Spring 2015

Human impacts on water systems: Biological assessment of water quality in the Bosque Protector Río Guajalito (BPRG) using aquatic macroinvertebrates

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***Human impacts on water systems*:**
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Submitted in partial fulfillment of the requirements for Ecuador: Comparative Ecology and Conservation, SIT Study Abroad, Spring 2015

Acknowledgements

To my parents, for their understanding, care and encouragement throughout my life.

To my academic director, Dr. Xavier Silva, for his encouragement, knowledge and guidance throughout this semester.

To my professor Javier Robayo, for his effort, knowledge and guidance this semester.

To my advisor Carolina Arroyo, for her help identifying macroinvertebrates and with the writing and editing of my paper.

To Dr. Vlastimil Zak, for showing me the Bosque Protector Rio Guajalito and for kind enough to bring me chocolates on the weekends.

To my host family the Verdesotos, for taking me in as one of their own and showing me around Ecuador.

To Doña Maria and her family, for the delicious meals she cooked me every day and for the company she provided.

To my family and friends, for their affection and love.

Abstract

Benthic macroinvertebrates have been extensively used as bioindicators for water quality due to their varying sensitivity to a diverse range of impacts on hydrographic sources. In this study a sampling of macroinvertebrates was carried out in the Guajalito and Brincador rivers and a small creek that runs through the private reserve Bosque Protector Rio Guajalito in order to analyze if the health of the rivers has changed in relation to previous studies. The sampling stations were located upstream and downstream of potential sources of disturbance in each of the three streams sampled in order to determine what level of impact the disturbance had on the stream. Additionally basic physiochemical parameters were characterized at each sampling station to validate the biological data. Significant differences were observed in the water quality biotic indexes of BMWP and IBMWP between the three streams sampled. There were no significant differences, however, in the abundance, richness, biological index scores, and diversity measures between the upstream and downstream stations. According to the biological index scores of BMWP and IBMWP, the three streams sampled have fair and good water quality. According to the BMWP/Col, Sensibilidad, FBI and %EPT indexes, however, the streams rank as good, very good, and excellent. These results are consistent with the previous study conducted by Arroyo (2007) and indicate that the water quality conditions have not significantly changed in the time since that study.

ISP Topic Codes: 614, 615, 627
Keywords: bioindication, macroinvertebrates, rivers, water quality

Resumen

Macroinvertebrados bentónicos han sido ampliamente utilizados como bioindicadores de la calidad del agua debido a su sensibilidad a la variación de una amplia gama de impactos sobre las fuentes hidrográficas. En este estudio un muestreo de macroinvertebrados se realizó en los ríos Guajalito y Brincador y un pequeño arroyo que corre a través de la reserva privada Bosque Protector Río Guajalito, para analizar si la salud de los ríos ha cambiado en relación a los estudios anteriores. Las estaciones de muestreo fueron ubicados arriba y abajo de las fuentes posibles de perturbación en cada uno de las tres ríos para determinar el nivel de impacto que la perturbación tuvo en cada río. Además parámetros fisicoquímicos básicos se caracterizaron en cada estación de muestreo para validar los datos biológicos. Se observaron diferencias significativas en los índices bióticos de calidad del agua de BMWP y IBMWP entre los tres ríos muestreados. No hubo diferencias significativas, sin embargo, en la abundancia, la riqueza, las puntuaciones del índice biológicos, y índices de diversidad entre las estaciones arribe y debajo de las fuentes posibles de perturbación. De acuerdo con las puntuaciones del índice biológico de BMWP y IBMWP, los tres ríos tienen la calidad del agua razonable y buena. Según el BMWP / Col, Sensibilidad, el FBI y el índice %EPT, sin embargo, los aguas son de calidad buena, muy buena, y excelente. Estos resultados son consistentes con el estudio realizado previamente por Arroyo (2007) e indican que las condiciones de calidad del agua no han cambiado de manera significativa en el tiempo transcurrido desde el estudio anterior.

ISP códigos tema: 614, 615, 627
Palabras clave: bioindicación , macroinvertebrados , ríos , calidad del agua

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Introduction

The preservation of water quality in naturally occurring streams and rivers has become an issue of growing concern in recent decades. Access to clean water is an important resource for many urban, industrial, and agricultural activities, among others. However, the use of water sources for human needs has had negative impacts on the health of aquatic ecosystems, and has contributed to decreasing water quality in many streams and rivers through contamination, alteration and overexploitation (Baron *et al*. 2002). This decline in clean water resources has been the cause of worry for many people in recent decades, the result of which is that there is now much interest in ensuring water quality is monitored and protected (Toro *et al.* 2003).

Initially monitoring of water quality was heavily based in chemical parameters, but increasingly biomonitoring has become a popular way of assessing and preservering water. The basic theory being biomonitoring is that because organisms live in the aquatic system under question, they are subject to pollutants and other impacts. Therefore the health of aquatic organisms reflects the quality of the water they live in (Byl *et al.* 1994). One effective means of assessing water quality is through observation of benthic macro-invertebrate community structure. Combined with the measurement of physical and chemical parameters as reference conditions, macroinvertebrates provide a valuable tool for monitoring water quality conditions (Hilsenhoff, 1987; Justus et al, 2010; Smith et al., 2007; Washington, 1984).

At this point in time benthic macros-invertebrate sampling is one of the most abundant types of biomonitoring techniques. This is largely due to the many advangaes that these organisms have for bioassessment purposes. First of all, they are abundant and easy to collect given their sedentary lifestyle. Additionally, most can be viewed easily with the naked eye(Alba-Tercedor 1996, Toro *et al.* 2003). Second, Benthic macro-invertebrate communities are diverse and susceptible to change in environmental quality. (Klemm *et al.* 1990, Alba-Tercedor 1996, Merrit and Cummings 1996)The diversity and varying sensitivity among benthic macroinvertebrates makes them ideal for studies involving stream water integrity because various taxa are tolerant of a variety of different pollutants. Assessment of benthic community structure may reveal the absence of a pollution intolerant taxa, dominance of particular taxon, low taxon richness, or measureable changes in community structure. Disturbances can be detected in macro-invertebrate community structure up to a period of a few weeks to months (Alba-Tercedor 1996). When compared with some reference condition, such as the presence or absence of a given stressor, results can provide a quantified measure of stream integrity. (Barbour et al., 1999; Lazorchak et al., 1998; Plafkin et al., 1989; USEPA, 2006). Lastly, there are a variety of different methodologies for assessment of aquatic invertebrates that are specialized for regions throughout the globe (Toro *et al.* 2003).

Many methodologies are already developed and well established, such as the Biological Monitoring Working Party (BMWP), the Family-level Biotic Index (FBI), among others. (Zimmerman 1993, Alba-Tercedor 1996, Roldán 2003) Most of these methodologies, however, have been specialized for specific regions, mostly temperate regions in Europe. Few studies of this kind have been carried out in Latin America (Segnini 2003). The result is that most studies have been developed in temperate regions not in tropical regions, and these methodologies do not work in areas outside where they were created.

The area where this study will take place is known as the Bosque Protector Rio Guajalito (BPRG). The BPRG is a private reserve of 710 ha located in the central eastern zone of Ecaudor at the 59 kilometer mark of the old Quito-San Juan-Chiriboga-Santo Domingo road (00º 14' 57''S 78º 48'22''O) in the Pinchincha region of Ecuador (Figure 1). The reserve is located in the ecosystem known as Andean cloud forest and occupies an altitudinal range of 1800 to 2300 m. The BPRG has a two seasons, wet and dry. The wet season lasts from around December until May, and the dry season from June until November. Annual precipitation varies between 3700 and 2800 mm, and the average temperature is 16.4 ˚C with slight variation during the year (Robayo *et al.* 2004).

BPRG has a wide variety of animal and plant diversity. The reserve is home to 236 species of birds, 14 of which are endemic to the zone and four of which are listed as in danger of extinction by the IUCN. Additionally it has 45 mammal species and 47 species of amphibians and reptiles. In terms of plant species, 85 families of Angiosperms have been reported corresponding to 217 genuses and 345 species. 22 families of Pteridophytes have been reported from 42 genuses and 74 species (Robayo *et al.*2004).

The water basin within the BPRG sustains diverse animal and plant life in the area, not to mention the people who use the rivers as a source of water. Three rivers are present within the reserve, the Rio Guajalito, Rio Palmeras and Rio Brincador. Very few studies have characterized the water conditions in the area or characterized the lotic ecosystems within the reserve (Robayo *et al.* 2004, Arroyo 2007.). One previous water quality study using macroinvertebrates as bioindicators demonstrated that the rivers Guajalito, Palmera and Brincador are of good health and that a gradient of human impacts exists between the three rivers. According to this gradient, Rio Guajalito is the most impacted by human use, Rio Palmeras next, and Rio Brincador is the least impacted by humans (Arroyo 2007).

The BPRG is one of the few areas in the surroundings of the city of Quito that still contains well conserved ecosystems. Therefore it is very important that the area is conserved and managed sustainable. The results of this study will contribute to a body of knowledge that will help those managing the BPRG make informed decisions regarding how to best conserve resources in the area.

The overarching objective of this study is to assess the health of Rios Brincador and Guajalito using macro invertebrates as bioindicators of water quality. The results found will be compared to the previous study conducted by Arroyo (2007) in order to determine if water quality conditions have changed. Additionally, a study will be carried out regarding possible correlations between the population abundances of benthic macroinvertebrates and human impacts on water sources in the BPRG. Specifically this study will look at the impacts of recently established trout farming on the Rio Brincador and the alteration of the flow of a nearby creek to provide a water source for BPRG house. The trout farming on Rio Brincador was established after the study by Arroyo (2007) was carried out, and the creek flow was altered as a water source approximately three years ago.

The Rios Guajalito and Brincador are likely still in good health. While trout farming and water removal do affect health of aquatic ecosystems, it is hypothesized that these stresses do not yet exceed the capacity of the stream's ability to recover and that all the streams will exhibit good water quality. The gradient of human impact established previously by Arroyo (2007) is still expected to be accurate, with Rio Brincador less impacted than Rio Guajalito. Samples taken from sites downstream of the sources of stress, however, are expected to be less healthy than those upstream. Downstream samples are also expected to have different physiochemical properties as those from upstream. Additionally, a higher diversity of aquatic life is expected than was seen in the study by Arroyo (2007) due to the fact that this study takes place during the rainy season whereas the previous study was during the dry season.

Materials and Methods

Two sampling stations were established along Rio Brincador (B), two on the Rio Guajalito (G) and two in the creek used as a water source for the BPRG house (C), resulting in six sampling stations in total. Sampling stations were located both upstream (U) and downstream (D) of potential disturbances in each of the bodies of water. For B, one station was established upstream of the trout farming activities in prime forest and another downstream adjacent to the BPRG house. The sampling stations along G were positioned both upstream and downstream of the confluence with Rio Brincador. In the case of C, one sampling station was established upstream of the dam where water is removed for the BPRG house and another downstream. Each sampling station consists of a single riffle, from which all samples for that station were collected. The location of each station was selected based on accessibility and location in relation to potential sources of disturbance or pollution. All sampling was carried out in the month of April 2015, during the rainy season.

Physiochemical parameters were assessed in order to characterize each of sampling stations. The length of each riffle was measured prior to sampling. The depth and width were measured along two transects in each riffle, where the depth was measured at three equidistance points along each transect. The superficial velocity of the current was determined by timing a floating object in the water as it traveled 10 meters. Total volume of water (discharge) was then estimated by multiplying the width, depth, and velocity of the current. Chemical analysis of each stream was performed using a PondCare® Master Liquid Test Kit. The pH, Ammonia (NH_3/NH_4^+), Nitrate (NO_2^-) and Phosphate (PO_4^{3-}) levels were assessed at each sampling station.

Sampling locations within each riffle were selected strategically in order to achieve a high diversity of habitat types. Samples were taken near both banks, the center of the stream, and from the initial, middle, and terminal portions of each riffle. The percentage of canopy coverage was characterized at each sampling location using visual estimation. Based on the degree of coverage each site was assigned a number between one and five, with one being completely exposed and five being totally shaded. Additionally the degree of embeddedness, presence of organic material in the substrate, amount of aquatic vegetation and amount of algae was ranked on a scale of one to five at each of the sampling sites based on visual estimation.

At each of the four sampling station in rivers B and G a total of six samples of macroinvertebrates were collected. Due to the relatively small size of C only three samples were taken from each of the two stations located there, resulting in thirty samples total from all sampling stations. Sampling was done with a modified kick net measuring 0.65 by 0.65 meters with a mesh size of 1.0 millimeter. The net was placed upstream of the person sampling. All large rocks within arm's reach upstream of the net were scrubbed for a period of at least thirty seconds or until clean. Next the ground in the area upstream of the net within arm's reach was disturbed for a period of one minute, allowing all debris to flow downstream into the net. The same amount of effort was used at each of the sites.

Once collected the macroinvertebrates were identified using an Olympus SZ40 microscope with 10X - 40X magnification. Invertebrates were identified to the level of family where possible. The invertebrate families were characterized according to their method of acquiring food, or functional feeding group (FFG) (Wallace 1996). These groups included:

Scrapers – organisms that graze or scrape their food from mineral and organic substrates.

Shredders – organisms that break up large decomposing plant tissue or wood and associated microflora and fauna and living vascular macrophytes.

Gatherers – organisms that feed on particulate organic matter deposited in streams.

Filterers – organisms that have specialized body parts that act as sieves to remove particulate matter in suspension.

Predators – organisms that feed on animal tissue by engulfing, prey or piercing and sucking out body contents

Results from the sampling of macroinvertebrates were then evaluated using the following indexes:

BMWP (Biological Monitoring Working Party) (Armitage et al. 1983) – This biological index assigns a score between 1 and 10 to various taxa. The higher or lower the score is based on the taxa's sensitivity to contamination or oxygen deficits, with 1 being the least sensitive and 10 being the most sensitive.

IBMWP (Alba-Tercedor 1996) – This is the BMWP index thaws was adapted for Spain. It uses the same scoring system and criteria as the BMWP.

BMWP/Col. (Roldán 2003) – This index is the BMWP adapted for Colombia, with the same scoring system and criteria as the BMWP.

Sensibilidad (Carrera et al. 2001) – This index was developed in order to adapt the BMWP/Col index for macroinvertebrates found in rivers from the coast of Ecuador

FBI (Family-level biotic index) (Hilsenhoff 1987, Hilsenhoff 1988) – This index was developed by Hilsenhoff as a rapid assessment index based on richness families and relative abundance of macroinvertebrates. Each family is assigned a point between 1 and 10 with corresponding to its level of resistance to contamination, with 1 being the least resistant and 10 being the most resistant. That number is then multiplied by the number of individuals in that taxa are present in a sample and then divided by the total number of individuals from all taxa in that sample.

%EPT (Carrera et al. 2001) – This index is based on the relative abundance of macroinvertebrates from the orders Ephemeroptera, Plecoptera and Trichoptera present in a sample. The abundance of all individuals from these three orders is divided by the total abundance of all individuals in the sample to give the percentage of EPT.

In addition to the indexes described above, the total richness at the level of family, Shannon diversity (H'), exponential Shannon diversity (exp(H')), Simpson diversity index (1-D) were calculated for each sampling station and each river.

All of the biotic and abiotic variables assessed in this study were subjected to the Shapiro-Wilk and Anderson-Darling normality tests (Hammer et al. 2001). The data sets that did not display normal distribution were transformed using the natural log. If the natural log of the variables continued to be non-normally distributed, the variables were analyzed using non-parametric tests.

A one way analysis of variance (ANOVA) was performed in order to determine if any relationships existed between the physiochemical data sets and the three streams or the upstream and downstream sampling stations (Hammer et al. 2001). For the data sets that were not normally distributed, the non-parametric Kruskal-Wallis test was used. In both cases the response variables were depth, width, velocity, discharge, degree of embeddedness, amount of organics in the substrate, canopy coverage, pH and Ammonia. The explicatory variables were the stream sources (three levels G, B and C) and the location in relation to a potential disturbance (two levels upstream and downstream). One way analysis of variance (ANOVA) and Kruskal-Wallis tests were also carried out in order to analyze the macro-invertebrate communities sampled. The response variables in this case were richness, diversity indexes, abundance, BMWP, IBMWP, BMWP/Col and Sensibilidad, FBI, and %EPT and the explicatory variables were the same as above.

Additionally Pearson correlations were carried out in order to measure any correlation between the physiochemical variables and abundance, richness and diversity indexes for the three streams (B, G and C) and for the different categories of sampling stations (upstream and downstream). In the case of non-normally distributed data, Spearman correlations were used.

Results

The physical parameters that were assessed varied significantly between the three sources. The depth of the water ranged from 0.147 to 0.745 m (Table 1) and showed significant variation between the three sources ($F=9.296$, $P=0.001$). Of the three sources of water, G was the most profound in depth and C was the least. The depth also varied significantly between upstream and downstream sampling stations ($F=4.444$, $P=0.044$). In all three of the streams, the average depth increased in downstream sources as compared to upstream sources. The width of the streams ranged from 1.079 to 9.2 m (Table 1) and also varied significantly between the three sources ($H=7.731$, $P=0.021$), with G having the widest and C having the most narrow width. The width of the streams did not show significant differences between upstream and downstream stations. Superficial stream velocity varied between 0.54 and 1.085 m/s (Table 1) and was significantly different between the three streams ($F=20.49$, $P=1.95E-04$). The approximate discharge ranged from 0.095 to 7.438 m^3/s (Table 1) and also displayed significant variation between the three sources ($H=18.7$, $P=8.68E-05$). G was measured as having both the highest superficial stream velocity and approximate discharge, where as C had the lowest. In both B and C, the approximate discharge decreased in downstream sampling stations, but in G the discharge increased downstream (Table 1). However, the superficial stream velocity and discharge did not vary significantly between upstream and downstream sources.

The ranking of the degree of embeddedness of the substrate ranged from 1.5 to 3.33 out of 5 on average (Table 3). Embeddedness did not vary significantly between the three sources, but did vary significantly between upstream and downstream sampling stations (H=7.381, P=0.005). In all three streams, the degree of embeddedness decreased in downstream sampling stations as compared to those upstream. All three streams were dominated by cobble and gravel on average, with occasional sand or boulder/bedrock. The dominant substrate at each sampling station did not show significant variation between the three streams or between upstream and downstream sampling stations.

The ranking of the presence of algae ranged from 1.5 to 2.667 out of 5 (Table 3) and did not vary significantly between the three streams or the upstream and downstream sampling stations. Similarly, the ranking of aquatic vegetation, with a range of 1 to 2.333 out of 5 (Table 3), did not vary significantly between the three sources nor the upstream downstream stations. Ranking of the presence of organics in the substrate was between 1.833 and 3.667 (Table 3). Organics did not vary significantly between the three streams or the upstream downstream areas.

The canopy coverage of the areas sampled ranged from a ranking of 2 to 4 out of 5 (Table 3). Canopy coverage did not vary between upstream and downstream stations, but did show significant differences between the three streams, with C being the most covered and G being the most exposed.

With respect to the chemical parameters measured, the pH of the streams was for the most part basic, ranging from 7.625 to 8.25 on average (Table 4). The pH did not show significant variation between the three streams nor the upstream and downstream stations. Ammonia levels in the water ranged from 0.125 to 0.5 ppm (Table 4), and did not vary significantly between any of the streams or stations sampled. The level of phosphates and the level of nitrates in the streams observed was 0.0 ppm in every station sampled.

The Pearson correlations performed demonstrated that there was a significant negative correlation between Shannon diversity and pH (r=-0.892, P=0.017), exponential Shannon diversity and pH (r= -0.86, p=0.028) and Simpson diversity and pH (r= -0.83, P=0.041). (Figure 6) Additionally a significant positive correlation was observed between richness at the family level and width of the stream (r=0.851, P=0.032) (Figure 7). None of the other parameters tested produced significant correlations.

In terms of the aquatic invertebrates sampled in this study, a total of 2081 individuals were sampled, belonging to 42 different families from 14 orders (Tables 5 and 6). At the level of order, the most abundant were Trichoptera with 803 individuals (39%), Ephemeroptera with 547 individuals (26%) and Diptera with 444 individuals (21%).

The three most abundant groups at the level of families were Helicopsychidae with 318 individuals (15%), Baetidae with 279 individuals (13%) and Simuliidae with 227 individuals (11%) (Figure 2).

In the BU station a total of 598 individuals were collected, corresponding to 29 families and 11 orders. The most abundant families were Helicopsychidae (40%), Leptohyphidae (9%), and Psephenidae (8%). BD registered a total of 430 individuals from 25 families and 9 different orders. The most abundant families from BD were Simuliidae (25%), Baetidae (13%), and Glossosomatidae (12%) (Tables 5 and 6).

Samples collected at the GU station resulted in 546 individuals from 25 families and 11 orders . The families Baetidae (26%), Simuliidae (17%) and Hydropsychidae (9%) were the most abundant. 166 individuals were collected at the GD station corresponding to 24 families and 9 orders. Of these the most represented were Baetidae (24%), Glossosomatidae (14%) and Simuliidae (10%) (Tables 5 and 6).

In the CU station a total of 176 individuals were collected from 18 different families and 7 orders. The most representative species from CU were Elmidae (26%) and Leptophlebiidae (18%) and Hydropsychidae (14%). The station CD registered a total of 165 individuals from 21 families and 8 orders. The most abundant families were Hydropsychidae (24%), Helicopsychidae (20%) and Leptoceridae (10%) (Tables 5 and 6).

The richness at the level of family was highest in B and lowest in C, but did not vary significantly between the three rivers. Richness was higher in BU than BD and in GU than in GD, but lower in CU than in CD. Richness did not vary significantly between upstream and downstream stations. Abundance was highest in B and lowest in C, but did not vary significantly between the three streams. Abundance was higher in all upstream stations relative to the corresponding downstream station, but the difference was not significant (Table 5).

The functional feeding groups that were the most abundant were scrapers (35%), collectors (31%) and filterers (24%). No significant variances were observed in functional feeding groups between the three streams or between the upstream and downstream sampling stations. All of the functional feeding groups (scraper, shredder, predator, collector and filterer) were present in every sampling station. Shredders were consistently the least abundant (Figure 3).

Shannon diversity ranged from 1.974 to 2.246 across all the sampling stations, exponential Shannon diversity had a range of 7.313 to 9.627 and the Simpson index of diversity ranged from 0.779 to 0.859. In terms of the streams, the creek ranked highest in all three of the diversity measures (Figure 4), but the difference between the three streams was not significant. Likewise, the variation between the diversity measures in upstream and downstream sampling stations was not significant (Table 7).

The indexes BMWP, IBMWP, BMWP/Col and Sensibilidad had ranges from 35.9 to 61.767, 38.333 to 66.667, 89 to 105.333 and 55 to 88.2, respectively. There was a significant difference between the three streams in the indexes of BMWP ($H=6.554$, $P=0.037$) and IBMWP ($H=6.103$, $P=0.047$), but not between upstream and downstream stations. The indexes BMWP/Col and Sensibilidad did not vary significantly across the three streams or between upstream and downstream stations (Table 8).

The maximum value for the family-level biotic index (FBI) was 3.588 and the minimum was 2.825, with C scoring the lowest and B the highest (Table 9). The FBI did not vary significantly between the three sources or between upstream and downstream stations. The range of percentages of EPT across all three streams was 57.837% to 79.766% (Table 10). Percent EPT did not vary significantly between the streams or the stations sampled.

Of the biotic indexes used to analyze the aquatic invertebrates in this study, the index that covered the most families was the BMWP/Col (90%). The other indexes rank as follows: FBI (69%), Sensibilidad (67%), IBMWP (64%) and BMWP (36%).

Discussion

The Rio Brincador (B), Rio Guajalito (G) and creek used as a water source for the BPRG house (C) are fairly consistent in the physical and chemical parameters assessed in this study. The major notable differences are largely physical in nature and relate to the relative sizes of the different streams. C differs greatly from the B and G both in terms of size and volume of water. The creek is located at a higher altitude than the B and G, therefore it is unable to accumulate large amounts of water and instead feeds G further below. G, which is fed by both B and C, naturally is the largest of the three streams. The depth increased significantly in downstream stations (D) compared to upstream stations (U) in all three streams. This may be because as one advances downstream the waters are fed by tributaries. This is certainly the case for G, since one station was placed upstream of the convergence with B and one station was placed downstream, accounting for the difference in size and discharge in GD relative to GU. In the case of B and C however, the streams are not fed by any large tributaries between upstream and downstream stations. In fact, in B and C the water is being diverted in order for human uses. Between BU and BD is a trout farm that includes several aquaculture pools filled with water from the river. After it is cycled through the pools the water is returned to the river. In C, the stream is dammed with concrete between CU and CD and a small proportion of the water is diverted to tanks where it is used as a water supply for the BPRG house. In both B and C the width decreases slightly from downstream compared to upstream, yet the depth has increased significantly. This could be a sign that these streams are undergoing a process of channelization, whereby the impacts of human alterations cause the stream to width to narrow and depth to increase. Channelization can have detrimental effects on the natural flow regime of streams and rivers, and can compromise the ecology of the stream. In order to ensure a healthy stream ecosystem, the natural dynamic flow regime must be maintained (Poff et al. 1997).

The variation in the relative amount of canopy cover over each stream can be explained by the relative size of each stream. G, which is the widest, has the least coverage due to the fact that it is more difficult for trees to reach over the water. C has the most coverage due to the fact that it is the most narrow of the three streams and therefore vegetation may easily grow alongside the stream and provide coverage it without risk of being washed away.

Of the chemical parameters measured, only pH and Ammonia tests provided results of any use. The tests for phosphate and nitrate concentrations came up 0 ppm at every sampling station. The pH values measured did not exceed the maximum acceptable values as put forth by in the environmental legislation of Ecuador (Libro IV, sections: 4.1.20 Criterios de calidad para aguas de consumo humano y uso doméstico, and 4.12 Criterios de calidad de las aguas para la preservación de flora y fauna en aguas dulces frías o cálidas, y en aguas marinas y de estuarios) (Tulas 2003). However, the pH was mostly basic which is in contrast to the results published by Arroyo (2007). The chemical assessment performed in 2007 measured pH values from 6.07 to 6.41, making the water mostly acidic. Further studies are needed in order to determine if this increase in pH is a trend or if it is simply a regular fluctuation.

Ammonia levels in this study were surprisingly high, especially in areas not exposed to aquaculture. The highest levels of ammonia were observed in BD, the station just downstream of the trout farm. This indicates that the trout farm is likely contributing some input of ammonia into B and thus into G. Ammonia is toxic to all vertebrates and excess levels could be problematic for the aquatic ecosystem and for the people who rely on B and G as a water source (Randall 2002). It is important that future studies monitor the ammonia levels in areas near aquaculture operations in order to make sure they are within the capacity of the stream to absorb. The chemical analysis performed in this study utilized very basic equipment that does not offer sufficient data in order to make serious conclusions about the water quality in B, G and C. Extensive long term studies that utilize more advanced chemical equipment are necessary in order to sufficiently characterize these streams.

The functional feeding groups of scraper, shredder, predator, collector and filterer were present throughout the sampling stations, with scrapers being the most abundant and shredders being the least abundant. In the station BU the relative abundance of scrapers is very large, due to the extremely high abundance of Helicopsychidae. The abundance of predators was the second lowest throughout the stations. This may be explained by the fact that predators rely on the presence of prey to survive, therefore their populations are limited by the abundance of other organisms lower in the food web.

The most abundant family in this study was Helicopsychidae, from the order Trichoptera. This family is known to prefer meso-oligotrphic conditions (Roldan 2003). The second most abundant family was Baetidae, from the order Ephemeroptera. Baetidae is considered an indicator of clean waters (Roldan 2003). Simuliidae was the third most abundant, and this family from the Diptera order is considered an indicator of oligotrophic waters (Roldan 2003). The large abundance of these organisms may indicate that the waters in BPRG are for the most part clean and low in nutrient content. What is more, the order Trichoptera was the most abundant order in this investigation and is considered to be an indicator of good water quality due to its sensitivity to pollution, along with the order Ephemeroptera, which was the second most abundant order (Carrera *et. al* 2001).

The family Euthyplociidae from the order Ephemeroptera was present in both B and C but not in G. Euthyplociidae is known to be a good indicator of clean waters (Roldan 2003). Additionally the family Calamoceratidae, from the order Trichoptera was present in B and C but not in G. Calamoceratidae is known to prefer oligotrophic waters (Roldan 2003). These results correspond to the gradient of impact established by Arroyo (2007), which ranks B as less impacted than G.

The richness at the level of family and abundance also corresponds to the gradient established by Arroyo (2007). B displayed both a higher abundance and a higher richness than G. C was lowest in richness and abundance (Table 6). In terms of %EPT, B also ranks higher than G, but in this index C has the rank highest of all (Table 10). In terms of BMWP, IBMWP, BMWP/Col, and Sensibilidad, B ranks the highest out of the three streams sampled and G ranks lowest in all except for the IBMWP index (Table 8). These results seem to indicate that the gradient established by Arroyo (2007) still holds true and the impact on B since the 2007 study has not surpassed the impacts already present in G. The diversity measures, however, confuse these results. In the Shannon, exponential Shannon, and Simpson diversity indexes, G ranks slightly higher than B on average. C, in contrast, ranks the highest in all three diversity indexes (Figure 4). Furthermore, it is the same in the case of the family based biotic index (FBI), with B raking the highest (most impacted) and C the lowest (lease impacted) (Table 9). Taken together, these results indicate that the differences between the three streams are not significant enough to develop a clear pattern and that more long term studies will be needed to fully establish the gradient.

With respect to the difference between upstream and downstream stations, BU and GU both have higher richness and abundance than BD and GD. CU, however, displays a lower richness yet higher abundance relative to CD (Table 5). Similarly, %EPT is higher in BU and GU than in BD and GD, but CD has a higher %EPT than CU (Table 10). BMWP/Col and Sensibilidad indexes produced the same results, with

BU and GU ranking higher than BD and GD, but CU ranking lower than CD. BMWP and IBMWP, however, conflict with the BMWP/Col and Sensibilidad scores in that they both rank BU below BD yet CU above CD. Only the station GU ranked above GD across all the biological monitoring working party indexes (Table 8). The FBI scores, in contrast rank all upstream stations lower (less impacted) than downstream stations (Table 9). Much like in the case of describing the differences between the three streams sampled, the lack of significant trends throughout the indexes makes it difficult to make a claim about the difference in water quality in upstream and downstream stations. It seems to be that the downstream water is slightly more impacted than upstream, but some of the indexes are contradictory. Based on the relative coverage of the indexes, the FBI, BMWP/Col and Sensibilidad seem to be the best suited for studies in this region. If results for future studies in this region are assessed using just these indexes, contradictions would be less frequent and results will be more conclusive.

Conclusion

Overall the water sources of B, G and C in the BPRG are in very good health, despite increases in potential threats in recent years. According to all the indexes used the water quality ranks between fair and excellent, and according to the indexes better adapted for this region water quality at the BPRG ranks from good to very good. Continued monitoring of BPRG in the future is vital in order to maintain the quality of water and ensure it is not degraded. In particular, more extensive baseline data should be established in order to make future studies more easily interpreted. The only major study of aquatic invertebrates was only carried out during the dry season (Arroyo 2007). The current study was done in the wet season, but it was not as extensive as the previous. Extensive baseline data is needed for both the wet and dry seasons regarding variations in aquatic invertebrate community composition as well as changes in physical and chemical variables. With a solid baseline in place, future studies can much more readily draw conclusions about the quality of water in BPRG.

An additional necessity is the establishment of nutrient gradient criteria for the Andes of Ecuador that could be applied to BPRG. Establishment of acceptable nutrient criteria is a vital first step in the effort to effectively manage water quality. The establishment of acceptable nutrient criteria for a body of water incorporates many variables that may change depending on the biotic and abiotic environmental factors of the body of water in question. Defining acceptable nutrient criteria therefore requires case-by-case scrutiny of the ability of a system to absorb nutrients and of the threshold at which nutrient loads become excessive or damaging to the system. Additionally, comparison of benthic macro-invertebrate community structure across a nutrient gradient provides a possible method for establishing community responses to varying nutrient loads within a given region. In streams exhibiting poor biological conditions, elevated levels of nitrogen (N), phosphorus (P) and fine sediments are considered to be significant sources of stress to ecosystems (Paulsen et al., 2008;

USEPA, 2006). The study by Arroyo (2007) started the work of establishing an acceptable nutrient criteria for BPRG, but future studies across a larger nutrient gradient will be needed in order to ensure that the nutrient criteria for this region reflects the needs and nuances of the area. Lastly, the development of Ecuador specific biotic index is necessary in order to ensure that studies of aquatic invertebrates are accurate in their assessments of water quality. Based on the results of this study, the FBI, BMWP/Col and Sensibilidad indexes seem to be the most accurate and best fit for macro-invertebrate assessment in this region. Despite the relative utility of these indexes, an index specific to the Andes region of Ecuador is a vital next step in ensuring good water quality for the BPRG.

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Tables

Table 1 Average values and standard deviation (in parentheses) of depth, width, velocity and discharge in upstream (U) and downstream (D) stations of Rio Brincador (B), Rio Guajalito (G) and creek used for water source (C) in the BPRG Ecuador

Source	Station	Depth (m)	Width (m)	Velocity (m/s)	Discharge (m^3/s)
Brincador	BU	0.277(±0.304)	8.735(±1.761)	0.806(±0.139)	1.937
	BD	0.298(±0.068)	6.97(±0.382)	0.847(±0.074)	1.761
Guajalito	GU	0.372(±0.159)	7.565(±0.389)	0.946(±0.025)	2.66
	GD	0.745(±0.266)	9.2(±0.283)	1.085(±0.055)	7.438
Creek	CU	0.147(±0.049)	1.21(±0.127)	0.691(±0.083)	0.123
	CD	0.163(±0.035)	1.079(±0.171)	0.54(±0.127)	0.095

Table 2 Dominant substrate for each sample in upstream (U) and downstream (D) stations of Rio Brincador (B), Rio Guajalito (G) and creek used for water source (C) in the BPRG Ecuador

Source	Station	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6
Brincador	BU	Boulder	Cobble	Sand	Cobble	Cobble	Gravel
	BD	Cobble	Sand	Cobble	Gravel	Cobble	Gravel
Guajalito	GU	Boulder	Cobble	Sand	Cobble	Boulder	Cobble
	GD	Cobble	Cobble	Sand	Cobble	Cobble	Boulder
Creek	CU	Boulder	Cobble	Cobble	-	-	-
	CD	Gravel	Sand	Cobble	-	-	-

Table 3 Average ranking and standard deviation (in parentheses) of degree of substrate embeddedness, presence of organic materials, amount of aquatic vegetation, amount of algae and degree of canopy coverage in upstream (U) and downstream (D) stations of Rio Brincador (B), Rio Guajalito (G) and creek used for water source (C) in the BPRG Ecuador. Each category ranked on a scale of 1-5

Source	Station	Embeddedness	Organics	Vegetation	Algae	Canopy Coverage
Brincador	BU	2.833(±1.329)	1.833(±0.983)	1.5(±0.837)	2.333(±0.816)	3(±1)
	BD	1.5(±0.548)	3.167(±0.753)	1.333(±0.516)	1.833(±0.753)	2(±0)
Guajalito	GU	3.333(±0.816)	3.333(±1.211)	2.333(±0.816)	2.167(±0.983)	3(±1)
	GD	2.667(±1.033)	1.833(±0.753)	1(±0.632)	1.5(±0.837)	1.667(±0.577)
Creek	CU	3.333(±0.577)	3.333(±0.577)	1.333(±0.577)	2.667(±0.577)	3.667(±0.577)
	CD	1.667(±0.577)	3.667(±1.155)	3.333(±1.155)	2.333(±1.528)	4(±1)

Table 4 Average values and standard deviation (in parentheses) of chemical characteristics in upstream (U) and downstream (D) stations of Rio Brincador (B), Rio Guajalito (G) and creek used for water source (C) in the BPRG Ecuador

Source	Station	pH	Ammonia	Nitrate	Phosphate
Brincador	BU	8.25(±1.061)	0.125(±0.178)	0	0
	BD	8(±1.414)	0.5(±0.707)	0	0
Guajalito	GU	8(±0.707)	0.125(±0.177)	0	0
	GD	8.25(±1.061)	0.125(±0.177)	0	0
Creek	CU	8(±1.414)	0.125(±0.177)	0	0
	CD	7.625(±0.884)	0.125(±0.177)	0	0

Table 5 Richness of orders, richness of families and number of individuals collected in upstream (U) and downstream (D) stations of Rio Brincador (B), Rio Guajalito (G) and creek used for water source (C) in the BPRG Ecuador

Source	Station	Orders	Families	Number of Individuals
Brincador	BU	11	29	598
	BD	9	25	430
Guajalito	GU	11	25	546
	GD	9	24	166
Creek	CU	7	18	176
	CD	8	21	165

Table 6 Total number of individuals of each taxa collected in upstream (U) and downstream (D) stations of Rio Brincador (B), Rio Guajalito (G) and creek used for water source (C) in the BPRG Ecuador. Characterized by functional feeding group (FFG) at the level of family. ?=Functional feeding group undetermined

Order	Family	BU	BD	GU	GD	CU	CD	FFG
Hidracarina		2	-	-	1	1	-	Predator
Coleoptera	Elmidae	31	24	12	10	46	8	Scraper
	Lampyridae	-	-	-	1	-	-	Predator
	Psephenidae	49	-	4	1	4	3	Scraper
	Ptilodactylidae	1	2	-	-	-	5	Shredder
	Staphylinidae	1	-	-	-	-	-	?
Crustacea	Pseudothelpusidae	-	-	-	-	-	1	?
Diptera	Athericidae	14	4	-	-	-	-	Predator
	Blepharoceridae	1	2	40	9	-	-	Scraper
	Ceratopogonidae	1	4	11	3	-	1	Predator
	Chironomidae	16	33	37	3	8	11	Collector/Filterer
	Dixidae	-	-	-	-	1	-	Filterer
	Ephydridae	-	-	-	1	-	-	Shredder/Scraper
	Psychodidae	-	-	1	1	-	1	Collector
	Simuliidae	5	109	94	17	1	1	Filterer
	Stratiomyidae	-	-	-	2	1	-	Collector
	Tabanidae	2	-	-	-	-	-	Predator
	Tipulidae	2	1	-	1	5	-	Shredder
Ephemeroptera	Baetidae	27	54	140	40	11	7	Collector
	Euthyplociidae	1	-	-	-	-	1	Filterer
	Leptohyphidae	52	37	33	11	13	7	Collector
	Leptophlebiidae	17	5	32	2	32	12	Collector
	Oligoneuriidae	1	11	1	-	-	-	Filterer
Haplotaxida	Tubificidae	1	15	2	2	-	-	Collector
Hemiptera	Veliidae	-	-	1	-	-	-	Shredder
Hirudinea	Glossiphonidae	1	-	-	-	-	-	Predator
Lepidoptera	Pyralidae	2	1	3	3	-	-	Shredder
Megaloptera	Corydalidae	2	-	1	-	-	1	Predator
Odonata	Calopterygydae	1	1	-	-	-	-	Predator
	Gomphidae	1	-	1	-	1	-	Predator
	Libellulidae	-	3	-	-	-	-	Predator
	Polythoridae	-	1	-	-	2	2	Predator
Plecoptera	Perlidae	6	5	9	1	4	2	Predator
Pulmonata	Hydrobiidae	-	2	1	-	-	-	Scraper
	Planorbidae	-	-	1	1	-	-	Scraper
Trichoptera	Calamoceratidae	-	1	-	-	-	5	Shredder
	Glossosomatidae	47	51	45	24	1	6	Scraper
	Hydropsychidae	47	26	52	2	24	40	Filterer
	Helicopsychidae	239	16	10	11	9	33	Scraper
	Hydrobiosidae	-	-	1	-	-	1	Predator
	Hydroptilidae	17	8	1	3	-	-	Scraper
	Leptoceridae	11	14	13	16	12	17	Predator
Total		598	430	546	166	176	165	

Table 7 Average values and standard deviation (in parentheses) of Shannon (H'). exponential Shannon (exp(H')), Simpson (1-D) diversity indexes in upstream (U) and downstream (D) stations of Rio Brincador (B), Rio Guajalito (G) and creek used for water source (C) in the BPRG Ecuador.

Source	Station	H'	exp(H')	S
Brincador	BU	1.974(±0.196)	7.313(±1.419)	0.779(±0.067)
	BD	2.162(±0.356)	9.117(±2.858)	0.837(±0.076)
Guajalito	GU	2.125(±0.26)	8.615(±2.271)	0.83(±0.056)
	GD	2.032(±0.321)	7.937(±2.253)	0.821(±0.071)
Creek	CU	2.035(±0.302)	7.894(±2.468)	0.819(±0.059)
	CD	2.246(±0.239)	9.627(±2.212)	0.859(±0.021)

Table 8 a) Significance of scores for the biological indexes of BMWP, IBMWP, BMWP/Col and Sensibilidad (Carrera et al., 2000). b) Average score and standard deviation (in parentheses) of the biological indexes BMWP, IBMWP, BMWP/Col and Sensibilidad for Rio Brincador, Rio Guajalito and creek used as water source for BPRG house. c) Average values and standard deviation (in parentheses) of scores for the biological indexes of BMWP, IBMWP, BMWP/Col and Sensibilidad in upstream (U) and downstream (D) stations of Rio Brincador (B), Rio Guajalito (G) and creek used for water source (C) in the BPRG Ecuador.

a)

Score	Water quality	Significance	color
>150, 101-120	Very good	Unpolluted, unimpacted	Blue
61-100	Good	Clean but slightly impacted	Green
36-60	Fair	Moderately impacted	Yellow
16-35	Poor	Polluted or impacted	Orange
<15	Very poor	Heavily polluted	Red

b)

Source	BMWP	IBMWP	BMWP/Col	Sensibilidad
Brincador	60.003(±9.298)	63.833(±14.186)	101.917(±16.534)	84.1(±9.879)
Guajalito	45.067(±11.247)	51.417(±13.235)	85.333(±21.256)	65.667(±15.616)
Creek	52(±8.974)	44.5(±12.412)	97.167(±33.583)	77(±25.432)

c)

Source	Station	BMWP	IBMWP	BMWP/Col	Sensibilidad
Brincador	BU	58.24(±9.708)	61(±12.39)	105(±14.089)	88.2(±6.797)
	BD	61.767(±8.888)	66.667(±15.983)	98.833(±18.978)	80(±12.961)
Guajalito	GU	54.233(±8.303)	61.333(±8.641)	96.5(±20.482)	76.333(±16.305)
	GD	35.9(±14.191)	41.5(±17.83)	74.167(±22.031)	55(±14.926)
Creek	CU	56.8(±5.651)	50.667(±10.599)	89(±32.234)	75(±23.643)
	CD	47.2(±12.298)	38.333(±14.224)	105.333(±34.933)	79(±27.221)

Table 9 a) Classes of water quality and significance of scores for the family-level biotic index (Hilsenhoff, 1988). b) Average score and standard deviation (in parentheses) of the family-level biotic index for Rio Brincador, Rio Guajalito and creek used as water source for BPRG house. c) Average values and standard deviation (in parentheses) of family-level biotic index score in upstream (U) and downstream (D) stations of Rio Brincador (B), Rio Guajalito (G) and creek used for water source (C) in the BPRG Ecuador.

a)

Family Biotic Index	Water quality	Degree of organic Pollution
0.00-3.75	Excellent	Organic pollution unlikely
3.76-4.25	Very Good	Possible slight organic pollution
4.26-5.00	Good	Some organic pollution probable
5.01-5.75	Fair	Fairly substantial pollution likely
5.76-6.50	Fairly Poor	Substantial pollution likely
6.51-7.25	Poor	Very substantial pollution likely
7.26-10.0	Very Poor	Severe organic pollution likely

b)

Source	FBI Score
Brincador	3.231(±0.572)
Guajalito	3.223(±0.762)
Creek	3.018(±0.413)

c)

Source	Station	FBI Score
Brincador	BU	2.875(±0.272)
	BD	3.588(±0.873)
Guajalito	GU	3.174(±0.674)
	GD	3.273(±0.85)
Creek	CU	2.887(±0.591)
	CD	3.149(±0.236)

Table 10 a) Significance of percentages of EPT index (Carrera et al, 2000). b) Average %EPT and standard deviation (in parentheses) for Rio Brincador, Rio Guajalito and creek used as water source for BPRG house. c) Average %EPT and standard deviation (in parentheses) in upstream (U) and downstream (D) stations of Rio Brincador (B), Rio Guajalito (G) and creek used for water source (C) in the BPRG Ecuador.

a)

Percentage	Water Quality
75-100%	Very good
50-74%	Good
25-49%	Fair
0-24%	Poor

b)

Source	%EPT
Brincador	68.255(±9.772)
Guajalito	65.108(±9.319)
Creek	68.787(±5.346)

c)

Source	Station	%EPT
Brincador	BU	76.766(±3.86)
	BD	59.744(±15.685)
Guajalito	GU	66.744(±10.467)
	GD	63.472(±8.172)
Creek	CU	57.837(±6.174)
	CD	79.736(±4.519)

Figures

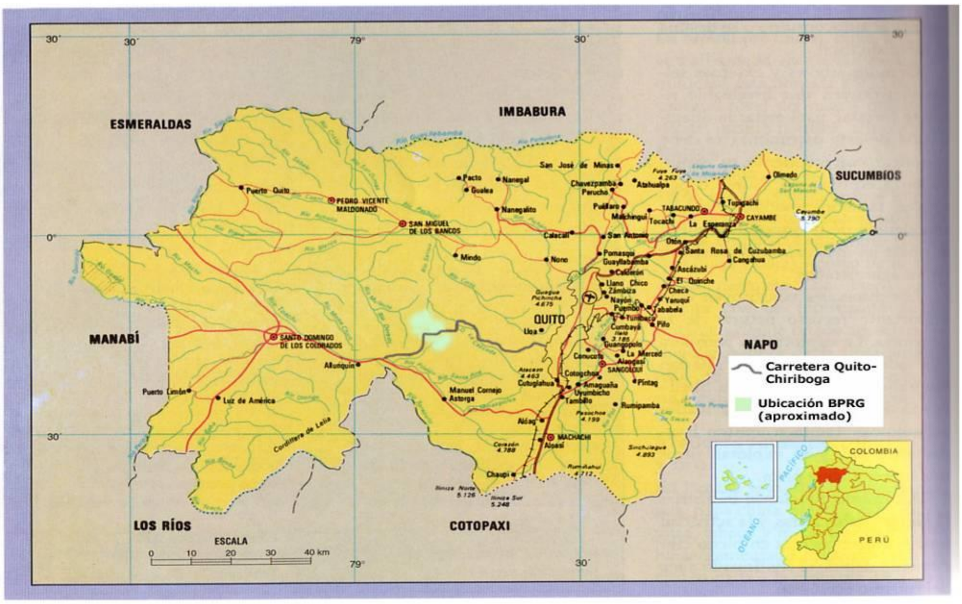


Figure 1 Map of Pinchincha region demonstrating approximate location of BPRG

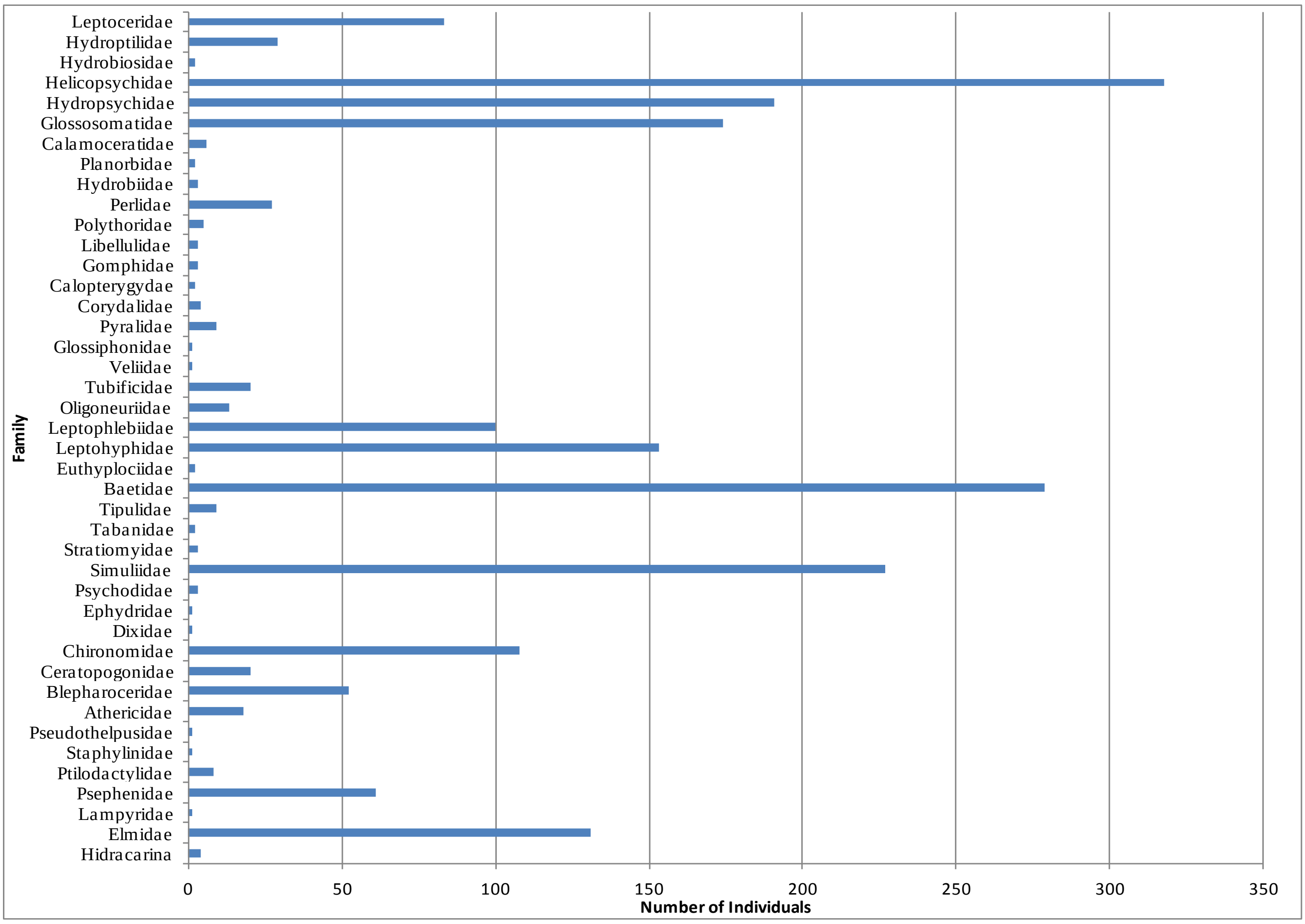


Figure 2 Richness and relative abundance of individuals of macroinvertebrates collected the Rio Brincador (B), Rio Guajalito (G) and creek used for water source (C) in the BPRG Ecuador.

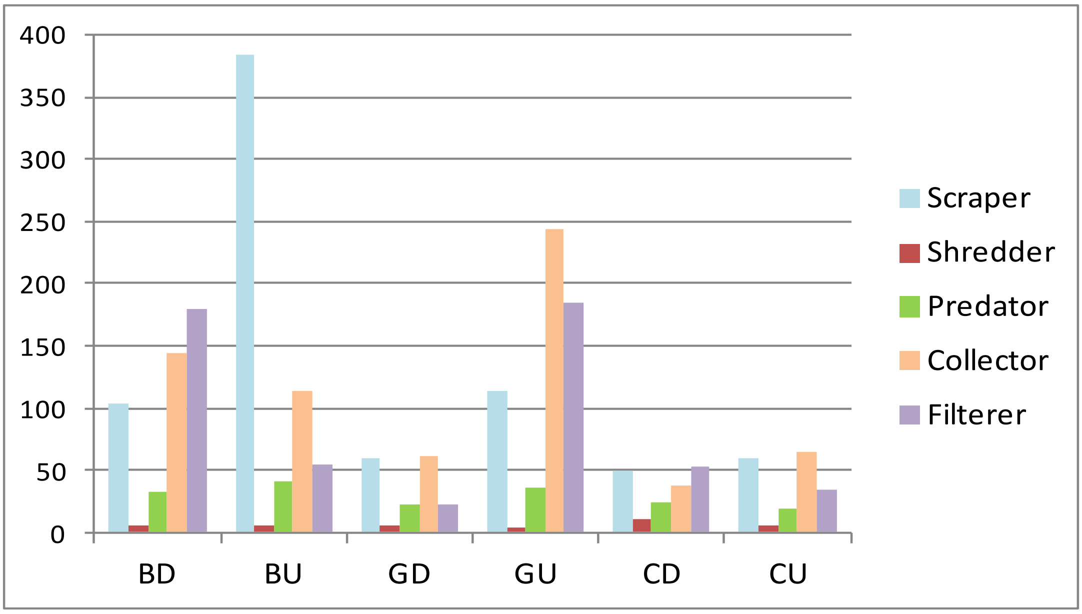
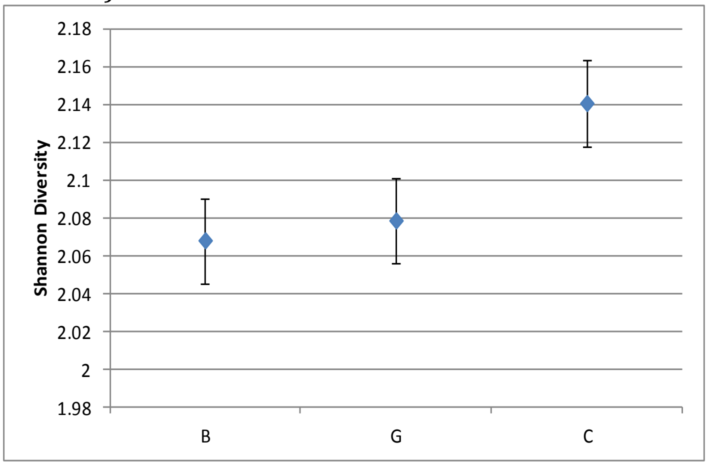
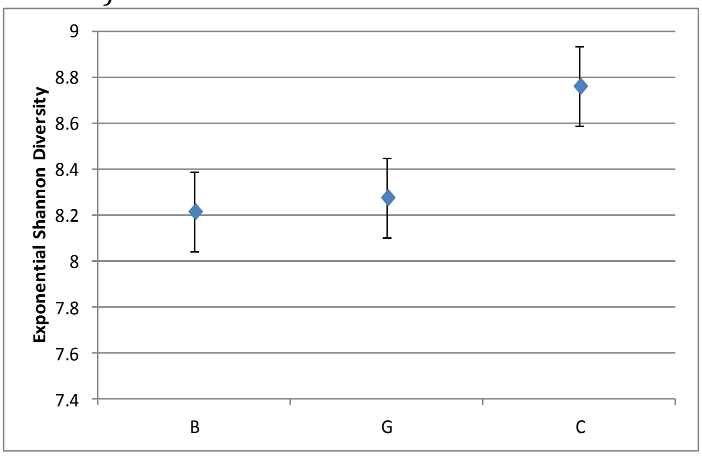


Figure 3 Proportions of functional feeding groups present in upstream (U) and downstream (D) stations in Rio Brincador (B), Rio Guajalito (G) and creek used for water source (C) in the BPRG Ecuador in terms of abundance.

a)



b)



c)

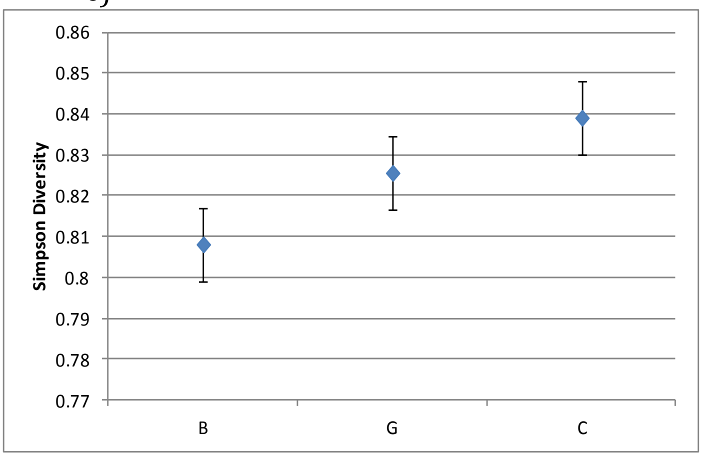


Figure 4 a)Shannon diversity index b)Exponential Shannon diversity index c) Simpson diversity index in Rio Brincador (B), Rio Guajalito (G) and creek used for water source (C) in the BPRG Ecuador. Standard error represented by bars.

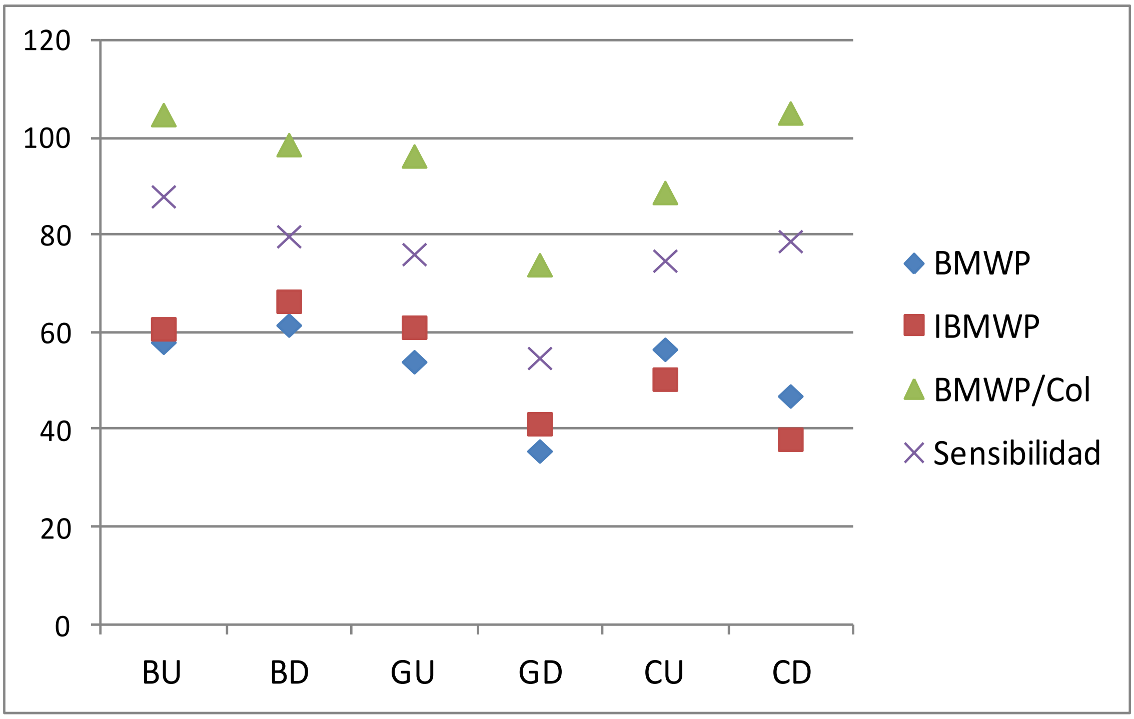


Figure 5 Scores of the biological indexes BMWP, IBMWP, BMWP/Col and Sensibilidad in upstream (U) and downstream (D) stations of Rio Brincador (B), Rio Guajalito (G) and creek used for water source (C) in the BPRG Ecuador.

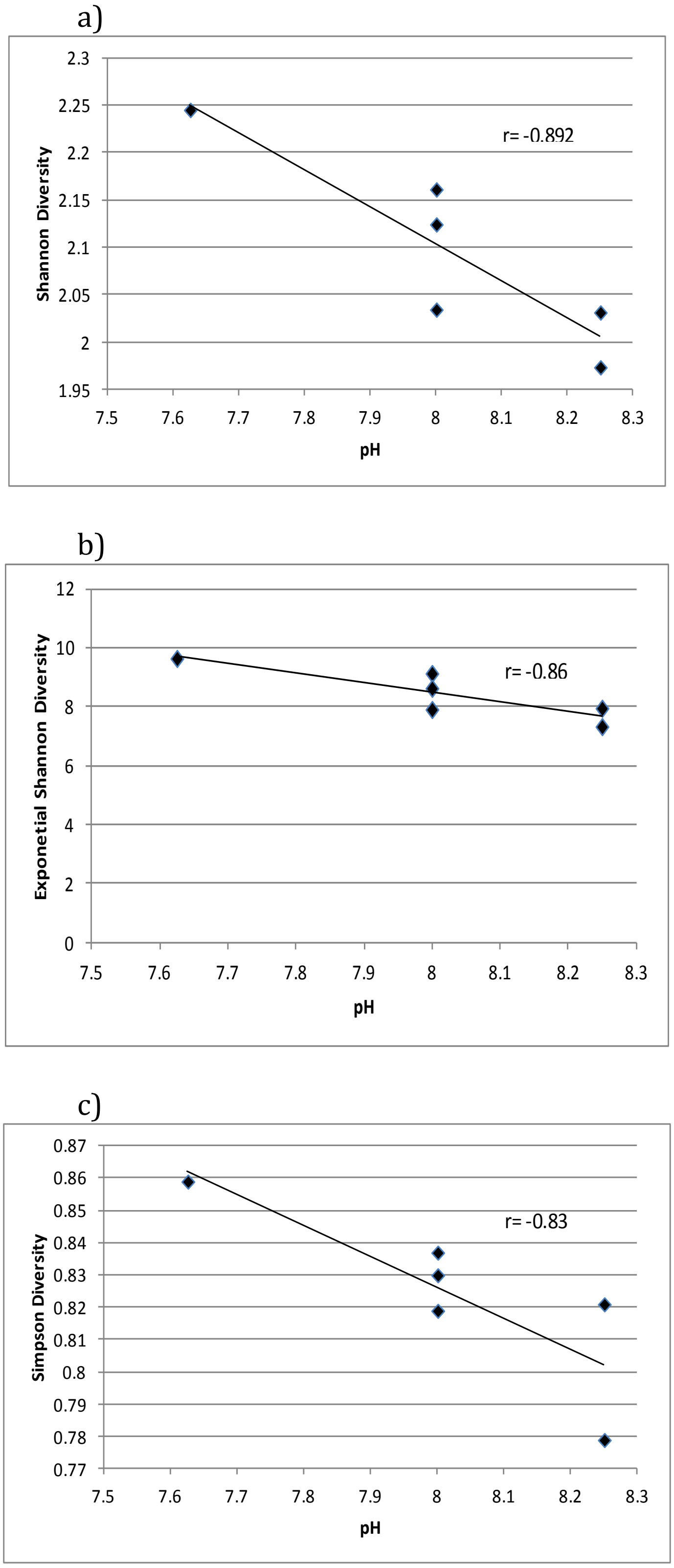


Figure 6 Pearson correlations between a) Shannon diversity and pH. b) Exponential Shannon diversity and pH. c) Simpson diversity and pH

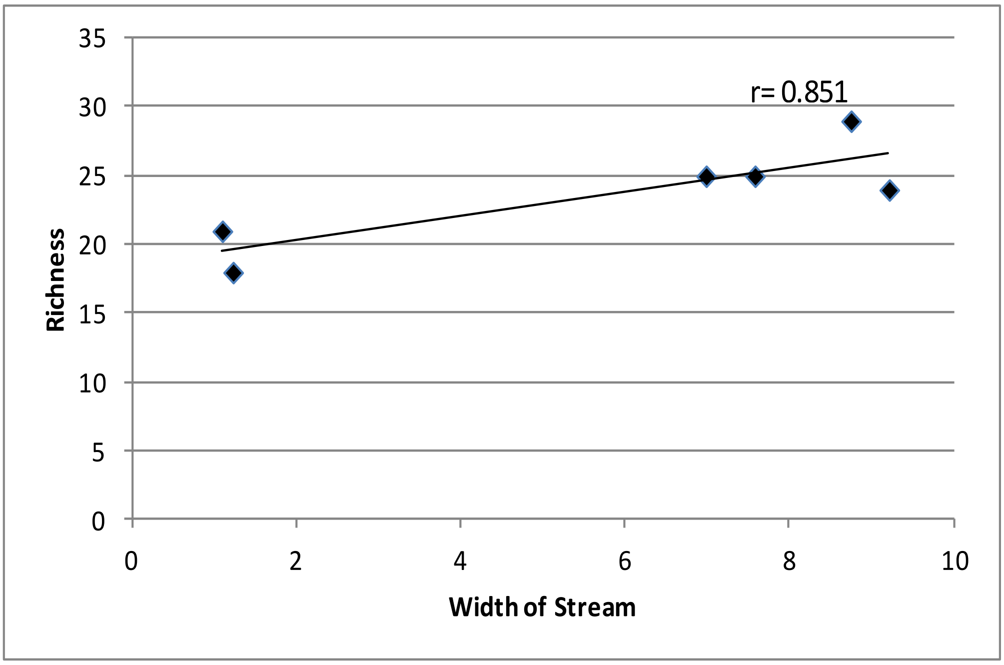


Figure 7 Pearson correlation between richness and width of stream

Appendix

Appendix I. P values from ANOVA and Kruskal-Wallis analysis between Rio Brincador (B), Rio Guajalito (G) and the creek (C) used as a water source for the BPRG house. P= P value DF=Degrees of freedomH/F= H value(Kruskal-Wallis) and F value (ANOVA). a) Abiotic variables. b) Biotic variables. Variables assessed using non-parametric Kruskal-Wallis marked with an asterisk*.

a)

Variable	DF	H/F	P
Width*	2	7.731	0.021
Depth	2	9.296	0.001
Velocity	2	20.49	1.95E-04
Discharge*	2	18.7	8.68E-05
Embeddedness*	2	3.295	0.171
Coverage*	2	6.442	0.029
Organics	2	2.637	0.241
pH	2	0.429	0.791
Ammonia	2	0.736	0.637

b)

Variable	DF	H/F	P
Vegetation*	2	2.245	0.258
Algae*	2	1.679	0.393
Simpson*	2	0.632	0.729
Inverse Simpson*	2	0.632	0.729
Shannon*	2	0.498	0.78
Exponential Shannon	2	0.322	0.728
BMWP*	2	6.554	0.037
IBMWP*	2	6.103	0.047
BMWP/Col*	2	1.42	0.491
Sensibilidad	2	1.017	0.375
HBI	2	0.314	0.733
%EPT*	2	0.401	0.818
Families*	2	2.307	0.312
Abundance*	2	0.295	0.747

Appendix II. P values from ANOVA and Kruskal-Wallis analysis between upsream (U) and downstream (D) stations in Rio Brincador (B), Rio Guajalito (G) and the creek (C) used as a water source for the BPRG house. P= P value DF=Degrees of freedom H/F= H value(Kruskal-Wallis) and F value (ANOVA). a) Abiotic variables. b) Biotic variables. Variables assessed using non-parametric Kruskal-Wallis marked with an asterisk*.

a)

Variable	DF	H/F	P
Width	1	0.231	0.631
Depth	1	4.444	0.044
Velocity	1	0.026	0.874
Discharge*	1	1.602	0.206
Embeddedness*	1	7.381	0.005
Coverage*	1	1.996	0.139
Organics*	1	0.052	0.813
pH*	1	0.098	0.743
Ammonia*	1	0.098	0.729

b)

Variable	DF	H/F	P
Vegetation*	1	0.387	0.494
Algae*	1	2.753	0.08
Simpson*	1	2.822	0.093
Inverse Simpson*	1	2.822	0.093
Shannon*	1	1.6	0.206
Exponential Shannon	1	1.664	0.208
BMWP*	1	1.772	0.182
IBMWP*	1	1.44	0.23
BMWP/Col*	1	0.155	0.693
Sensibilidad	1	0.494	0.488
HBI	1	2.007	0.168
%EPT*	1	0.043	0.836
Families*	1	0.035	0.851
Abundance*	1	1.419	0.244