Chauvet).

In addition to the impact of riparian vegetation, the water quality was tested using a test kit to determine the pond's water quality and to observe the abiotic factors that impact the environment. Testing the abiotic factors of the water allowed for a more structured conclusion to be made regarding the quality of the pond than with just the riparian vegetation alone. The occurrence of unfavorable conditions may reduce the amount of species found in the leaf packs, and alter the EPT and Biotic indices. A study conducted involving Chinese rivers emphasized the direct correlation between lessened biodiversity in the presence of pollution by testing for factors including nitrogen levels, phosphorus levels and dissolved oxygen in multiple rivers and observing macroinvertebrate samples from each (Xu, Wang, Duan and Pan). Overall, while conducting the experiment of the toop property pond, it was thought that the macroinvertebrates would benefit more from one leaf pack, while not being as diverse in the other, and they would also be affected by the abiotic factors of the environment's water, allowing a general conclusion to be made regarding the overall water quality of the pond.

Materials and Methods:

- 20 G Oak leaves
- 20 G Mixed leaves
- Waterproof marker
- 2 Tags
- 2 Mesh bags
- Scale
- Twine/rope
- Thermometer
- 2 Zipper-top bags
- Scissors
- Bucket
- 2 Sorting trays
- Sorting sheets
- 2 Spoons
- 2 Brushes
- Magnifying glass
- Strainer
- 10 Petri dishes
- Macro lenses
- Leaf Pack Invertebrate Record Sheet

- Water Quality Test Kit and result key
- Water sample container

To initially set up the experiment, leaves were collected from the ground for two separate leaf packs. Leaf pack one contained 20 grams of mixed leaves, while leaf pack two contained 20 grams of exclusively Oak leaves. The leaf packs were created by first weighing the mesh bag, and then adding in the respective leaves. Using the scale provided, the mesh bag was clipped onto the scale, and the scale was held by a ring allowing the mesh bag to hang uninhibited from the scale. The weight of the bags were noted, and the leaves were slowly added to the mesh bags until the weight increased by 20 grams. It was noted that the leaves should be dry to avoid inaccurate measurements, but there was light, inconsistent rainfall/mist which made collecting dry leaves difficult. Once the proper amount of leaves were added to each bag, two tags were made using a waterproof marker which specified the date, leaf pack number (one or two), location, experimental variable (mixed leaves verses Oak leaves) and the school's name. The tags were placed in the bags with the proper leaves, and then tied shut at the top.

The leaf packs were then taken to the bank of the pond to be fastened and submerged fully under water. Pieces of brick were found on site, and were fastened closely to both leaf packs using rope to add enough weight to keep the packs in place and submerged. The item used will vary depending on availability but a heavy object such as bricks, rocks or cinder blocks is recommended. Additionally, a long extension of rope was tied to the top of the bag, so it could be fastened to a surrounding structure. The leaf packs were placed in the pond so that the brick was not covering the pack, allowing for as much of the bag to be exposed to the water as possible. The ropes attached to both leaf packs were fastened to a nearby tree stump and positioned relatively close to each other. Once fully submerged, a sketch of the position of the leaf packs was drawn and a photo of the area was taken. The temperature of the water, which was also taken the day the leaf packs were placed, was 13 °C. A thermometer attached to a yard stick was used to determine the temperature. The packs were then left undisturbed for three weeks.

After three weeks had passed, the leaf packs were located for removal and the water temperature was taken again and observed to be 14 °C. Using a zipper bag, a small amount of stream water was collected and the leaf pack was slowly dragged up from the water. While the leaf pack was still mostly submerged, rope was untied from the tree. In one swift motion, the leaf pack was brought up and placed into the bag. The procedure was repeated for the second leaf pack and was quickly taken into the lab along with an extra bucket of pond water. Once brought inside, the leaf packets were prepared for the macroinvertebrate sorting process one by one. A tray was placed in the sink, and the leaf pack was slowly removed from it's bag onto a strainer over the tray. The leaf pack was carefully rinsed until much of the sediment was removed. The leaves were then removed from the mesh bags, rinsed again briefly, and then divided into two sorting trays along with about 1/4 inch of the pond water that was collected in the bucket. After the leaves were fully removed from the strainer, the tray in the sink was observed for any macroinvertebrates that may have fallen in. If any organisms were seen in the tray, they were placed in a sorting tray along with the leaves. This process was repeated for both leaf packs.

Once the leaf pack was divided into two sorting trays, each leaf was closely inspected for the presence of macroinvertebrates by observing the leaf's surface and brushing each leaf gently with a brush. The water in the sorting trays was also closely inspected for any macroinvertebrates that were not on the leaves. Once a macroinvertebrate was spotted, they were carefully removed from the leaf or tray with the brush and a spoon and placed on a petri dish with water near the type of organism it looks most like on the sorting sheet. Many organisms were found in the water of the sorting trays, so the water was observed especially close. To get a closer look at the organisms, a magnifying glass and macro lense were used during the sorting process. Once the sorting process was complete, the amount of organisms in each petri dish was counted and recorded. This process was repeated for each leaf pack.

While the leaf packs were in the pond, the water was tested to assess the abiotic factors that impact the environment. The tests began by collecting samples of water in a container. When collecting the water, care was taken to ensure there was as little debris as possible in the sample container. The temperature of the water was taken (measured to be 13 °C) using the same method used when placing the leaf packets. Once the samples were taken, the water was tested to observe various factors using a water quality test kit. A method used to assess the possible pollutants that could be found in the water was to observe the odor. Any unusual scent could indicate pollutants such as chlorine, sewage, decomposing material and pesticides. The water's appearance was also observed by filling a clear glass jar with the pond water and holding it in front of a white background to further assess the water's quality. To test the dissolved oxygen level (DO), test tube 0125 filled to the top, and two dissolved oxygen test tabs were added. The tube was sealed, observed to confirm that there was no air bubbles, and slowly inverted until both tablets were fully dissolved. After the tablets dissolved, the test tube was left for five minutes. Once the test was complete, the color of the water was compared to the Dissolved Oxygen Color Chart provided. Using the dissolved oxygen value, dissolved oxygen percent saturation was determined using the chart provided by comparing the temperature of the water and the dissolved oxygen level.

The biochemical oxygen demand (BOD) was determined by filling a small test tube with pond water until the tube was overflowing, The tube was capped and covered with aluminum foil and left in a dark area for one week. The tube was then uncovered and the dissolved oxygen test was performed again using the same tablets. Once the dissolved oxygen level was determined for the sample that was left in the dark. The value was subtracted from the dissolved oxygen level taken the previous week to obtain the biochemical oxygen demand. The water's nitrate levels were tested by filling test tube 0106 with 5 mL of pond water, placing one Nitrate #1 TesTab in the tube and mixing. After the first tablet has dissolved, one Nitrate #2 CTA TesTab was added and the test tube was mixed until the tablet dissolved, the tube was then left for five minutes. The color of the water was then compared to the chart provided to determine the nitrate level of the water.

The pH of the water was tested by filling a test tube with 10 mL of water and adding one Wide Range TesTab and mixing until disintegrated. The color of the water was compared to the chart provided to obtain the pH number. The water's phosphate level was tested by filling a test tube with 5 mL of water and adding one Phosphorous TesTab and mixing until dissolved. After five minutes, the sample was compared to the Phosphate Color Chart to determine the phosphate present in the sample. Finally, the turbidity of the water was determined by observing the Secchi disk icons on the bottom of the water sample bucket. Additionally, the turbidity tube provided was filled and placed on the outline on the turbidity chart to look at the Secchi disk icon through the tube. The appearance of the Secchi disk icon was compared to the various secchi disks to confirm the turbidity of the sample. When all calculations were completed, the results were interpreted in the discussion using the water quality packet provided.

To complete the calculations, a chart was made for each leaf pack, showing the types of macroinvertebrates found, the amount of each, their pollution tolerance level, and the total pollution tolerance level for each group of macroinvertebrate. The total pollution tolerance level was calculated by multiplying the pollution tolerance level by the number of each type of organism found. With this information, the biotic index was calculated by taking the sum of all of the total tolerance values for both leaf packs and then dividing that value by the total amount of macroinvertebrates found. The value was then compared to the biotic index value chart provided. The EPT index was calculated by taking the total sum of mayflies, stoneflies and caddisflies (excluding netspinning caddisflies) from both leaf packs and then dividing that value by the total amount of macroinvertebrates found and multiplying the result by 100 to obtain the percent value. The percent was then compared to the chart provided. These calculations, along with the results of the leaf packs and abiotic factors testing was finally used to determine the overall water quality and impact on the macroinvertebrates.

Results:

The following table illustrates the type and quantity of macroinvertebrates found in both leaf packs, which were extracted on October 24th 2019. The Macroinvertebrates were compared to their pollution tolerance value to determine the total tolerance value for each species.

Leaf Pack 1:

Macroinvertebrates	**Number Found**	**Pollution Tolerance Value**	**Total Tolerance Value**
Ephemeroptera (mayflies)	5	3.6	18.0
Plecoptera (stoneflies)	0	1.0	0.0
Trichoptera (caddisflies)	2	2.8	5.6
*Most caddisflies	0	2.8	0.0
*Hydropsychidae (netspinning, excluded from EPT)	0	5.0	0.0
Odonata	0	7.0	0.0
*Anisoptera (dragonflies)	4	4.0	16.0
*Zygoptera (damselflies)	0	7.0	0.0
Megaloptera	0	2.0	0.0
*(dobsonflies)	0	2.0	0.0
*(alderflies)	0	2.0	0.0
Coleoptera (beetles)	0	4.6	0.0
*(beetle larvae)	2	4.6	9.2
*(adult beetles)	1	4.6	4.6
*(water pennies)	0		0.0
Hemiptera (true bugs)	0		0.0
Diptera (true flies)	0		0.0
*Chironomidae (midges)	0	6.0	0.0
*Simullidae (black flies)	0	6.0	0.0

*Tipulidae (crane flies)	0	3.0	0.0
*Other Diptera	0	6.0	0.0
Amphipoda (scuds)	3	6.0	18.0
Isopoda (sowbugs)	1	8.0	8.0
Oligochaeta (aquatic earthworms)	11	8.0	88.0
Hirudinea (leeches)	2	10.0	20.0
Turbellaria (planarians)	0	4.0	0.0
Gastropoda (snails)	0	7.0	0.0
Misc. Non-insect Invertebrates	4		0.0
Total	**35**		**187.4**
Total # of Macros - EPT Macros	**35-7=28**		

Leaf Pack 2:

Macroinvertebrates	**Number Found**	**Pollution Tolerance Value**	**Total Tolerance Value**
Ephemeroptera (mayflies)	6	3.6	21.6
Plecoptera (stoneflies)	1	1.0	1.0
Trichoptera (caddisflies)	3	2.8	8.4
*Most caddisflies	2	2.8	5.6
*Hydropsychidae (netspinning, excluded from EPT)	1	5.0	5.0
Odonata	7	7.0	49.0
*Anisoptera (dragonflies)	7	4.0	28.0
*Zygoptera (damselflies)	0	7.0	0.0

Megaloptera	12	2.0	24.0
*(dobsonflies)	1	2.0	2.0
*(alderflies)	11	2.0	22.0
Coleoptera (beetles)	2	4.6	9.2
*(beetle larvae)	1	4.6	4.6
*(adult beetles)	1	4.6	4.6
*(water pennies)	0		0.0
Hemiptera (true bugs)	0		0.0
Diptera (true flies)	0		0.0
*Chironomidae (midges)	0	6.0	0.0
*Simullidae (black flies)	0	6.0	0.0
*Tipulidae (crane flies)	0	3.0	0.0
*Other Diptera	0	6.0	0.0
Amphipoda (scuds)	2	6.0	12.0
Isopoda (sowbugs)	2	8.0	16.0
Oligochaeta (aquatic earthworms)	1	8.0	8.0
Hirudinea (leeches)	2	10.0	20.0
Turbellaria (planarians)	1	4.0	4.0
Gastropoda (snails)	0	7.0	0.0
Misc. Non-insect Invertebrates	0		0.0
Total	**63**		**245**
Total # of Macros - EPT Macros	**63-12= 51**		

Using the tables generated and the combined data from both leaf packs, the EPT index and Biotic index were calculated to assess the water quality. The EPT Index was calculated to be 19.38%, indicating that the water quality is poor in regards to species richness. The Biotic Index was calculated to be 4.41 indicating that the water quality is good regarding types of species present.

EPT Index: Biotic Index:

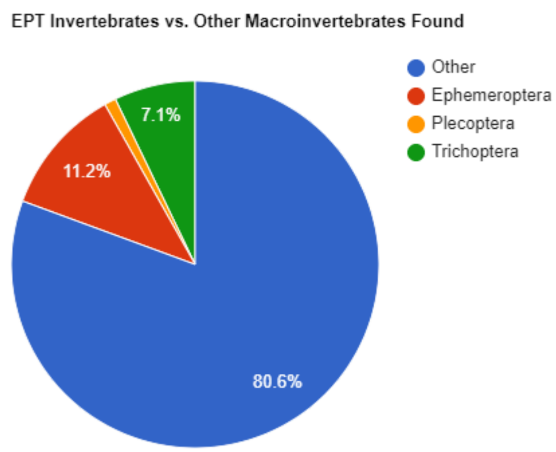
$$\frac{E+P+T}{Total\ \#\ of\ macros} * 100 \qquad \frac{Sum\ of\ total\ tolerance\ value}{Sum\ of\ number\ found} = Biotic\ Index$$

$$\frac{5+2+6+1+3+2}{98} * 100 \qquad \frac{187.4+245}{35+63} \rightarrow \frac{432.4}{98}$$

$EPT = \frac{19}{98} * 100$= 19.38% Biotic Index= 4.41

EPT Index Values in Relation to Water Quality:	
EPT ≥ 50%	Good
25% ≤ EPT ≤ 50%	Moderate
EPT < 25%	Poor

Biotic Index Values in Relation to Water Quality:		
Biotic Index Value	Water Quality	Degree of Organic Pollution
< 3.75	Excellent	Organic pollution unlikely
3.76-5.0	Good	Some organic pollution present
5.1-6.5	Fair	Substantial organic pollution present
6.6-10.0	Poor	Severe organic pollution present



Abiotic Factors:

Study Site: Toop Pond

Water Temperature: 13 °C

Abiotic Factors			
	Excellent	Good	Poor
Water Odor	Musky		
Water Appearance	Cloudy, slight brown color.		
Dissolved Oxygen (ppm): 4 ppm Percent Saturation= 38%	91-100	51-90	**38%** <50

Bod ppm DO Original Sample: 4 ppm Incubated Sample: 0	0	**4** 4-8	>8
Nitrate (ppm)	<4	**5** 4-19	>20
pH	7	**6** 6 or 8	4,5,9,10 or 11
Phosphate (ppm)	<1	**2** 2-4	>4
Turbidity (JTU)	0	**40** 40-100	>100

Discussion:

Looking at the results of the various tests and calculations performed, the abundance and richness of the macroinvertebrates does seem to be affected by both riparian vegetation, as well as the abiotic factors of the water and surrounding environment. Testing the water quality using the test kit showed that the water had a musky scent and a cloudy/slight brown appearance. The musky scent could indicate the possible presence of pollution, as well as the presence of decaying vegetation. It was noted that the leaf packs were placed in a shallow area with a detritus already present, which could explain this scent. The color of the water indicates the presence of sediments suspended in the water, possibly due to runoff caused by precipitation. The measurements for BOD, nitrate, pH, phosphate and turbidity all indicated that the water supply can be considered good to fair. Nitrate content measured to 5 ppm. According to the water quality test pack, waters with no pollution typically have nitrate levels at around 4 ppm, indicating that the sample water may have a small amount of pollution. The pH of the water was measured to be around 6, making the water slightly acidic but still in the optimal pH range. Increased vegetation growth may lead to a higher pH, and despite the pH only being slightly acidic, it may affect the more sensitive macroinvertebrates.

The phosphate level found in the water was at 2 ppm, which indicates generally healthy phosphate level. The turbidity of the water was determined to be around 40 JTU which indicates that the water clarity was ideal. The suspended particles were most likely sediment and erosion. The biochemical oxygen demand (BOD) was calculated at 4 ppm, indicating that bacteria are not utilizing the majority of the oxygen available in the water. The only abiotic factor that displayed poor results was the dissolved oxygen level (DO) at 4 ppm, and 38% saturation. The low (DO) level may indicate increased levels of decaying plants and other matter, or the presence of pollution. The results may have been affected by the area where the water was taken. As previously mentioned, the water samples were taken in an area with high levels of rotting vegetation, which may have affected the results.

The extraction of the packets and calculation of the biotic index concluded that the water quality can be considered good. The calculation using both leaf packs came out to 4.41, which indicates that only a small amount of organic pollution may be present. However, the EPT index was determined to be 19.38% (a low value), which indicates that of the various species found, the vast majority were not sensitive to pollution. Due to the low EPT index, it must be inferred that there is most certainly pollution or high levels of decay present. In the study assessing the impact of pollutants on macroinvertebrates in Chinese rivers, it was determined that in the rivers which had pollution, the only macroinvertebrates that were found were the ones that were, to some extent, tolerant of pollution. On the other hand, species sensitive to pollution were few and far between (Xu, Wang, Duan and Pan). Between both packets, there was a diverse group of organisms that included the pollution sensitive macroinvertebrates used in the EPT calculations. The various types of macroinvertebrates collected indicates that the environment is healthy enough to support a plethora of different organisms, however it cannot support high densities of sensitive macroinvertebrates.

The leaf pack extraction also showed a very vast difference in the diversity of species between the oak and mixed leaf packs. Leaf pack one, which contained mixed vegetation has a significantly smaller amount of macroinvertebrates (a total of 35 found) and was far less diverse than leaf pack two. Leaf pack two, which contained only oak leaves had a much more diverse group of macroinvertebrates at higher densities (a total of 63 found). Moreover, leaf pack two had 12 EPT macroinvertebrates while leaf pack one had 7. The results of the effects of riparian vegetation on the macroinvertebrates in the Ruisseau de Peyreblanque support the notion that the diversity and composition of the riparian vegetation impact the leaf litter that is found in the freshwater. It was shown that according to the type of leaf litter, decomposition rates vary greatly, which in turn affects the macroinvertebrates (Sanpera-Calbet, Lecerf and Chauvet). The fact that the macroinvertebrates preferred the less diverse leaf pack came as a surprise, but the results were consistent with other mixed leaf packs. However the results may have been altered due to the position of the leaf pack in the pond, the manner in which the leaf pack was extracted and limited time to sort the packs.

In summation, according to the results obtained by observing the leaf pack contents, calculating the EPT and Biotic Index and measuring the pond water's abiotic characteristics, it can be confirmed that riparian vegetation and abiotic factors do indeed impact freshwater macroinvertebrate populations. The macroinvertebrates showed a definite preference to the type of vegetation used and were more diverse in one type of leaf pack. The impact of abiotic factors on the general presence of macroinvertebrates also allowed for a general consensus to be made about the water's quality. Maintaining and protecting freshwater ecosystems is critical in both preserving supply of fresh water and protecting the abundant species that can be found in the water. As pollution levels continue to rise, the freshwater ecosystems become more and more vulnerable. Species that depend on the water are increasingly threatened and there is a growing threat to public health. The macroinvertebrates that are found in these environments are an extremely efficient way to test water quality and work toward maintaining healthy water.

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