A biological assessment of water quality in El Placer, Ecuador: The effect of agriculture on stream health and the quality of historical versus current drinking water sources

Danielle Kleinberg
SIT Study Abroad

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Danielle Kleinberg

Project Advisor: Ana María Ortega
Academic Director: Xavier Silva

Gettysburg College
Chemistry and Environmental Studies

El Placer, Tungurahua Province, Ecuador, South America

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Abstract

Although fresh water is one of Ecuador's most abundant resources, high quality drinking water for its inhabitants is scarce (Wingfield et al., 2021). The most prevalent sources of water pollution in Ecuador are domestic waste, silver and gold mining, oil production, and agricultural chemicals (Buckalew et al., 1997). El Placer, a village located in Tungurahua, Ecuador, is highly dependent on agriculture as a source of income. The first objective of this study was to determine the effect of agriculture on the El Placer's Tía Anita Stream through comparing the water quality at three sites with varying agricultural influence. The second objective was to investigate the quality of El Placer's historical and current drinking water sources. These objectives were carried out through comparison of chemical factors, physical factors, hydromorphological quality, and macroinvertebrate composition at each of the study sites.

Macroinvertebrate and hydromorphological analyses agree that agriculture did have an effect on water quality in the Tía Anita Stream, suggesting that Site 3 (no agricultural influence) had higher water quality than Sites 1 and 2 (both were influenced by agriculture). Chemical analysis did not agree with these biological findings, but suggested that Site 1 had the lowest water quality, whereas Site 2 had the highest water quality. This inconsistency may have been due to physical factors such as stream velocity causing a higher sediment load in Site 1 (EPA, 1997). Macroinvertebrate, hydromorphological, and chemical analyses agree that Site 4, El Placer's historical water source, had moderate water quality. It is important to note that, in the past, Site 4's water quality was likely higher. Macroinvertebrate, hydromorphological, and chemical analyses agree that Site 5, a stream from El Placer's current potable water source, had very good water quality.

Keywords: Water quality, potable water, benthic macroinvertebrates, bioindication, agriculture, EPT Index, Andean Biotic Index, BMWP/Col Index, chemical water quality indicators, physical water quality indicators, hydromorphological quality.

Resumen

Aunque el agua dulce es uno de los recursos más abundantes de Ecuador, el agua potable de alta calidad para sus habitantes es escasa (Wingfield et al., 2021). Las fuentes principales de contaminación del agua dulce en Ecuador son los desechos domésticos, la minería de plata y oro, la producción de petróleo, y los químicos agrícolas (Buckalew et al., 1997). El Placer, una parroquia ubicada en Tungurahua, Ecuador, depende en gran medida de la agricultura como fuente de ingresos. El primer objetivo de este estudio fue determinar el efecto de la agricultura en la Quebrada de Tía Anita de El Placer mediante la comparación de la calidad del agua en tres sitios adyacentes a diferentes usos del suelo. El segundo objetivo fue investigar la calidad de las fuentes de agua potable históricas y actuales de El Placer.

Los análisis de macroinvertebrados e hidromorfológicos coinciden en que la agricultura tuvo un efecto en la calidad del agua en la Quebrada de Tía Anita, lo que sugiere que el Sitio 3 (sin influencia agrícola) tuvo una calidad de agua más alta que los Sitios 1 y 2 (ambos fueron influenciados por la agricultura). El análisis químico no estuvo de acuerdo con estos hallazgos biológicos, pero sugirió que el Sitio 1 tenía la calidad de agua más baja, y que el Sitio 2 tenía la calidad de agua más alta. Esta inconsistencia puede deberse a factores físicos, como la velocidad de la corriente, que provocan una mayor carga de sedimentos. Los análisis de macroinvertebrados, hidromorfológicos, y químicos coinciden en que el Sitio 4, la fuente histórica de agua de El Placer, tenía una calidad de agua moderada. Es importante mencionar que, en el pasado, la calidad del agua del Sitio 4 probablemente era más alta. Los análisis de macroinvertebrados, hidromorfológicos, y químicos coinciden en que el Sitio 5, una quebrada de la actual fuente de agua potable de El Placer, tenía agua de muy buena calidad.

Palabras Claves: Calidad del agua, agua potable, macroinvertebrados bentónicos, bioindicación, agricultura, EPT índice, Índice Biótico Andino, BMWP/Col índice, indicadores químicos de calidad del agua, indicadores físicos de calidad del agua, calidad hidromorfológica.

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Statement of Ethics

All necessary permissions for conducting research were attained by academic advisor, Xavier Silva and project advisor, Ana María Ortega. Informal interviews, regarding the use of agricultural chemicals and the history of El Placer's drinking water sources, were conducted with full consent of the interviewee. Macroinvertebrates captured for the purpose of identification were killed with 70% alcohol to minimize suffering. The macroinvertebrate sample size was insignificant compared to the overall local macroinvertebrate community and is therefore unlikely to make a significant ecological impact.

Introduction

Fresh water is one of South America's most abundant resources. About 30% of our planet's freshwater supply is reserved in three of South America's watersheds: the Amazon, the Parana-Plata, and the Orinoco (United Nations, 2021) .The Amazon Basin alone, the largest river system on the planet, delivers approximately 15% of the global freshwater run-off (Wildlife Conservation Society, 2021). In terms of rainfall, South America receives an average of 1,600 mm annually -- more than any other continent and over double the global land average (Linacre & Geerts, 1999). Despite high accessibility to fresh water sources, safe drinking water is limited in Latin America. About one third of the population in Latin America does not have sustained access to safe drinking water (United Nations, 2021). Safe and available water, used for drinking, domestic use, food production, and recreational purposes, is vital to public health. In addition, improved water supply has been found to boost countries' economic growth and reduce poverty (World Health Organization, 2021).

Even though it is located in the largest river basin in the world and receives an average annual rainfall of 1,200 mm, Ecuador struggles to supply safe and readily available water to its inhabitants (Buckalew et al., 1997). Before Ecuador's 2007 National Development Plan prioritizing water management, the country's fresh water sources were completely contaminated with organic loads and toxic substances (Balicanta, 2020). Despite years of progress in water sanitation, as of 2020, only 73% of the Ecuadorian population has access to standard quality water. Higher quality water resources are even more scarce, accessible to only 68% of Ecuador's population (Wingfield et al., 2021). The most prevalent sources of water pollution in Ecuador are domestic waste, silver and gold mining, oil production, and agricultural chemicals (Buckalew et al., 1997).

The Effect of Agricultural Run-off on Water Quality and Human Health

Between 2010 and 2014, Ecuador used more than 15,000 metric tons of agricultural chemicals (Deknock et al., 2019). Pesticides, a class of agrichemicals including fungicides, insecticides, herbicides, and rodenticides, are used to protect plants and humans from various diseases (Nicolopoulou-Stamati et al., 2016). Fertilizers, used to increase the speed of plant growth as well as the quantity of food produced, are necessary in order to feed the world population (Frontiers for Young Minds, 2021). Although these chemicals help to boost Ecuador's economy through increasing food production and exports, they accumulate in the natural environment. In fact, the majority of pesticides never even reach their target, but sink into the surrounding environment (Deknock et al., 2019). Fertilizers, which contain high levels of nitrogen and phosphorus to support plant growth, are often not fully utilized by plants. Excess fertilizer flows into surrounding bodies of water, a process known as run-off, increasing nitrogen and phosphorus levels in river water (*Sources and Solutions*, 2021). An imbalance of these nutrients in freshwater can lead to excess plant growth and decrease water quality. A build-up of these chemicals can lead to hypoxia, a water condition where oxygen levels are too low to sustain aquatic life. Affecting the bottom fauna first, hypoxia works its way up through the trophic cascade, eventually affecting all the entire aquatic ecosystem (NOAA, 2021). Furthermore, deforestation from agricultural activity reduces hydromorphological quality through effects on structure and naturalness of vegetation on riverbank, continuity of riverbank, connectivity of riparian vegetation with other adjacent/nearby landscape elements, and naturalness of the river channel (Encalada et al., 2011).

Run-off of agrichemicals into potable water sources can have a detrimental effect on human health. Depending on the type of chemical, duration of exposure, and individual health status, acute and chronic health effects can occur (Nicolopoulou-Stamati et al., 2016). Exposure to agrichemicals can lead to lung damage, chemical burns, infant methemoglobinemia, and acute and chronic neurotoxicity (Weisenburger, 1993). Due to its devastating effect on both the environment and human health, agricultural pollution of freshwater sources should be a top priority of study.

Indicators of Water Quality

Photo 1. Macroinvertebrates from the pollution-sensitive order Trichoptera, family Leptoceridae.

Macroinvertebrates refer to organisms without backbones that are visible to the human eye. Benthic, "bottom-dwelling", refers to the habitat of these macroinvertebrates -- they live in leaf litter, on rocks, and in sand of aquatic ecosystems. Benthic macroinvertebrates include small aquatic organisms such as snails, crabs, worms, and beetles, as well as the aquatic larvae of insects (*Indicators: Benthic Macroinvertebrates*, 2021). Macroinvertebrates serve as useful bioindicators because they respond to human changes of the physical and chemical conditions of their habitats. Due to evolutionary adaptations to environmental conditions, macroinvertebrates vary in their tolerances to environmental disturbances. Some orders of macroinvertebrates are more sensitive to contamination than others (Photo 1). The relative abundance of highly-sensitive orders provides insight into water quality. Several indices exist that assign scores to different macroinvertebrate families based on their tolerance to pollution; the sum of family scores at a given study site can indicate its water quality. Furthermore, bioassessments of macroinvertebrate populations are favored because these organisms are almost universally found in freshwater ecosystems, collection methodology is well-defined, and there are countless resources for identification (Stark et al., 2001).

Along with macroinvertebrate composition, hydromorphology of the river's surrounding ecosystem is also an important indicator of water quality. At times, even if a water source is chemically and physically high quality, the surrounding environment can negatively affect the ecosystem. Alteration of the natural river channel affects biological communities and reduces diversity. Factors such as continuity, connectivity, and naturalness of the riverbank can be qualitatively assessed and can contribute to conclusions of water quality (Encalada et al., 2011).

Chemical indicators, such as pH, total dissolved solids (TDS), electrical conductivity (EC), and oxidation-reduction potential (ORP) can also give insight into water quality. The acidity or basicity of a solution, pH, can indicate accumulation of agrichemicals. Agrichemicals with bicarbonates and carbonates increase the basicity of water through bonding to hydrogen ions from water, creating basic hydroxide. In contrast, agrichemicals high in ammonium increase water acidity though donating a hydrogen ion to water, creating acidic hydronium ions (Dickson, 2017). High levels of TDS, the total concentration of solids in water, can indicate that harmful contaminants, such as iron, manganese, sulfate, bromide and arsenic, may have been added to the water through runoff and wastewater discharges (Safe Drinking Water Foundation, 2021). Although EC, the measure of the ability of a solution to conduct an electrical current, does not give insight into the pollution source, elevated EC values can indicate pollution due to higher levels of dissolved salts (*School of Geography*, 2021). ORP measures the ability of a freshwater source to break down waste products, such as pollutants and decomposing matter. Higher ORP values indicate higher concentrations of dissolved oxygen. Since this oxygen is used to cleanse the river of contaminants, higher ORP values generally indicate higher quality water (James, 2004).

In addition, physical variables, such as temperature, velocity, and discharge are important in assessing the quality of freshwater systems. In terms of temperature, dissolved oxygen (DO) is more soluble in cold water than in warm water. In polluted areas with lower levels of DO, warmer temperatures may exacerbate the deficiency, leading to the suffocation of sensible organisms. Stream velocity is also important to the health of aquatic communities. High stream velocities may make it difficult for benthic organisms to hold to their substrate. Slow stream velocities limit aerations and, therefore, dissolved oxygen quantities; they also limit the stream's potential to carry sediments. At a given velocity, depth and width can be used to calculate discharge, another physical indicator of water quality. Rivers with higher dischargers can receive pollution with little effect, whereas smaller streams have less capacity to dilute and degrade wastes (EPA, 1997).

Site Overview: El, Placer, Ecuador

This study will take place in El Placer, a small village located in Tungurahua, Ecuador. El Placer was established in 1971 and has a population of about 420 residents. Located in the eastern Andean cloud forest, the slopes surrounding this village have fertile soils perfect for agriculture. Agriculture is the principal source of income for the village; the most popular products are naranjilla, tree tomato, blackberry, and mandarin (J. Recalde, Personal Communication, December 5, 2021).

Little is known about the effect of agriculture on El Placer's water quality. In a previous unpublished macroinvertebrate study of El Placer's Chinchín River, it was found that agriculture caused light levels of chemical pollution. In addition, it was found that agriculture impacted the stream bank architecture, nutrient retention, presence of microhabitats, water temperature, and stream depth (Chang, 2019). Since agriculture is vital to El Placer's economy, further studies regarding its effect on water quality would benefit the village. In terms of El Placer's drinking water sources, water is currently delivered to the village from a natural source on the nearby mountain, Nueva Libertad. Before 2006, water came from a closer natural source across from the main road, Vía a Baños. Macroinvertebrate studies regarding water quality in El Placer's historical and current potable water sources have not been completed previously, so information regarding the quality of these water sources would be important for the village.

The objectives of this study are twofold. The first objective is to determine the effect of agriculture on the Tía Anita Stream through comparing the water quality at three sites with varying agricultural influence. The second objective is to investigate the quality of El Placer's historical and current drinking water sources.

The first objective will be carried out through comparison of chemical factors, hydromorphological quality, and macroinvertebrate composition at each of the study sites. Physical factors will be measured to indicate water quality differences independent of agricultural influence between sites. The second objective will be carried out through the comparison of chemical factors, physical factors, hydromorphological quality, and macroinvertebrate composition. In terms of the agricultural comparison, it is hypothesized that the most downstream site of the Tía Anita Stream, located near a naranjilla grove, will have the lowest water quality due to the accumulation of agrichemicals and agricultural deforestation. This predicted effect of agrichemical accumulation would be reflected by low scores in macroinvertebrate water quality indices, high values for TDS and EC, a low value for ORP, and a non-neutral pH in the agricultural sites. The predicted effect of agricultural deforestation would be reflected in lower hydromorphological quality in agricultural sites. Site 3, the upstream Tía

Anita Stream site located in secondary forest, is hypothesized to have the highest water quality out of the three Tía Anita Stream sites, as reflected by optimal values for macroinvertebrate indices, TDS, EC, ORP, pH, and hydromorphological quality.

Materials and Methods

Site Description: El Placer

El Placer is located along the Pastaza River, a large tributary to the Marañón River with headwaters in the Andean highlands (Bernal, 2011). Although this river contains an immense quantity of fresh water, it is not utilized by the village due to high levels of contamination from the neighboring city of Baños. The Chinchín River, a tributary to the Pastaza, is another large source of freshwater in close proximity to the community. Its main uses include recreation, washing dogs, and drinking for those working in the field. Along with several small tributaries, there are three main streams that contribute to the Chinchín River: Tía Anita Stream, Amarilla Stream, and Cascada Fantasma Stream. Water from these streams is utilized for irrigating agricultural plots as well as providing water to livestock. While working in the field, farmers often drink from these streams as well. It is estimated that approximately 12 workers drink from the Tía Anita Stream regularly. One family uses stream water from the Tía Anita Stream as drinking water for their home (J. Recalde, Personal Communication, December 5, 2021).

When the village was first established in 1971, nearby freshwater sources were utilized for drinking water. The two main historical water sources are located across the main road, Vía a Baños. In the year 2006, these water sources were depleted due to the San Francisco Hydroelectric Project, which constructed tunnels under El Placer in order to supply water to the Pastaza River's Agoyán Dam. Currently, the 100 families in El Placer receive their potable water through pipes coming from the Nueva Libertad Mountain. On this mountain, there are three natural sources of surface water that are combined and distributed to the village. Although there is no formal protection of these water sources, their steep slopes naturally protect them through making it impossible for agricultural activity to occur in the surrounding areas. In the past, there have not been concerns with the protection or regulation of these water sources (J. Recalde, Personal Communication, December 5, 2021).

Site Description: Study Sites

In order to investigate the effect of agriculture on water quality in the Tía Anita Stream, water quality in the following three sites were compared:

Site 1 is located at an altitude of 1544 meters, the lowest altitude of the three sites. Although its direct surroundings are secondary forest, it is about 100 meters from a naranjilla grove (Photo 2). Fungicides, insecticides, and herbicides are applied every three weeks to the hybrid species of naranjilla. Every two years, the location of the grove shifts slightly, so it is possible that in the past the grove was closer to the stream.

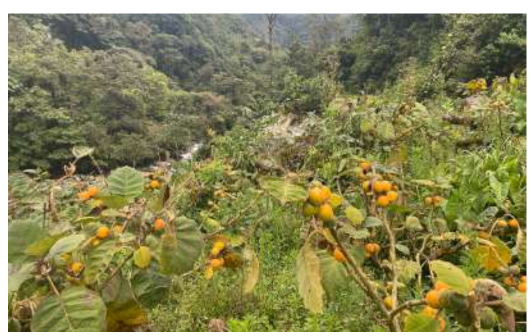

Photo 2. Naranjilla groves near Site 1 of Tía Anita Stream.

Photo 3. Tree tomato groves near Site 2 of Tía Anita Stream.

Site 2 is located at an altitude of 1745 meters. One side of this site is covered in tree tomato groves, while the other side is secondary forest (Photo 3). Fungicides and insecticides are applied monthly to the tree tomate grove, whereas herbicides are applied every two months.

Site 3 is located at an altitude of 1754 meters, the highest altitude of the three sites. This site is surrounded completely with secondary forest (Photo 4). There are no signs of alteration in the surrounding environment, except for a small path leading to the stream.

Photo 4. Site 3, secondary forest site of Tía Anita Stream.

In order to investigate drinking water quality in El Placer historically and currently, the two sites were chosen:

Site 4 is El Placer's historical drinking water source (Photo 5). It is located at an altitude of 1540 meters. This site is directly off the main road, Vía a Baños, and adjacent to a mandarin grove. Water flow in this stream was depleted almost completely after the San Francisco Hydroelectric Project.

Photo 5. Site 4, El Placer's historical water source.

Photo 6. Site 5, El Placer's current water source.

Site 5, part of El Placer's current drinking water source, is located at an altitude of 1692 meters. This site is a stream from one of the three natural water sources that are combined in order to supply El Placer with water. The pipes that bring this water to the village are visible in Photo 6. Located on the steep slopes of Nueva Libertad Mountain, Site 5 is surrounded completely with secondary forest.

A map of all study sites can be seen below (Figure 1):

Figure 1. Map of study sites, mapped using Google Earth Pro.

Data Collection

At each study site, physical, chemical, and biological data were collected. In terms of physical data, velocity was measured through averaging three trials of time it took for an object to travel a specified distance (between 1 and 2 meters, depending on the study site). In addition, the width of the stream was measured at the widest point, and the depths at various points along overall width were measured in order to create a cross-section of the stream. The value for the cross-sectional area at each site was calculated using the formula for the area of a trapezoid:

$$1) \quad \frac{(a+b)h}{2}$$

where *a* and *b* are are the depths on either side of the region and *h* is the width of the region. All trapezoidal areas within a cross-section were summed to calculate the total cross-sectional area. Through multiplying the velocity and the total cross-sectional area, the discharge was calculated for each site.

In terms of chemical measurements, a water quality meter (Multifunction Model C-600) was used to measure water temperature, pH, total dissolved solids (TDS), electrical conductivity (EC), and oxidation-reduction potential (ORP). Chemical variables were measured in four different areas at each study site and averaged. For each measurement, time was given for the instrument to calibrate. SenSafe® John's Copper Check disposable test strips were used to estimate concentration of dissolved Cu^{+1}/Cu^{+2}. At each site, 2 test strips were used and averaged.

To assess the biological quality of each study site in its entirety, the Hydromorphological Quality Index (HQI) was utilized, which uses surrounding vegetation as a bioindicator (Appendix A). Through adding the scores of several criteria, a total score is obtained for each study site. Based on its HQI value, a site's water quality can be classified as excellent, good, moderate, or bad (Table 1). In addition, the surrounding half hectare of each study site was identified (primary forest, secondary forest, agricultural area, etc.) and described.

Table 1. Hydromorphological quality based on total score (Encalada et al., 2011)

Total Score	Hydromorphological Quality
>35	Excellent
29-35	Good
20-28	Moderate
10-19	Bad

Along with biologically assessing the area, macroinvertebrates present in the water were collected to serve as bioindicators of water quality. Using a square-framed surber net, macroinvertebrates were collected along 10 meter stretches of each study site. At each site, six samples were taken with different benthic substrates in order to ensure representative sampling. Two samples were taken in each of the following substrates: rock, sand, and leaf litter. At each site, sampling began downstream in order to not disturb future samples. The protocol for macroinvertebrate collection for each sample was as follows:

1. The suber net* was placed on the bottom of the stream and for 3 minutes while the substrate was aggressively disturbed. Any larger rocks were rubbed.
2. The contents of the net were placed onto a collecting tray and macroinvertebrates were sorted out of the debris into petri dishes based on morphospecies using tweezers and sifters.
3. The samples were preserved in a 70% solution of alcohol.

*At Site 5, flow was not strong enough to use the surber net for collection, so macroinvertebrates were collected manually for 2.5 hours.

Macroinvertebrates were identified down to the family ex-situ using a hand lens and three macroinvertebrate identification guides (Encalada et al., 2011; Andino et al., 2017; Pérez, 1996). When necessary, a stereo microscope with 10x magnification was used to identify families.

Data Analysis

The following three biotic indices were used in order to draw conclusions from the macroinvertebrate composition: Ephemeroptera, Plecoptera and Trichoptera (EPT), Andean Biotic Index (ABI), Biological Monitoring Working Party Colombia (BMWP/Col).

The EPT Index estimates water quality using the presence of the macroinvertebrate orders Ephemeroptera, Plecoptera and Trichoptera. These orders are very sensitive to pollutants, so their relative abundance at each site can be used to compare water pollution at each site. EPT is expressed as a percentage of the individuals in the sensitive orders over the total individuals found (Carrera & Fierro, 2001). Based on its EPT value, a site's water quality can be classified as very good, good, standard, or bad (Table 2).

Table 2. Water quality classifications based on EPT value (Carrera & Fierro, 2001).

EPT	Water Quality
75-100%	Very Good
50-74%	Good
25-49%	Standard
0-24%	Bad

The ABI is a family-level water quality index specific to the Andean Region, designed for altitudes greater than 2000 meters above sea level (Encalada et al., 2011). ABI assigns scores, within the range of 1-10, to families and orders based on their specific tolerance to contamination (Appendix B). Lower scores are assigned to the least sensitive families that have a high tolerance to pollution, whereas higher scores are assigned to more sensitive families with a low tolerance to pollution. Through adding the scores of each family/order found, the total ABI for a particular site can be calculated. Based on its ABI, a site's water quality can be classified as very good, good, standard, or bad (Table 3).

Table 3. Water quality classifications based on ABI value (Encalada et al., 2011).

ABI	Water Quality
>96	Very Good
59-96	Good
35-58	Standard
<35	Bad

Similar to the ABI, a score for the BMWP/Col Index is obtained through summing the individual scores of all families present. Individual families receive scores, within the range of 1-10, based on their pollution tolerance levels and knowledge on distribution and abundance (Appendix C). Similar to the ABI, pollution-tolerant families receive low scores and pollution-intolerant families receive high scores (Bueñano et al., 2018). Through adding the scores of each family found, the total BMWP/Col for a particular site can be calculated. Based on its BMWP/Col, a site's water quality can be classified as good, acceptable, doubtful, critical, or very critical (Table 4).

Table 4. Water quality classifications based on BMWP/Col (Bueñano et al., 2018).

BMWP/Col	Water Quality	Description
>120, 101-120	Good	Clean or very clean
61-100	Acceptable	Lightly contaminated
36-60	Doubtful	Moderately contaminated
16-35	Critical	Very contaminated
<15	Very Critical	Strongly contaminated

Using the program iNext, two family accumulation curves were created - one based on sample size, and the other based on sample coverage. In addition, a sample completeness curve was created in order to determine if sampling was representative. A numerical value for estimated sample coverage, also obtained from iNext, was tabulated.

Results

Physical and Chemical Data

Graphical representations of the cross-sectional areas of each study site can be seen Figures 2-5. Cross-sectional area was not measured at Site 5 due to very limited water flow.

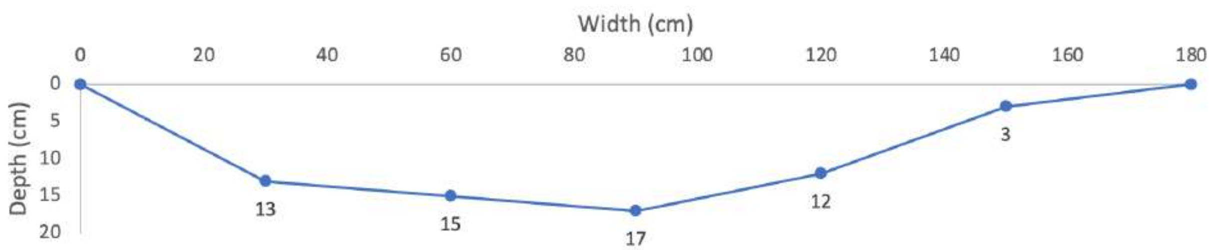

Figure 2. Cross-sectional area of Site 1.

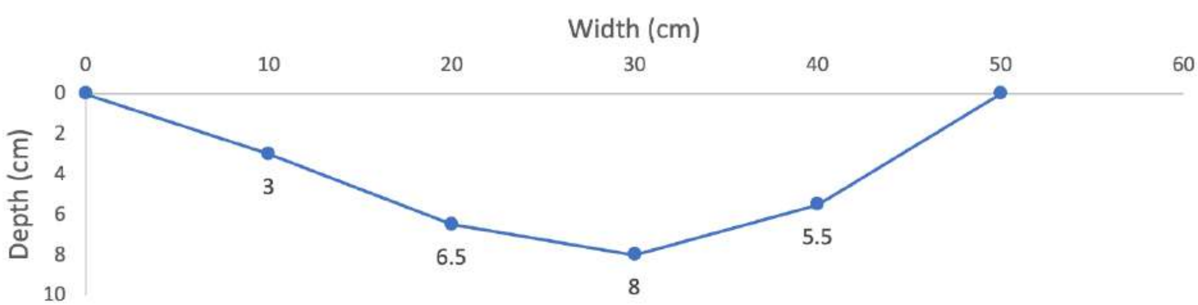

Figure 3. Cross-sectional area of Site 2.

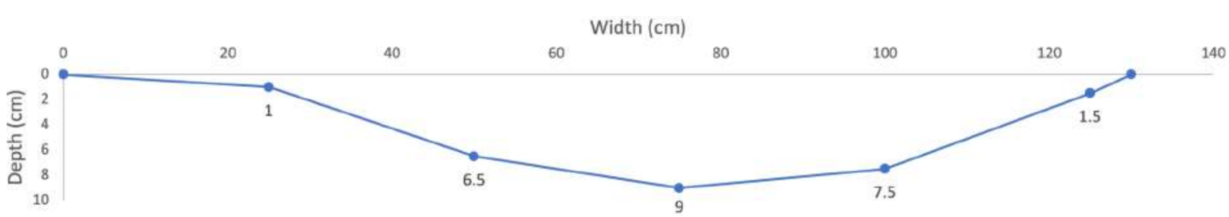

Figure 4. Cross-sectional area of Site 3.

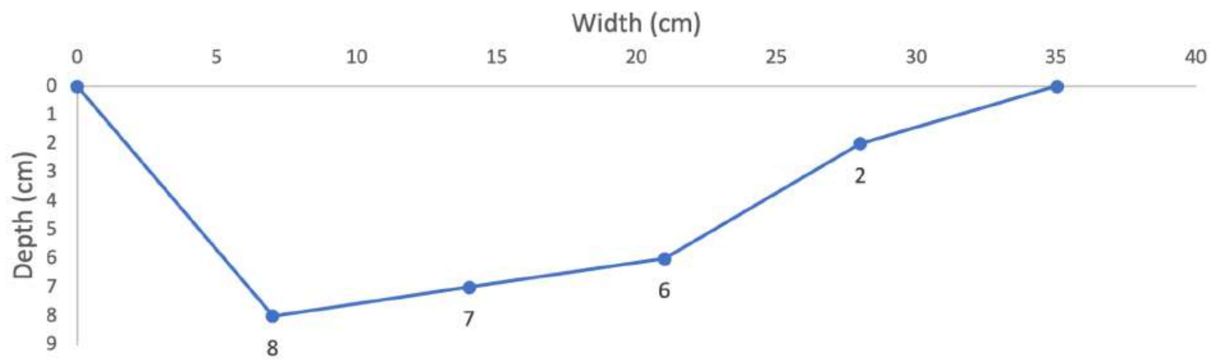

Figure 5. Cross-sectional area of Site 4.

Values for the cross-sectional area of each study site, calculated using Formula 1, can be seen in Table 5. Out of Sites 1-4, Site 1 had the largest cross-sectional area at 1800 cm^2, whereas Site 4 had the lowest cross-sectional area at 161 cm^2.

Table 5. Cross-sectional area at each study site.

Site	Cross-Sectional Area (cm^2)
1	1800
2	230
3	623
4	161

Velocity at each study site can be found in Table 6. Velocity was not measured at Site 5 due to very limited water flow. Site 1 had the highest velocity at 47.5 cm/s, whereas Site 4 had the lowest velocity at 12.0 cm/s.

Table 6. Velocity at each study site.

Site	Velocity (cm/s)
1	47.5
2	25.7
3	36.1
4	12.0

Note. Each value is an average of three trials.

Through multiplying the velocity and cross-sectional area at each study site, the discharge was calculated (Table 7). Site 1 had the highest discharge at 85,500 cm^3/s, whereas Site 4 had the lowest discharge at 1,939 cm^3/s.

Table 7. Discharge at each study site.

Site	Discharge (cm^3/s)
1	85,500
2	5,901
3	22,480
4	1,939

Chemical measurements at each site can be seen in Table 8. The temperature range across all sites was 1.8° C, with the highest in Site 4 (18.4° C), and the lowest in Site 2 (16.6° C). The range of pH across sites was 0.28, with the highest in Site 3 (7.23) and the lowest in Site 2 (6.95). TDS had a range of 39 ppm, with the highest in Site 5 (85 ppm) and the lowest in Site 2 (46 ppm). EC, with a range of 78 uS/cm, was correlated with TDS with the highest in Site 5 (171 uS/cm) and the lowest in Site 2 (93 uS/cm). ORP had a range of 300 mV, with the highest at Site 2 (382 mV) and the lowest at Site 4 (82 mV). Concentrations of Cu^{+1}/Cu^{+2} at all sites were between 0 and 0.05 ppm.

Table 8. Chemical measurements at each study site.

Site	Temperature (° C)	pH	TDS (ppm)	EC (uS/cm)	ORP (mV)
1	17.7	7.09	62	125	197
2	16.6	6.95	46	93	382
3	16.9	7.23	58	117	314
4	18.4	7.07	51	102	82
5	16.8	6.99	85	171	327

Note. Each value is an average of four trials

Hydromorphological Quality Index

Using the criteria Appendix A, a HQI score was assigned to each site. The scores for each criteria and the total score for each site can be seen in Table 9. The ranges of hydromorphological quality based on total score can be seen in Table 1. Site 3 was the only site with excellent hydromorphological quality. Site 5 had good hydromorphological quality, Sites 1 and 2 had moderate hydromorphological quality, while Site 4 had bad hydromorphological quality.

Table 9. Scoring for Hydromorphological Quality Index. Blue indicates "excellent" quality, green indicates "good" quality, orange indicates "moderate" quality, while red indicates "bad" quality.

Criteria	Site 1	Site 2	Site 3	Site 4	Site 5
Structure and naturalness of vegetation on riverbank	3	2	5	2	4
Continuity of riverbank	3	3	5	2	5
Connectivity of riparian vegetation with other adjacent or nearby landscape elements	3	2	5	1	5
The presence of trash and debris	4	4	5	5	4
Naturalness of river channel	4	4	5	3	4
Substrate composition	4	4	4	2	4
Velocity and depth of the river	2	2	3	1	1
Elements of heterogeneity	4	3	4	2	3
Total Score	**27**	**24**	**36**	**18**	**30**

Benthic Macroinvertebrate Composition

In total, 878 individuals were found across 13 different orders. In Site 5, the most individuals, orders, and families were found. In Site 4, the least individuals, orders, and families were found (Table 10). Photos of each order can be found in Appendix D.

Table 10. Individuals, orders, and families found at each study site.

Site	Number of Individuals	Order Richness	Family Richness
1	169	8	18
2	156	10	18
3	203	9	24
4	86	6	15
5	264	11	25

Order abundance across sites can be seen in Figure 6. Diptera was the most abundant order with 175 individuals, followed by Ephemeroptera with 170 individuals, and Trichoptera with 151 individuals. Order composition of each study site can be found in Appendix D. Family composition at each study site can be found in Appendix E.

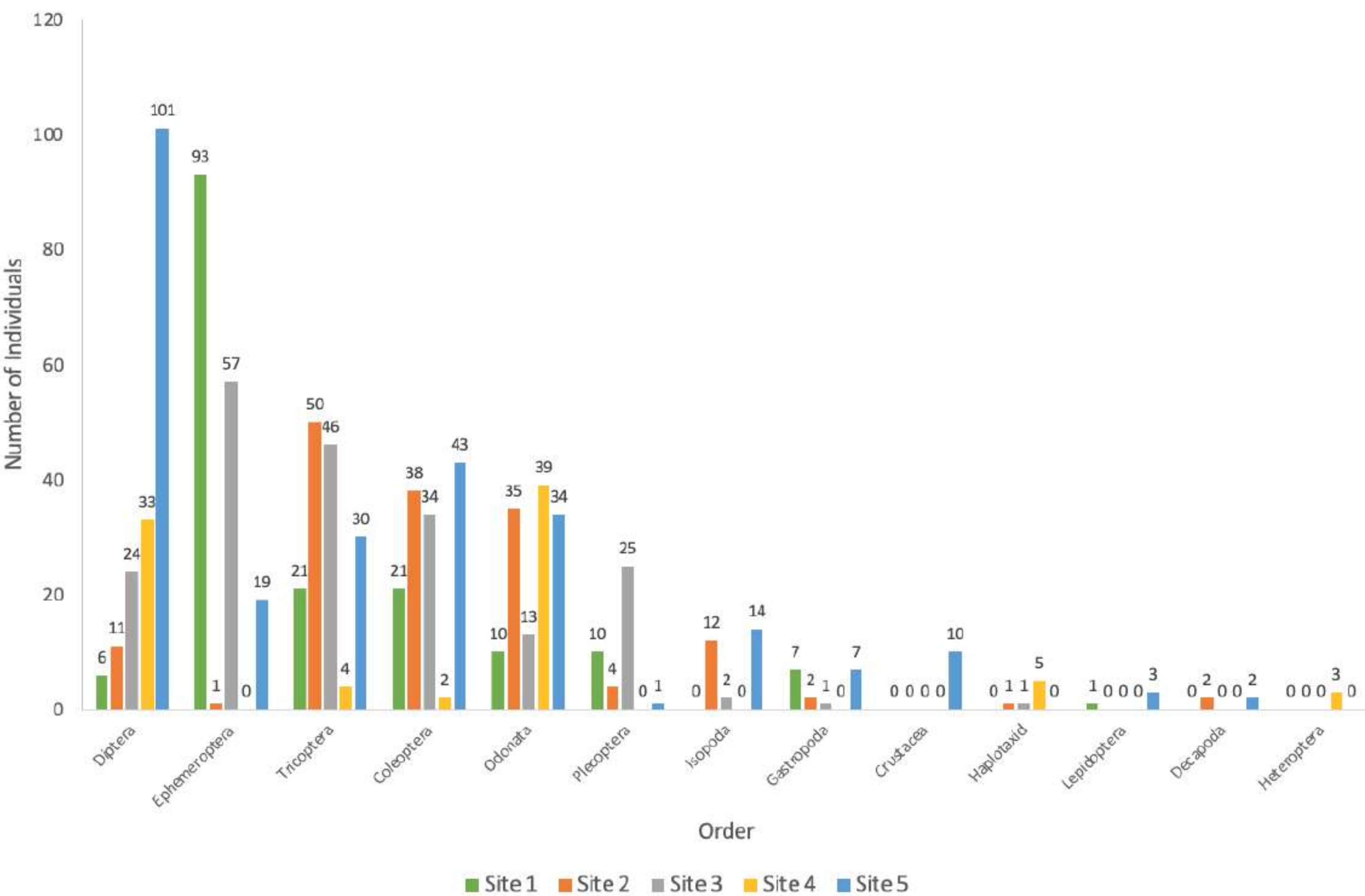

Figure 6. Order abundance at each study site. Total individuals found in each order decreases from left to right.

Ephemeroptera, Plecoptera and Trichoptera Index (EPT)

Table 11 shows the EPT score for each site. No sites scored "excellent" quality based on the EPT Index. Sites 1 and 3 were classified as "very good" quality. Site 2 was classified as "standard" quality, while Sites 4 and 5 were classified as "bad" quality. The ranges of EPT can be seen in Table 2.

Table 11. EPT scores for each site. Green indicates "very good" quality, yellow indicates "standard" quality, while red indicates "bad" quality.

Site	EPT (%)
1	73.3
2	35.3
3	63.1
4	4.7
5	18.9

Andean Biotic Index (ABI)

Table 12 shows the ABI score for each site. Based on the ABI, Sites 1, 3, and 5 were classified as "excellent" quality, while Sites 2 and 4 were classified "very good" quality. The ranges of ABI can be seen in Table 3. Calculations for the ABI can be found in Appendix F.

Table 12. ABI scores for each site. Blue indicates "excellent" quality, while green indicates "very good" quality.

Site	ABI
1	117
2	88
3	141
4	75
5	107

Biological Monitoring Working Party Colombia Index (BMWP/Col)

Table 13 shows the BMWP/Col score for each site. Sites 1, 2, 3, and 5 were all classified as "good" according to the BMWP/Col Index. Site 4 was classified as "acceptable". The ranges of BMWP/Col can be seen in Table 4. Calculations for the BMWP/Col Index can be found in Appendix G.

Table 13. BMWP/Col scores for each site. Blue indicates "good" quality, while green indicates "acceptable" quality.

Site	BMWP/Col
1	126
2	107
3	154
4	89
5	134

Sample Coverage Statistical Analysis

As seen in Figure 7, Sites 3 and 4 had the highset family diversity, and it is not expected to increase much with a higher sample size. Sites 1 and 2 had lower family diversities, but family diversity was still not expected to increase much with a higher sample size in these sites. In contrast, with more sampling in Site 4, family diversity is projected to increase significantly. As seen in Figure 8, all sites had a high sample coverage. The lowest sample coverage was Site 4 at 93.1%, while the highest sample coverage was Site 1 at 97.7% (Table 14). In terms of family accumulation based on sample coverage, it is clear that as sample coverage approaches 1, family diversity increases at an increasing rate for each of the five sites (Figure 9).

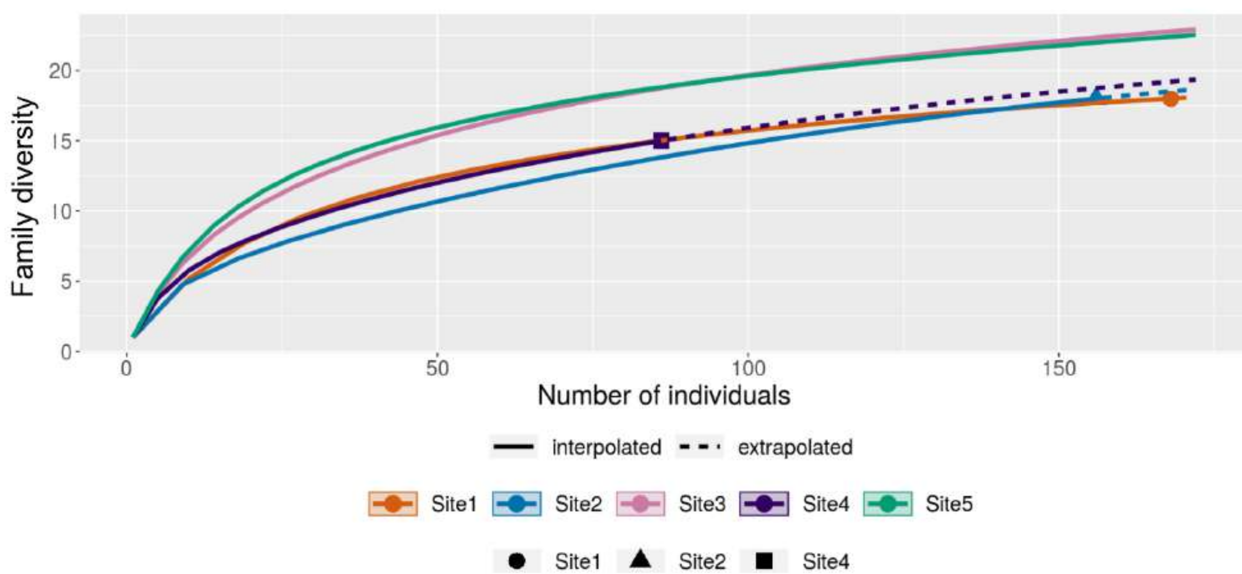

Figure 7. Family accumulation curve for each site, extrapolating family diversity based on sample size. Created using iNext.

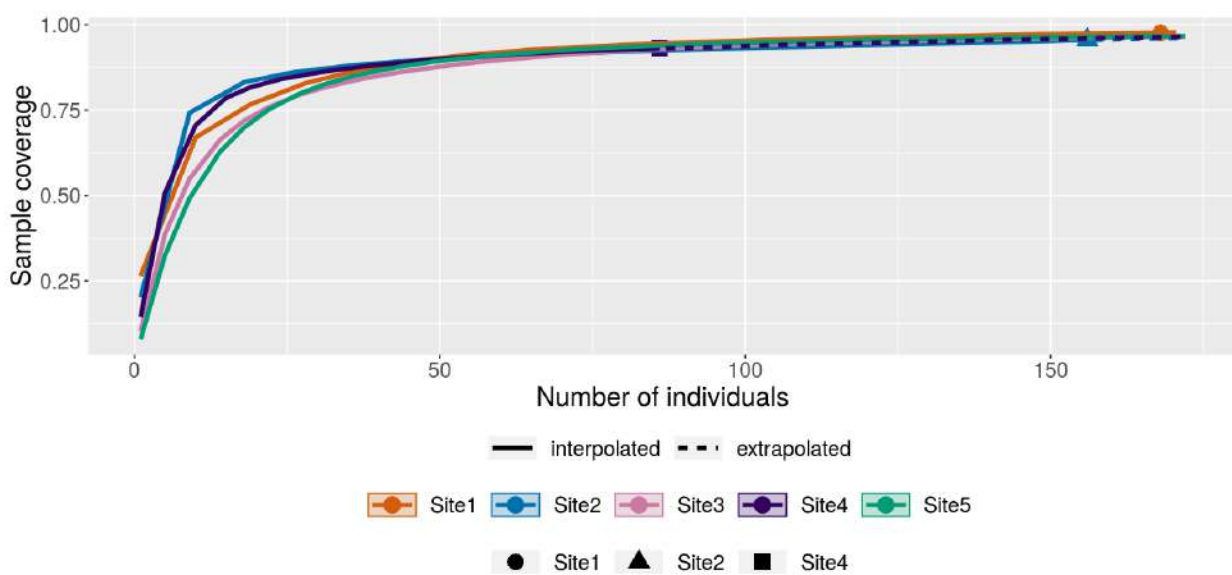

Figure 8. Sample completeness curve for each site. See Table 14 for each site's estimated sample coverage. Created using iNext.

Table 14. Estimate of sample coverage at each site derived from Figure 8. Calculated using iNext.

Site	Sample Coverage (%)
1	97.64
2	95.55
3	97.1
4	93.08
5	97.72

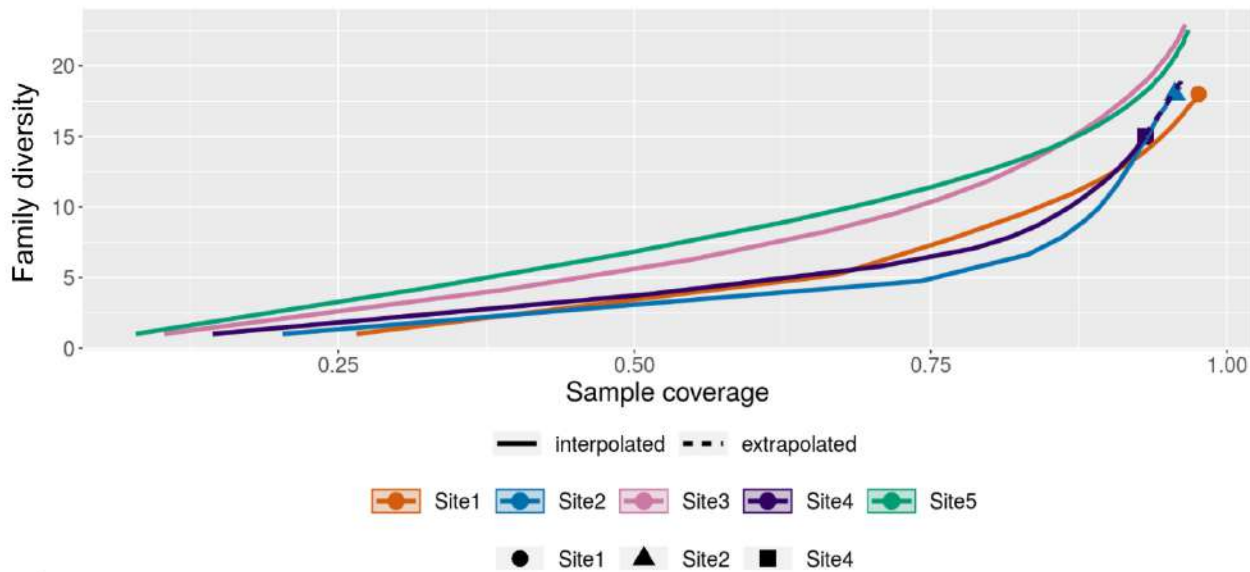

Figure 9. Family accumulation curve for each site, extrapolating family diversity based on sample coverage. Created using iNext.

Discussion

Effect of Agriculture on Tía Anita Stream

The first objective was to determine the effect of agriculture on the Tía Anita Stream through comparing the water quality at three sites with varying agricultural influence. Site 1, the most downstream Tía Anita Stream site located near the naranjilla grove, was hypothesized to have the lowest water quality out of the three Tía Anita Stream sites due to the accumulation of agrichemicals and agricultural deforestation, as reflected by poor values for macroinvertebrate indices, TDS, EC, ORP, pH, and hydromorphological quality. Site 3, the upstream Tía Anita Stream site located in secondary forest, was hypothesized to have the highest water quality out of the three Tía Anita Stream sites as reflected by optimal values for macroinvertebrate indices, TDS, EC, ORP, pH, and hydromorphological quality.

Using hydromorphology as an indicator of water quality, Site 3 had the highest water quality, which is consistent with the hypothesis. In the HQI, Site 3 scored "excellent", whereas Sites 1 and 2 scored "moderate". Specifically, Sites 1 and 2 were lacking in the following criteria: structure and naturalness of vegetation on riverbank, continuity of riverbank, and connectivity of riparian vegetation with other adjacent/nearby landscape elements. Site 2 had a slightly worse hydromorphological quality than Site 1, which is due to its closer proximity to agricultural activity, decreasing scores for connectivity and elements of heterogeneity (Table 9).

This finding suggests that agriculture does have an effect on water quality through altering the natural hydromorphology.

Macroinvertebrate analysis was also consistent with the hypothesis that Site 3 would have the highest water quality. Site 3 received the highest ABI and BMWP/Col scores (Tables 12, 13) Although Site 1 received the highest EPT score (Table 11), this index does not account for pollution-intolerant families outside of the orders Ephemeroptera, Plecoptera, and Trichoptera. Therefore, ABI and BMWP/Col may be more comprehensive and conclusive in terms of correlating their scores with water quality. Macroinvertebrate analysis was inconsistent with the hypothesis that Site 1 would have the lowest water quality -- all three of the indices agree that Site 2 has the lowest water quality (Tables 11, 12, 13). It is likely that Site 2 had the lowest quality water because it was directly adjacent to the tree tomato groves, which use fungicides, insecticides, and herbicides. Site 1, although more downstream than Site 2, was about 100 meters further from any agricultural activity. This difference in proximity to agricultural activity may account for why Site 1 has higher water quality than Site 2 based on macroinvertebrate analysis.

Chemical analysis supports the prediction that there may have been accumulation of agrichemicals in Site 1. Site 1 had the highest TDS (62 ppm) and EC (125 uS/cm) out of the three sites, indicating the highest presence of dissolved solvents in the water. These values are still within the acceptable range of TDS (0-500 ppm) and EC (0-200 uS/cm) in freshwater, but may indicate the presence of dissolved agricultural contaminants in Site 1 (DataStream, 2021). In addition, Site 1 has the lowest ORP (197 mV), suggesting lower oxygen content and therefore a lower ability to break down contaminants. In healthy lakes and rivers, ORP should be between 300-500 mV (DataStream, 2021). Site 1's low level of oxygen suggested by the ORP may be further amplified by its warmer temperature, 17.7^{o} C, which is the highest of all three sites (Table 8).

Although chemical analysis supports the first hypothesis, chemical analysis is not consistent with the hypothesis that Site 3 would have the highest water quality -- it indicates that Site 2 has the highest water quality. Site 2 has the lowest TDS (46 ppm) and EC (93 uS/cm) out of the three sites, indicating a lower quantity of dissolved contaminants in the water. In addition, Site 2 has the highest ORP (382 mV) out of the three sites, suggesting that it is best able to break down contaminants. Site 2 also has the lowest temperature, 16.6^{o} C, out of all three sites, allowing for the easiest dissolution of oxygen (Table 8).

Biological and chemical conclusions regarding the effect of agriculture on water quality are inconsistent. Hydromorphological and macroinvertebrate analysis agree that Site 3 has highest quality water, whereas Site 2 has the lowest quality water. Chemical analysis, on the other hand, suggests that Site 2 has the highest water quality, whereas Site 1 has the lowest water quality. Chemical analyses may have been skewed by each site's velocity. Higher velocities allow for the ability to carry more sediment load (EPA, 1997). It is possible that the high velocity, and therefore higher sediment load, at Site 1 (Table 6) may have caused higher TDS and EC values. If Site 1's TDS and EC values were worse due to an accumulation of agricultural chemicals, it is likely that pH would also be skewed. In reality, Site 1's pH was 7.09, which is very close to neutral (Table 8). Site 2 had the lowest velocity (Table 6), causing a lower sediment load which may have accounted for its lower TDS and EC values.

Another possible reason for the disagreements between biological and chemical conclusions is the sample coverage of macroinvertebrate collection at each site. Site 2 had the lowest sample coverage, 95.6%, of all three sites (Table 14). The most abundant order in Site 2 was Trichoptera (Appendix D). With more samples, it is likely that more of this pollution-sensitive order would be found, thereby increasing values of all three indices. With higher values for each of the macroinvertebrate indices, the biological conclusion may be more consistent with the chemical conclusion that Site 2 is of highest quality.

Furthermore, physical analysis suggests that Site 1 would have the highest tolerance to pollution. Its high velocity should allow for aeration, which would supply more oxygen to aquatic life. In addition, its high discharge should allow for the dispersion of contaminants (EPA 1997). Therefore, even though Site 1 may have higher levels of dissolved contaminants per the chemical tests, high velocity and discharge (Tables 6, 7) may decrease their effect on the macroinvertebrate community. Although Site 2 has the healthiest levels of TDS, EC, and ORP, its low velocity and discharge may lower its capacity to dilute pollutants. In Site 2, aquatic macroinvertebrate populations may be affected by these relatively concentrated polluants, which would be consistent with the finding that Site 2 scored the lowest on all three macroinvertebrate indices.

In a previous study regarding the impact of agricultural land-use on aquatic macroinvertebrates in Colombian streams, it was found that the Ephemeroptera, Trichoptera, and Plecoptera orders were found in high abundance in cropland and forested streams (Giraldo et al., 2014). This finding is consistent with the results of this study - Site 1, a cropland stream, had the highest abundance of these orders as represented by a “very good” EPT score of 73.3%. Site 3, a forested stream Site 1, also had a “very good” EPT score, of 63.1%. Site 2, another cropland stream, had a relatively lower “standard” EPT score of 35.3% (Table 11). Giraldo et al. (2014) also found that Diptera and Mollusca were the most abundant orders in streams impacted by cattle ranching. In the future, it would be interesting to see the effect of cattle ranching on macroinvertebrate communities in El Placer streams.

Another previous study, conducted in an agricultural river basin in southeast Brazil, supports this study’s finding that agriculture has an impact on hydromorphological quality, stream chemistry, and macroinvertebrate communities (Egler et al., 2012). They found that agricultural deforestation affected river morphology, which agrees with the low HQI scores of Sites 1 and 2 (Table 9). They also found that riverside erosion in agricultural areas increased sediments in the stream; this increase in sediment load agrees with the high TDS and EC values found in Site 1 (Table 8). In addition, they found that taxa richness was reduced in agricultural areas. When looking at family richness, Site 3 had the highest family richness out of the Tía Anita Stream sites (Table 10), which agrees with this previous study.

Investigation of Historical and Current Drinking Water Source

The second objective was to investigate the quality of El Placer’s historical and current drinking water sources.

Site 4, El Placer’s historical drinking water source, was found to have moderate water quality in terms of biological analysis. Site 4 scored “bad” according to the HQI, lacking in nearly all criteria (Table 9). Site 4’s low hydromorphological quality is due to the adjacent road and surrounding mandarin groves. In terms of macroinvertebrate analysis, Site 4 scored “bad” in EPT, “very good” in ABI, and “acceptable” in BMWP/Col (Tables 11, 12, 13). Although Site 4 scored “bad” in EPT, this index only accounts for three pollution-intolerant orders, but does not take into account other pollution intolerant families. Odonata was the most abundant order in Site 4, containing several pollution-sensitive families (Appendix E). Specifically, the families Polythoridae, Calopterygidae, and Gomphidae were found. These families of Odonata are highly sensitive to pollution, all scoring higher than a 7 out of 10 in both the ABI and BMWP/Col

Indices. Furthermore, in terms of sample coverage, Site 4 had the lowest of all five sites at 93.1% (Table 14). Only 86 individuals were found, which spanned 6 orders and 15 families (Table 10). As seen in Figures 7 and 9, more individuals or a higher sample coverage would lead to more family diversity in Site 4, allowing for stronger conclusions in terms of macroinvertebrate analysis. Based on these current biological findings, it can be concluded that water quality in Site 4 is moderate.

Chemical and physical analysis also suggests moderate water quality for Site 4. Site 4's TDS and EC values of 51 ppm and 102 uS/cm, respectively, are well below the maximum recommended values of 500 ppm and 200 uS/cm, respectively. These variables suggest that Site 4 does not contain many dissolved contaminants. Furthermore, Site 4's neutral pH of 7.07 suggests little influence from the adjacent mandarin grove's agrichemicals. ORP in Site 4 is 82 mV, much lower than the ORP range in healthy lakes and rivers, 300-500 mV (Table 8). This finding suggests that Site 4 likely has a lower levels of oxygen, and a lower ability to cleanse itself of contaminants. Site 4's low level of oxygen suggested by the ORP may be amplified by its warmer temperature, 18.4^o C, which is the highest of all five study sites. Although Site 4 has a good TDS and EC, it's ORP and high temperature lower its quality. Furthermore, Site 4's velocity was 12.0 cm/s (Table 6), which is considered slow based on the HQI (Appendix A). This slow velocity, along with the low discharge of 1,939 cm^3/s (Table 7), suggest that Site 4 would be unable to dilute contaminants. Combining these chemical and physical factors, it can be concluded that Site 4 has moderate water quality.

Although biological, chemical, and physical measurements agree that Site 4 has moderate water quality, it is likely that water quality was higher in the past. Before the San Francisco Hydroelectric Project in 2006, there was a much higher discharge in Site 4 (J. Recalde, Personal Communication, December 5, 2021). Discharge was depleted due to the construction of underground tunnels bringing water to the Pastaza River's Agoyán Dam. With a higher discharge, Site 4 would likely have a higher water quality due to the ability to disperse contaminants. Nevertheless, hydromorphological quality at Site 4 was likely still "bad" before 2006, due to its location along the main road. In the future, it would be interesting to investigate the history of the adjacent mandarin grove and its use of agrichemicals for more comprehensive conclusions.

Site 5, a stream from one of the three natural sources of El Placer's drinking water, was found to have very good water quality based on biological assessments. Of the five sites tested, Site 5 is the only with "excellent" hydromorphological quality (Table 9). Located within secondary forest, Site 5 had excellent structure and naturalness of vegetation on riverbank, continuity of riverbank, and connectivity of riparian vegetation with other adjacent or nearby landscape elements. The channel was not completely natural due to pipes constructed to bring this water to El Placer. Site 5 scored "bad", "excellent" and "good" on the EPT, ABI, and BMWP/Col Indices, respectively. Although Site 5 scored "bad" on the EPT Index, several pollution-sensitive families were found which allowed for Site 5 to score in the highest category in the other two indices. The families Polythoridae, Calomorceratidae, Blepharocidae, and Perlidae were found, all of which scored a 10/10 on both the ABI and BMWP/Col Indices. Considering Site 5's high sample coverage of 97.8% (Table 14), it is likely that macroinvertebrate analysis was representative of the current macroinvertebrate community. Based on these biological findings, it can be concluded that Site 5 has very good water quality.

Chemical analysis confirms these findings of very good water quality in Site 5. Site 5's TDS and EC values of 85 ppm and 171 uS/cm, respectively, are well below the maximum recommended values of 500 ppm and 200 uS/cm, respectively. These chemical variables suggest that Site 5 does not contain many dissolved contaminants. In addition, Site 5's neutral pH of 6.99 indicates good water quality. Furthermore, the ORP in Site 4 is 327 mV, which is within the ORP range of healthy lakes and rivers, 300-500 mV (Table 8).

Physically, Site 5's velocity and discharge were too small to measure accurately. With a higher velocity and discharge, it is possible that water quality would have been even higher due to more aeration along with dispersion of any contaminants. Site 5's limited water flow also affected collection techniques - macroinvertebrates were manually collected for 2.5 hours rather than collected with a surber net. Despite this change in methodology, 264 individuals were found in Site 5, which is more than any other study site. Nevertheless, it is possible that the sample was less representative due to this collection method. The richness of orders such as Ephemeroptera may not have been representative due to this order's smaller size and higher mobility, which is a possible reason for Site 5's "bad" score in the EPT index.

Sources of Error

This study contained several sources of error which likely had an influence on results. To begin, the water quality meter, Multifunction Model C-600, may have been inaccurate. At times, measurements within a certain site had high variability. Although each chemical value included in the study was an average of four measurements, it is possible that chemical data is inconclusive due to inaccuracy of the water quality meter. Furthermore, although testing of sites always occurred in the mid-morning or early-afternoon, weather conditions were variable. Variation in weather conditions, such as temperature and precipitation, may have influenced physical and chemical data. In streams with a smaller discharge, water temperature was likely more influenced by weather conditions. Another possible source of error is the mis-identification of macroinvertebrate families, which would influence values of the ABI and BMWP/Col Indices. Due to the fragile bodily structures of macroinvertebrates, their legs and tails were often lost before identification, leading to difficulty in accurately identifying their families. It is also important to note that the ABI is designed for rivers with altitudes greater than 2000 meters (Encalada et al., 2011), whereas this study's sites were between 1500 and 1800 meters. Since this study's sites were not within the altitudinal range of the ABI, it is possible that the ABI values may not be accurate.

Conclusion

Through the comparison of chemical factors, hydromorphological quality, and macroinvertebrate composition at each of the study sites, the objectives to determine the effect of agriculture on the Tía Anita Stream were achieved. Using the ABI and BMWP/Col Indices, it was concluded that Site 3, the secondary forest site, had higher water quality than Sites 1 and 2, the sites influenced by agriculture (Tables 12, 13). Although Site 1 had the highest EPT Index (Table 11), this index does not account for pollution-sensitive families in other orders. Chemical analysis, which was inconsistent with biological analysis, found that Site 1 had the lowest water quality and Site 2 had the highest water quality. This inconsistency may have been due to stream velocity affecting sediment load (which in turn affects TDS and EC) as well as incomplete sampling in Site 2 as determined by the sample coverage curves. Overall, the data show that agriculture does have an influence on water quality in terms of macroinvertebrate composition and hydromorphological quality.

The second objective to investigate the quality of El Placer's historical and current drinking water sources was also achieved through the analysis of chemical factors, physical factors, hydromorphological quality, and macroinvertebrate composition. Site 4, El Placer's historical potable water source, was found to have moderate quality based on biological and chemical factors. Scoring "bad" in EPT, "good" in ABI, and "acceptable" in BMWP/Col, macroinvertebrate composition suggests moderate water quality (Tables 11, 12, and 13). It is important to note that sampling coverage was the lowest at Site 4, at only 93.1% (Table 14); more sampling at this site would lead to stronger conclusions based on macroinvertebrate composition. Although Site 4's TDS, EC, and pH suggested good quality water, its low ORP suggests a low ability to cleanse itself from contaminants. Hydromorphological quality of Site 4 was "bad" due to the influence of the neighboring road and mandarin groves. It is important to note that in the past, Site 4's water quality was likely higher due to increased discharge. Site 5, El Placer's current potable water source, was found to have very good water quality. Scoring "bad" in EPT, "excellent" in ABI, and "good" in BMWP/Col, macroinvertebrate composition suggests very good water quality (Tables 11, 12, 13). Although Site 5 scored "bad" on the EPT Index, several pollution-sensitive families were found which allowed for Site 5 to score in the highest category in the other two indices. Chemical analysis confirmed very good water quality in Site 5 with healthy values for TDS, EC, ORP, and pH (Table 8). Scoring "excellent", Site 5 also had the best hydromorphology of all 5 sites, which is another confirmation of very good water quality.

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Appendix A: Criteria for the Hydromorphological Quality Index (Encalada et al., 2011). Each site was rated between 0 and 5 (inclusive) for each criterion.

Criterion	0 (terrible)	5 (excellent)	Other
Structure and naturalness of vegetation on riverbank	Banks are barren with stripped soil	Banks have mixed trees with native species and excellent amount of nature	
Continuity of riverbank	Discontinuous, small patches of vegetation	Continuous vegetation without patches of cattle ranching/farming	
Connectivity of riparian vegetation with other adjacent or nearby landscape elements	More than 50% of adjacent landscape is composed of agriculture/urban elements	Adjacent landscape is at least 75% natural vegetation without urban elements.	
The presence of trash and debris	Trash accumulated like a dump that would need to be removed by machinery	No trash/debris.	
Naturalness of river channel	Two sides of river channel are modified by solid structures and naturalness is nonexistent	No signs of modification such as cement/other structures and there is an excellent degree of naturalness.	
Substrate composition			Number of substrates found; for each one present, a point is added.
Velocity and depth of the river			Fast-shallow, fast-deep, slow-shallow, slow-deep; shallow is under 0.4 m and fast is a floating object traveling at least 30 cm/sec. For each combination present, 1 point was added. If all 4 were present, an extra point was added
Elements of heterogeneity			For each element present: leaf litter, trunks and branches, natural dams, submerged roots, submerged aquatic vegetation (mosses and plants) and submerged aquatic vegetation (algae), a point is added

Appendix B: Individual Family Scores for ABI (Encalada et al., 2011).

Order	Family	ABI Score
Turbellaria	Planariidae	5
Hirudinea		3
Oligochaeta		1
Gastropoda	Anclidae	6
	Physidae	3
	Hydrobiidae	3
	Limnaeidae	3
	Planorbidae	3
Bivalvia	Sphaeriidae	3
Amphipoda	Hyalellidae	6
Ostracoda		3
Hydracarina		4
Ephemeroptera	Baetidae	4
	Leptophlebiidae	10
	Leptohyphidae	7
	Oligoneuriidae	10
Odonata	Aeshnidae	6
	Gomphidae	8
	Libellulidae	6
	Coenagrionidae	6
	Calopterygidae	8
	Polythoridae	10
Plecoptera	Perlidae	10
	Gripopterygidae	10
Heteroptera	Veliidae	5
	Gerridae	5
	Corixidae	5
	Notonectidae	5
	Belostomatidae	4
	Naucoridae	5
Trichoptera	Helicopsychidae	10
	Calamoceratidae	10
	Odontoceridae	10
	Leptoceridae	8
	Polycentropodidae	8
	Hydroptilidae	6
	Xiphocentronidae	8
	Hydrobiosidae	8
	Glossosomatidae	7
	Hydropsychidae	5
	Anamalopsychidae	10
	Philopotamidae	8
	Limnephilidae	7
Lepidoptera	Pyralidae	4

Order	Family	ABI Score
Coleoptera	Ptilodactylidae	5
	Lampyridae	5
	Psephenidae	5
	Scirtidae	5
	Staphylinidae	3
	Elmidae	5
	Dryopidae	5
	Gyrinidae	3
	Dytiscidae	3
	Hydrophilidae	3
	Hydraenidae	5
Diptera	Blepharoceridae	10
	Simuliidae	5
	Tabanidae	4
	Tipulidae	5
	Limoniidae	4
	Ceratopogonidae	4
	Dixidae	4
	Psychodidae	3
	Dolichopodidae	4
	Stratiomyidae	4
	Empididae	4
	Chironomidae	2
	Culicidae	2
	Muscidae	2
	Ephydridae	2
	Athericidae	10
	Syrphidae	1

Appendix C: Individual Family Scores for BMWP/Col Index (Bueñano et al., 2018).

Families	Score
Anamalopsychidae, Atriplectididae, Blepharoceridae, Calamoceratidae, Ptilodactylidae, Chordodidae, Ghomphidae, Hydridae, Lampyridae, Lymessiidae, Odontoceridae, Oliigoneuridae, Perlidae, Polythoridae, Psephenidae	10
Ampullariidae, Dytiscidae, Ephemeridae, Euthyplociidae, Gyrinidae, Hydraenidae, Hydroboscidae, Leptophlebiidae, Phylopotamidae, Polycentropodidae, Polymitarcyidae, Xiphocentronidae	9
Gerridae, Hebridae, Helicopsychidae, Hydrobiidae, Leptoceridae, Lestidae, Palaemonidae, Pleidae, Pseudothelpusidae, Saldidae, Simulidae, Veliidae	8
Baetidae, Caenidae, Calopterygidae, Coenagrionidae, Corixidae, Dixidae, Dryopidae, Glossomatidae, Hyalellidae, Hydropsychidae, Leptohyphidae, Naucoridae, Notonectidae, PLanariidae, Psychodidae, Scirtidae	7
Aeshnidae, Ancylidae, Corydalidae, Elmidae, Libellulidae, Limnichidae, Lutrochidae, Megapodagrionidae, Sialidae, Staphylinidae	6
Belostomatidae, Gelastocoridae, Mesoveliidae, Nepidae, Planorbiidae, Pyralidae, Tabanidae, Thiaridae	5
Chrysomelidae, Stratiomyidae, Haliplidae, Empididae, Dolichopodidae, Sphaeridae, Lymnaeidae, Hydrometridae, Notoceridae	4
Ceratopogonidae, Glossiphonidae, Cyclobdellidae, Hydrophylidae, Physidae, Tipulidae	3
Culicidae, Chironomidae, Muscidae, Sciomyzidae, Syrphidae	2
Tubificidae	1

Appendix D: Photos of Each Order & Order Composition at Each Site.


Order Diptera
Family Tipulidae


Order Ephemeroptera
Family Baetidae


Order Trichoptera
Family Hydropsychidae


Order Coleoptera
Family Elmidae


Order Odonata
Family Aeshnidae


Order Plecoptera
Family Perlidae


Order Isopoda

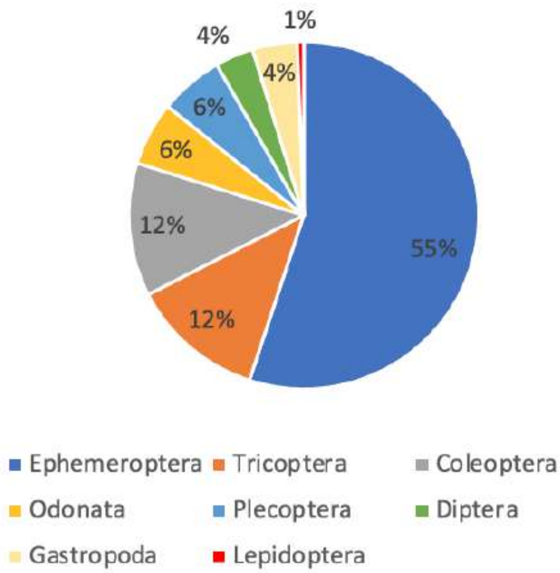

Order Gastropoda
Family Physidae


Order Crustacea
Family Hyalellidae

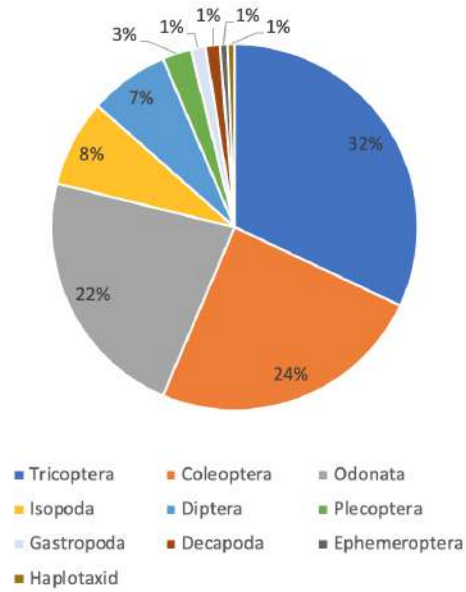

Order Haplotaxida


Order Lepidoptera

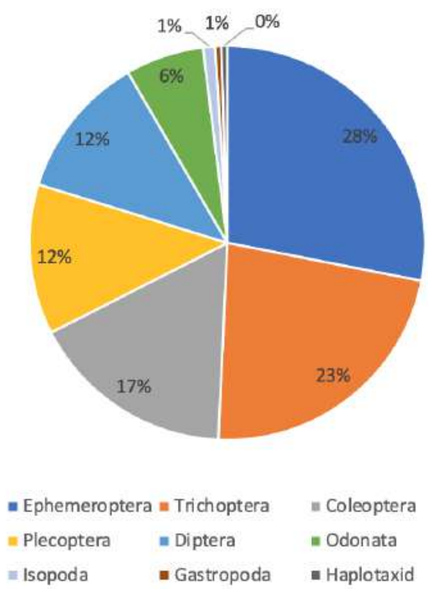

Order Decapoda
Family Brachyura



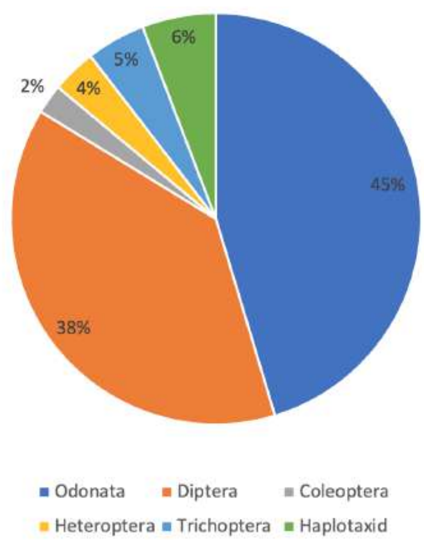
Site 1.



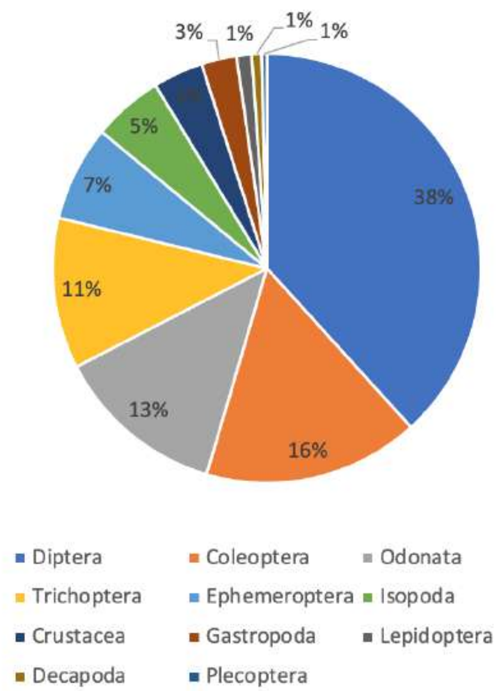
Site 2.



Site 3.



Site 4.


3%
1%
1%
1%
5%
7%
38%
11%
13%
16%
Diptera
Coleoptera
Odonata
Trichoptera
Ephemeroptera
Isopoda
Crustacea
Gastropoda
Lepidoptera
Decapoda
Plecoptera

Site 5.

Appendix E: Family Composition at Each Site.

Site 1.

Family	Count
Leptohyphidae	83
Hydropsychidae	13
Elmidae	12
Baetidae	10
Perlidae	10
Ptilodactylidae	8
Physidae	7
Gomphidae	5
Tipulidae	4
Calamoceratidae	2
Polythoridae	3
Hydrobiosidae	3
Leptoceridae	2
Empididae	2
Dytiscidae	1
Aeshnidae	1
Calopterygidae	1
Polycentropodidae	1

Site 2.

Family	Count
Hydropsychidae	49
Polythoridae	43
Emidae	24
Tipulidae	11
Ptilodactylidae	8
Perlidae	4
Dytiscidae	2
Planorbidae	2
Hydrophilidae	2
Calopterygidae	2
Brachyura	2
Calamoceratidae	1
Scirtidae	1
Hydrochidae	1
Libellulidae	1
Aeshnidae	1
Baetidae	1
Tubificidae	1

Site 3.

Family	Count
Leptohyphidae	42
Hydropsychidae	33
Perlidae	25
Tipulidae	19
Elmidae	18
Baetidae	9
Chironomidae	9
Gomphidae	7
Calamoceratidae	6
Ptilodactylidae	6
Scirtidae	5
Oligoneuriidae	5
Polythoridae	4
Lampyridae	3
Hydrobiosidae	3
Philopotamidae	2
Leptoceridae	2
Aeshnidae	2
Hydroscaphidae	1
Dytiscidae	1
Dixidae	1
Leptophlebiidae	1
Planorbidae	1
Tubificidae	1

Site 4.

Family	Count
Chironomidae	19
Gomphidae	17
Aeshnidae	17
Tipulidae	12
Hydropsychidae	4
Tubificidae	4
Veliidae	3
Libellulidae	2
Ptilodactylidae	2
Simuliidae	1
Dolichopodidae	1
Coenagrionidae	1
Polythoridae	1
Calopterygidae	1
Enchytraeidae	1

Site 5.

Family	Count
Simuliidae	38
Tipulidae	34
Polythoridae	24
Hydropsychidae	24
Chironomidae	23
Scirtidae	21
Baetidae	19
Styloniscidae	14
Libellulidae	10
Hyalellidae	10
Dytiscidae	7
Hydrophilidae	6
Physidae	6
Calamoceratidae	6
Elmidae	5
Dixidae	3
Blephariceridae	2
Chironomidae	2
Ceratopogonidae	1
Culicidae	1
Curculionidae	1
Staphylinidae	1
Perlidae	1
Ancylidae	1
Brachyura	2

Appendix F: ABI Calculations for Each Site.

Site 1 Families	Score
Leptohyphidae	7
Hydropsychidae	5
Elmidae	5
Baetidae	4
Perlidae	10
Ptilodactylidae	5
Physidae	3
Gomphidae	8
Tipulidae	5
Calamoceratidae	10
Polythoridae	10
Hydrobiosidae	8
Leptoceridae	8
Empididae	4
Dytiscidae	3
Aeshnidae	6
Calopterygidae	8
Polycentropodidae	8
ABI	**117**

Site 2 Families	Score
Hydropsychidae	5
Polythoridae	10
Emidae	5
Tipulidae	5
Ptilodactylidae	5
Perlidae	10
Dytiscidae	3
Planorbidae	3
Hydrophilidae	3
Calopterygidae	8
Brachyura	0
Calamoceratidae	10
Scirtidae	5
Hydrochidae	0
Libellulidae	6
Aeshnidae	6
Baetidae	4
Tubificidae	0
ABI	**88**

Site 3 Families	Score
Leptohyphidae	7
Hydropsychidae	5
Perlidae	10
Tipulidae	5
Elmidae	5
Baetidae	4
Chironomidae	2
Gomphidae	8
Calamoceratidae	10
Ptilodactylidae	5
Scirtidae	5
Oligoneuriidae	10
Polythoridae	10
Lampyridae	5
Hydrobiosidae	8
Philopotamidae	8
Leptoceridae	8
Aeshnidae	6
Hydroscaphidae	0
Dytiscidae	3
Dixidae	4
Leptophlebiidae	10
Planorbidae	3
Tubificidae	0
ABI	**141**

Site 4 Families	Score
Chironomidae	2
Gomphidae	8
Aeshnidae	6
Tipulidae	5
Hydropsychidae	5
Tubificidae	0
Veliidae	5
Libellulidae	6
Ptilodactylidae	5
Simuliidae	5
Dolichopodidae	4
Coenagrionidae	6
Polythoridae	10
Calopterygidae	8
Enchytraeidae	0
ABI	**75**

Site 5 Families	Score
Simuliidae	5
Tipulidae	5
Polythoridae	10
Hydropsychidae	5
Chironomidae	2
Scirtidae	5
Baetidae	4
Styloniscidae	0
Libellulidae	6
Hyalellidae	6
Dytiscidae	3
Hydrophilidae	3
Physidae	3
Calamoceratidae	10
Elmidae	5
Dixidae	4
Blepharocidae	10
Chironomidae	2
Ceratopogonidae	4
Culicidae	2
Curculionidae	0
Staphylinidae	3
Perlidae	10
Ancylidae	0
Brachyura	0
ABI	**107**

Appendix G: BMWP/Col Calculations for Each Site.

Site 1 Families	Score
Leptohyphidae	7
Hydropsychidae	7
Elmidae	6
Baetidae	7
Perlidae	10
Ptilodactylidae	10
Physidae	3
Gomphidae	10
Tipulidae	3
Calamoceratidae	10
Polythoridae	10
Hydrobiosidae	0
Leptoceridae	8
Empididae	4
Dytiscidae	9
Aeshnidae	6
Calopterygidae	7
Polycentropodidae	9
BMWP/Col	**126**

Site 2 Families	Score
Hydropsychidae	7
Polythoridae	10
Emidae	6
Tipulidae	3
Ptilodactylidae	10
Perlidae	10
Dytiscidae	9
Planorbidae	5
Hydrophilidae	3
Calopterygidae	7
Brachyura	0
Calamoceratidae	10
Scirtidae	7
Hydrochidae	0
Libellulidae	6
Aeshnidae	6
Baetidae	7
Tubificidae	1
BMWP/Col	**107**

Site 3 Families	Score
Leptohyphidae	7
Hydropsychidae	7
Perlidae	10
Tipulidae	3
Elmidae	6
Baetidae	7
Chironomidae	2
Gomphidae	10
Calamoceratidae	10
Ptilodactylidae	10
Scirtidae	7
Oligoneuriidae	10
Polythoridae	10
Lampyridae	10
Hydrobiosidae	0
Philopotamidae	0
Leptoceridae	8
Aeshnidae	6
Hydroscaphidae	0
Dytiscidae	9
Dixidae	7
Leptophlebiidae	9
Planorbidae	5
Tubificidae	1
BMWP/Col	**154**

Site 4 Families	Score
Chironomidae	2
Gomphidae	10
Aeshnidae	6
Tipulidae	3
Hydropsychidae	7
Tubificidae	1
Veliidae	8
Libellulidae	6
Ptilodactylidae	10
Simuliidae	8
Dolichopodidae	4
Coenagrionidae	7
Polythoridae	10
Calopterygidae	7
Enchytraeidae	0
BMWP/Col	**89**

Site 5 Families	Score
Simuliidae	8
Tipulidae	3
Polythoridae	10
Hydropsychidae	7
Chironomidae	2
Scirtidae	7
Baetidae	7
Styloniscidae	0
Libellulidae	6
Hyalellidae	7
Dytiscidae	9
Hydrophilidae	3
Physidae	3
Calamoceratidae	10
Elmidae	6
Dixidae	7
Blepharocidae	10
Chironomidae	2
Ceratopogonidae	3
Culicidae	2
Curculionidae	0
Staphylinidae	6
Perlidae	10
Ancylidae	6
Brachyura	0
BMWP/Col	**134**