UNIVERSIDAD REGIONAL AMAZÓNICA IKIAM

FACULTAD DE CIENCIAS DE LA TIERRA Y AGUA

CARRERA DE HIDROLOGÍA

DIVERSIDAD DE MACROINVERTEBRADOS ACUÁTICOS Y CALIDAD DEL AGUA A LO LARGO DE UN GRADIENTE ANTRÓPICO EN LA CUENCA ALTA DEL RÍO NAPO

Proyecto de investigación previo a la obtención del Título de:

INGENIERA EN CIENCIAS DEL AGUA

AUTORA

CYNTIA DANIELA ALVEAR SAYAVEDRA

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TUTOR: PhD. RODRIGO EDUARDO ESPINOSA BARRERA

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Ikiam Universidad Regional Amazónica

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Certifico que el trabajo de integración curricular titulado: “Diversidad de macroinvertebrados acuáticos y calidad del agua a lo largo de un gradiente antrópico en la cuenca alta del río Napo”, en la modalidad de: proyecto de investigación en formato artículo original, fue realizado por: Cyntia Daniela Alvear Sayavedra, bajo mi dirección.

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DEDICATORIA

A cualquier persona con pensamiento intergeneracional que desee profundamente un mundo mejor a futuro; y a cualquiera que le interese los temas de biomonitoreo de los ecosistemas para lograr entender a la PACHA MAMA.

A todas las personas que tratan a la naturaleza como un sujeto de derechos y buscan su protección, porque además disfrutan de su vida en cada Raymi que la madre tierra nos brinda.

A mi adorada ciudad de Pujilí, mi primer hogar y el lugar en donde crecí.

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RESUMEN

Varios afluentes de la cuenca del río Napo son fuertemente impactados por minería aurífera, acuicultura-agricultura intensiva, y descarga de aguas residuales. Algunos de ellos no han sido estudiados y carecen de información sobre calidad ecológica del agua y biodiversidad, limitando las acciones para la gestión del agua. Por tanto, los objetivos de investigación fueron (1) evaluar la calidad del agua en algunos tributarios sobre un gradiente antrópico, (2) relacionar la estructura de los macroinvertebrados con varios parámetros físico-químicos, y (3) determinar los impactos sobre la comunidad de macroinvertebrados y los ecosistemas acuáticos. Entonces se recolectaron datos fisicoquímicos y de macroinvertebrados en 36 sitios durante las épocas secas de 2020-2021; se evaluaron las características de la comunidad de macroinvertebrados y la calidad del agua. Los resultados mostraron impactos significativos sobre los ecosistemas acuáticos en sitios antrópicos, que comparados con FT muestran baja diversidad y calidad. La calidad del agua osciló entre moderada y muy mala para los índices AAMBI y BMWP-Col, clasificando los sitios como FT>CA>WD>GM; mientras que para CCE-A y CCE-B varió entre buena y pobre, categorizándose FT>CA>GM>WD. El análisis multivariado indicó que una mayor calidad de agua con pH y DO% altos caracteriza a FT; mientras TDS, turbidez y temperatura altos se relacionaron con GM. WD y CA no presentaron relaciones notables con los parámetros descritos. Con esta información, las partes interesadas tienen una línea base defendible para reducir el estrés en los afluentes, así como para desarrollar o mejorar las herramientas de gestión ambiental basadas en biomonitoreo.

Palabras clave: Biomonitoreo, Indicadores ecológicos, Macroinvertebrados, Calidad del agua, Amenazas humanas

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ABSTRACT

Several tributaries of the Napo River Basin are heavily impacted by gold mining, intensive aquaculture-agriculture, and wastewater discharge. Some of them haven´t been studied and lack information on ecological water quality and biodiversity, restraining actions for water management. Therefore, the aims research were (1) to assess the water quality in some waterways over an anthropic gradient, (2) to relate the macroinvertebrates structure with several physicochemical parameters, and (3) to ascertain impacts on the macroinvertebrates community and aquatic ecosystems. Thus, macroinvertebrate and physicochemical data were collected at 36 sites during dry seasons of 2020-2021; macroinvertebrate community characteristics and water quality were assessed. The results showed that there are significant impacts on aquatic habitats in the anthropic sites, as shown a low water quality and diversity compared to FT. The water quality in the anthropic gradient ranged from moderate to very bad for the AAMBI and BMWP-Col indices, ranked the sites as FT>CA>WD>GM in both; whereas in CCE-A and CCE-B the water quality varied between good and poor, categorized as FT>CA>GM>WD. Multivariable analysis showed that higher water quality and high pH and DO% values characterized FT, while higher TDS, turbidity, and temperature values were related to GM. WD and CA didn´t present a notable relationships with the described parameters. With this information, stakeholders have a defensible baseline to reduce stress on the streams, as well as to develop or improve environmental management tools based on biomonitoring.

Keywords: Biomonitoring, Ecological Indicators, Macroinvertebrates, Water quality, Human threats

1. INTRODUCTION

River water quality status is an important factor influencing the distribution and abundance of aquatic fauna and flora[1]. However, many factors related to anthropogenic activities (extractive and polluting) threatened the aquatic ecosystem's state as well as their use and exploitation [2]. River systems, provide a multitude of products and services for human consumption and enjoyment in the form of ecosystem services [3]. The benefits obtained directly or indirectly from ecosystem functions are described as ecosystem services that societies can manage according to their needs and choices [4]. These services are essential for human well-being but depend mainly on the state of the environment and effective ecosystem management related to a river basin[5,6]. This creates an urgency to guarantee sustainable use of water resources, developing approaches or methods that integrate the concept of ecosystem services and the integrated water quality monitoring of basins [3,7]. Assessment and classification of ecological water quality using index-based approaches can contribute to the conservation and management of rivers [8]. Nowadays, indicators based on the presence or absence of aquatic organisms (biological monitoring) have been developed to assess water quality and for the classification of the ecological status[9].

Biological monitoring helps to document the evolvement of the water quality through ecological data of indicator species (bioindicator) [10,11]. The biota gives an insight of the conditions existing in an aquatic ecosystems, and therefore, many living organisms (small mammals, fish, aquatic plants, algae, or invertebrate) can be used to assess ecological water quality [8,12]. The use of aquatic fauna as bioindicator at its structural or functional level (population, community, and ecosystem) is usual for environmental impact studies, and one of the most frequently used bioindicators is the community of aquatic macroinvertebrates [11,13]. The composition of macroinvertebrate communities are very sensitive to any kind of disturbance in an aquatic ecosystem [14,15]. Different kind of pollutants could vanish many species of macroinvertebrates and increase the abundance of other species, as a result of decreased competition [16] and survival of the most tolerant organisms. Therefore, the distribution pattern of macroinvertebrates to their ecosystem alteration provide information for assessing water quality[17]. Besides that, benthic macroinvertebrates are often preferred in water quality studies because: 1) they have a longer life cycle and respond to environmental changes in a shorter time than other organisms (e.g. algae or fish), and 2) they are easy to collect and they are generally diagnosed at the level of family[18,19].

Using biological approaches to determine the ecological effects of pollution has been preferred widely for decades [20]. Water quality and biodiversity indices developed for aquatic macroinvertebrate communities are useful parameters to qualify the condition of any fluvial ecosystems in the face of human intervention [9,21,22]. Some researchers have attempted to develop more robust biotic indices that account for the variable response of invertebrate taxon to water pollution, and they concluded that most biotic indices were sufficiently sensitive to ecological impairment to enable discrimination between impacted and non-impacted sites [23,24]. The most evident principle of these works is that in disturbed areas, a disappearance of sensitive macroinvertebrate species occurs resulting in the dominance of resistant species [25]. The calculation of these indices occurs by means of the ordering and weighting of the species obtained in the sampling campaigns [26], in which of the most prominent indices for the neotropical region are the Biological monitoring working party adapted in Colombia (BMWP-Col), Average Score Per Taxon (ASTP), and lately the Andean-Amazon Biotic Index (AAMBI) [23,26,27].

The Ecuadorian Amazon region is cataloged as a hotspot due to its high biodiversity and natural resources; besides providing several ecosystem services, it also is described as the most important source of fresh water and sink greenhouse gases on a global scale [28,29]. This region has historically been known for its oil and timber exploitation, but in recent decades mining activities have increased mainly in the northern zone in the provinces of Sucumbíos, Orellana, and Napo and the southern zone in the provinces of Morona Santiago and Zamora Chinchipe [30]. In addition, deforestation derived from agriculture and cattle has impaired Amazon streams; whereas, industrial and domestic sewage are also the main causes of degradation in mid to high elevation Andean Ecuadorian streams since only 20% of municipalities treat wastewater to the secondary level [31]. Napo as well as several of these provinces presents a high degree of vulnerability due to anthropic activities associated with the expansion of extractive and demographic frontiers, the exploitation of non-renewable resources, deforestation, and unsustainable agro-productive systems [28,32].

The Napo is an anastomosing and binational river, between Ecuador and Perú and is also a direct tributary of the Amazon river [33]. In the Napo River Basin (hereafter NRB), a large part of the water for consumption that supplies more than 2.5 million people from Quito is collected, and also sustains the production of 40% of the electrical energy consumed in Ecuador; in its lower parts, the rivers of this basin provide fishing and support the agriculture that feeds a considerable portion of the ecuadorian amazon population [23]. Then this research aimed to assess the ecological quality of some waterways impacted by different human activities to ascertain any impact on the freshwater ecosystem, by analyzing the diversity and composition of aquatic macroinvertebrates and their relation to several physicochemical factors. In addition, the NRB is one of the Napo province rivers but some of its small tributaries have not yet been studied. That is why this work also looks to fill gaps in river fauna monitoring and lay the foundation for dynamics, ecology, biodiversity, and control of water quality in the study area. Finally, the outcomes of this study can be used to steer baseline information for future water management strategies and priorities for restoration activities locally and in similar river systems at the Amazon region of Ecuador and neighboring countries.

2. MATERIAL AND METHODS

1.1. Study area

The whole Napo River drainage basin area covers 100500 km2, distributed among Ecuador (59.6%), Peru (40%) and Colombia (0.4%), with an altitude ranging from 100 to 6300 m.a.s.l [34]. The Ecuadorian part of the Napo basin crosses the “Cordillera Real”, whose eastern side has steep slopes that descend from 6000 to 500 m.a.s.l over only 100 Km (Figure 1), and covers an area equivalent to 20% of the eastern part of this country [34,35]. The NRB presents a strong climate gradient where several ecosystems can be found, from the higher to lower elevations (paramo, mountain forest, and piedmont rainforest) [36]. In addition, this basin is particularly important for its high density of river systems, which flow through protected areas and drain from the Andean slopes towards the tributaries of the Amazon River [37,38].The Napo river is a main eastern river and an important tributary of the Amazon river since provides a mean annual discharge of about 6300 m^3/s, with a drainage area at the Andean basin outlet of 12400 km^2 and mean precipitation rates of ˜290 mm/yr [33,36]. The most common anthropic pressure factors in NRB and its micro-basins are 1) the deforestation of the riverbanks, 2) the development of infrastructure (near and over) the rivers, 3) the release of untreated wastewater from homes and agricultural systems, 4) as well as gold and stone material mining in the river or its surroundings [7,39]. In fact several freshwater ecosystems impact studies due to population growth and the flexibility in the application of the environmental regulations [28,29,40] have been described: as the widespread contamination with emerging pollutants (EP) like microplastic [41,42], or the impact on water quality by high metal concentration (Cd, Pb, Cu, Zn, and Hg) [43,44]. In addition, these studies have also emphasized the importance of knowing the consequences of the pollution effects on water bodies in NRB, through standardized and long-term monitoring of these aquatic ecosystems [40–42].

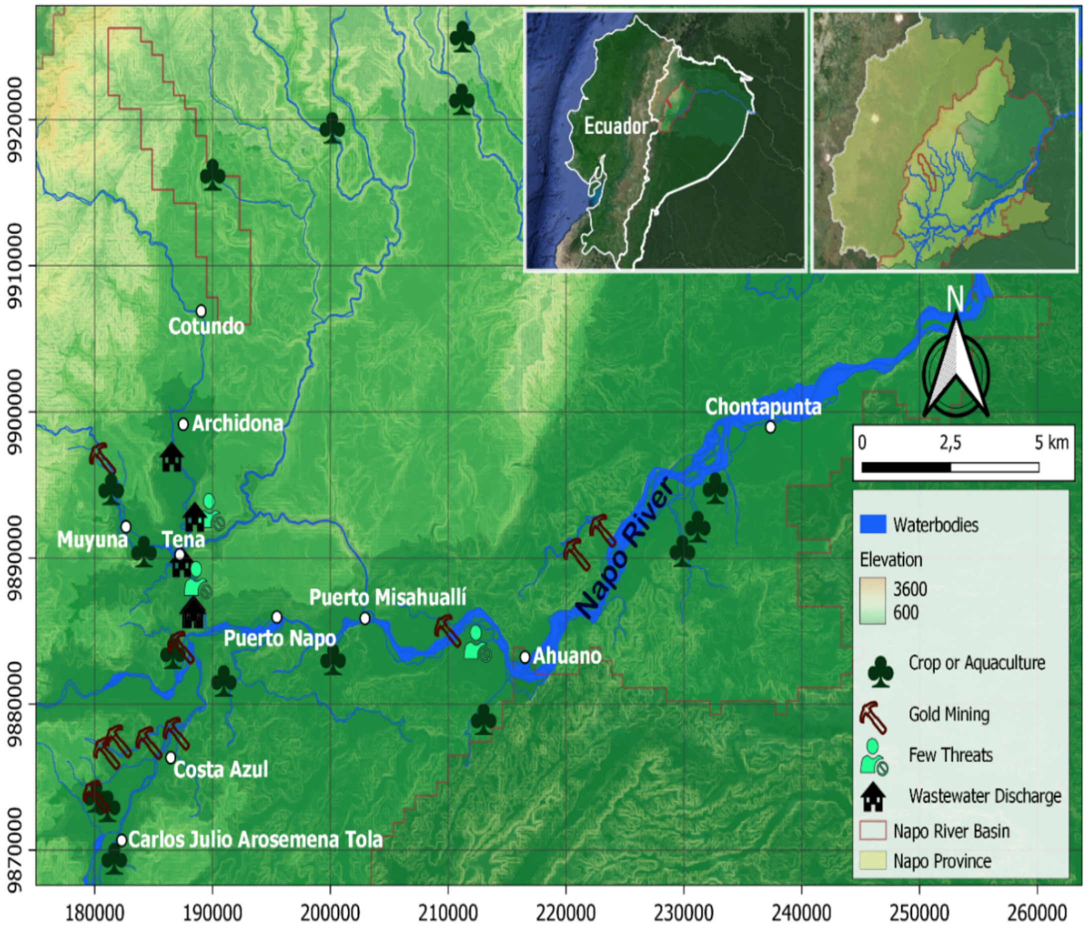

Figure 1. Study area: Map indication of the sampling sites in the NRB, the blue lines show the main Location of the Napo Basin and the study site.
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1.2. Data Collection

Samplings (called S) for S1 [25], S2 [40], and S3 [44] were made between February to December of 2020, then, a final sampling "S4" was done in November of 2021. All samplings were done at the beginning of the dry seasons to avoid high flows in the rivers since the flood current redistributes the substrate material, as well as drag and move the macroinvertebrates resulting in their composition changes [45,46]. In total, 36 samples were collected in some water bodies at the NRB (Figure 1; Table T1 of Supplementary Materials) in places with Crop or Aquaculture (CA), Gold Mining (GM), Wastewater Discharge (WD), and Sites with Few Threats (FT) as a way of classification an anthropic gradient. Samples of macroinvertebrates were collected according to the multi-habitat method, recommended to evaluate water quality in Ecuador by Chancay et al.,[47]; Celi et al.,[7]; Cornejo et al.,[6]; González et al.,[48].

Macroinvertebrates were collected with a D-frame dip net (500μm) considering each microhabitat and substrate present at each site as a sub-sample and covering a stretch of 10 meters for 3 minutes. All the subsamples were placed in the same plastic cover and treated as a composite sample to ensure a thorough biodiversity assessment at each sampling site. Macroinvertebrates were identified to the lowest taxonomic level possible (family), using the identification keys of Domínguez et al.,[19]; Hamada et al.,[49] and Palma.,[50]. Physicochemical parameters were obtained *in situ* simultaneously with the collection of macroinvertebrates in each study; for these parameters, a YSI professional plus multiparameter (YSI Incorporated, USA) was used to measure the temperature, dissolved oxygen (DO), turbidity, total dissolved solids (TDS), and pH.

1.3. Data analyses

1.3.1. *Ecological Water Quality Index*

In the present study, four biotic indexes were calculated for each sample site. First, the Andean-Amazon Biotic Index (AAMBI) was calculated and assigned a numerical value to each macroinvertebrate family; 1 for contamination-tolerant families to 10 for highly sensitive ones [6,40]. The sum of these numerical values were assigned to five water quality categories [44]; where the quality ranges were reclassified according to the site with the highest score (AAMBI=102 reflected in Table T5 of Supplementary Materials), so the classification was defined as: excellent (>100), very good (75-90), good (50-74), regular (25-49), and bad (0-24). Then, the Biological Monitoring Working Party adapted for Colombia (BMWP-Col) was used because Ecuador does not have its ecological water quality index, and Colombia has relatively similar environmental conditions to Ecuador [51].The BMWP-Col was performed based on the macroinvertebrate community composition, wherein each macroinvertebrate taxon is associated with a certain tolerance score [26,52]. The tolerance score ranged from 1 for tolerant taxa and 10 for representing sensitive taxa; the water quality score classification was: good (≥100), moderate (61-100), poor (36-60), bad (16-35), and very bad (0-15) ecological quality [26,53]. Finally, following the concept of the EU Water Framework Directive; the index of the ecological quality quotients (CCE) was calculated for AAMBI and BMWP-Col indexes, respectively [54]. Both calculus estimations were established on the deviation between the observed index (form CA, WD, and GM zones) and the expected index for the reference state (from FT sites); the ranges of these quotients are: High (0,90-1,00), good (0,75-0,89), average (0,60-0,74), poor (0,45-0,59), and bad (0-0,44) ecological quality [55].

1.3.2. *Statistical analysis*

To evaluate the differences between the alpha diversity to the efficiency of sampling, and to compare the equitability of species in each gradient (CA, GM, FT, and WD) the abundance data of macroinvertebrate families for these sites were obtained [56,57]. First, the sample coverage for each site was estimated by plotting the sample completeness curve, as a function of sample size for each of the four treatment zones [58]. Next for each site, the Hill diversity estimators were plotted through rarefaction and extrapolation integrate curves; Hill numbers (or an effective number of species) work to quantify the species/taxonomic diversity of an assemblage [59]. The sample size and coverage-based integrations of rarefaction (interpolation) and extrapolation (prediction) of Hill numbers represent a unified standardization method for quantifying and comparing species diversity across multiple assemblages [60]. This parameter tell us the number of effective or equally abundant families found at the site, so, a weight (q) was added to the specific abundances of each family, where q0 refers to the richness of families, q1 gives more importance to common families (Shannon diversity), and q2 gives more importance to the dominant families in the ecosystems (inverse Simpson diversity) with a confidence interval of 95% [61,62]. An estimator based to the lowest sample coverage value was applied in order to make reliable comparisons between the sites; these coverage values were obtained with the sample completeness curve [63]. Furthermore, the quantitative structure of the community was evaluated through rank-abundance curves, elaborated from the logarithms (log_{10}) abundance value of each family. Next, to know how complete the sampling was for each site completeness curves were made in the sites CA, GM, FT, and WD data, respectively. According to Jiménez-Valverde et al.,[64] and Lou et al.,[65] extrapolation and rarefaction curves based on sample coverage for diversity were plotted, and then the diversities of each site based on the coverage of the sample for each q value were compared, after calculating the base coverage [62]. Finally, Principal Component Analysis (PCA) clustering based on study zones data CA, GM, FT and WD (Tables T1, T2 and T5 of Supplementary Materials) was used to analyze the correlations between abiotic (physicochemical = Temperature, DO%, Turbidity, pH, and TDS) parameters and biotic (macroinvertebrates structure = Species richness, Shannon diversity, and Simpson diversity; and ecological water quality indexes = AAMBI, BMWP-Col) community [8]. The eigenvalues to determine the number of principal components to be considered according to Figure 8 (of Supplementary Materials).

The curves, the rarefaction and extrapolation estimators along with their confidence intervals, and all the statistical analyses were obtained using the R studio (version 4.0.3) packages *iNEXT()* and *BiodiversityR()*; then, for computing PCA the *prcomp()* and *PCA()* packages were used. These packages can be also found and downloaded from RDocumentation website.

3. RESULTS

3.1. Macroinvertebrate assemblage characteristics

A total of 1.218 benthic macroinvertebrates were counted and identified. All the specimens belong to 20 orders shared in 44 families respectively (Table 1). No benthic organisms were recorded at all sample points (Table T4 of Supplementary Materials) varying the richness from 0 to 16 families. Most of the aquatic organisms collected corresponded mainly to larvae, followed by adults. Diptera that represented the 50.98% *(*621 individuals*)* present in all community, followed by *148* Ephemeroptera (12.15%), 126 Coleoptera (10.34%) and the 26.52% remaining were distributed in Trichoptera, Odonata, Neotaenigloossa, Rhynchobdellida, Plecoptera, Pulmonata, Hemiptera, Megaloptera, and other nine orders. Chironomidae (547 individuals), Elmidae (110), and Leptophlebiidae (59) were the most abundant families and were present in almost every sample sites (see Figures 9-12).

Table 1. Species abundance within families at all sites of this study

Order	Family	Sample sites				Number of individuals
		CA	GM	FT	WD	
Coleoptera	Dytiscidae				1	1
	Elmidae	33	2	75	0	110
	Hydrophilidae			1		1
	Psephenidae	1		4		5
	Ptilodactylidae	2		1		3
	Scirtidae				6	6
Decapoda	Palaemonidae	6		1		7
Diptera	Ceratopogonidae		3	9		12
	Chironomidae	303	4	80	160	547
	Empididae			1	1	2
	Culicidae			1	2	3
	Simuliidae			57		57
Ephemeroptera	Baetidae	22		13		35
	Euthyplociidae	10		2	6	18
	Caenidae	1		2		3
	Leptohyphidae	10		23		33
	Leptophlebiidae	11	6	34	8	59
Hemiptera	Belostomatidae				2	2
	Hebridae			1		1
	Naucoridae	5	1			6
	Notonectidae				1	1
	Veliidae	1				1
Lepidoptera	Crambidae			1	2	3
Megaloptera	Corydalidae	2	4	2	1	9
Neotaenioglossa	Thiaridae				41	41
Odonata	Aeshnidae			2		2
	Coenagrionidae	4	4	6		14
	Gomphidae		3	15		18
	Libellulidae	10	1		1	12
	Megapodagrionidae			3	1	4
	Platystictidae			1		1
Plecoptera	Perlidae	3	5	8		16
Pulmonata	Planorbidae			1	13	14
Rhynchobdellida	Glossiphoniidae				8	8
	Piscicolidae				24	24
Seriata	Planariidae			4		4
Sorbeoconcha	Hydrobiidae				1	1
Sphaeriida	Sphaeriidae			50	3	53
Trichoptera	Calamoceratidae			1		1
	Glossosomatidae				24	24
	Hydrobiosidae			2	1	3
	Hydropsychidae	35		13	3	51
	Hydroptilidae	1				1
Tricladida	Dendrocoelidae				1	1
Number of individuals		460	33	414	311	**1218**
Richness		18	10	30	23	**44**

By: Daniela Alvear-S, 2022

The estimated sample coverage was high for all samples. The sample size for CA was 0.991, followed by FT (0.976), WD (0.971), and GM (0.9412). Indicating that sampling was likely to have covered the full taxonomic diversity almost completely for all studies zones (Figure 2).

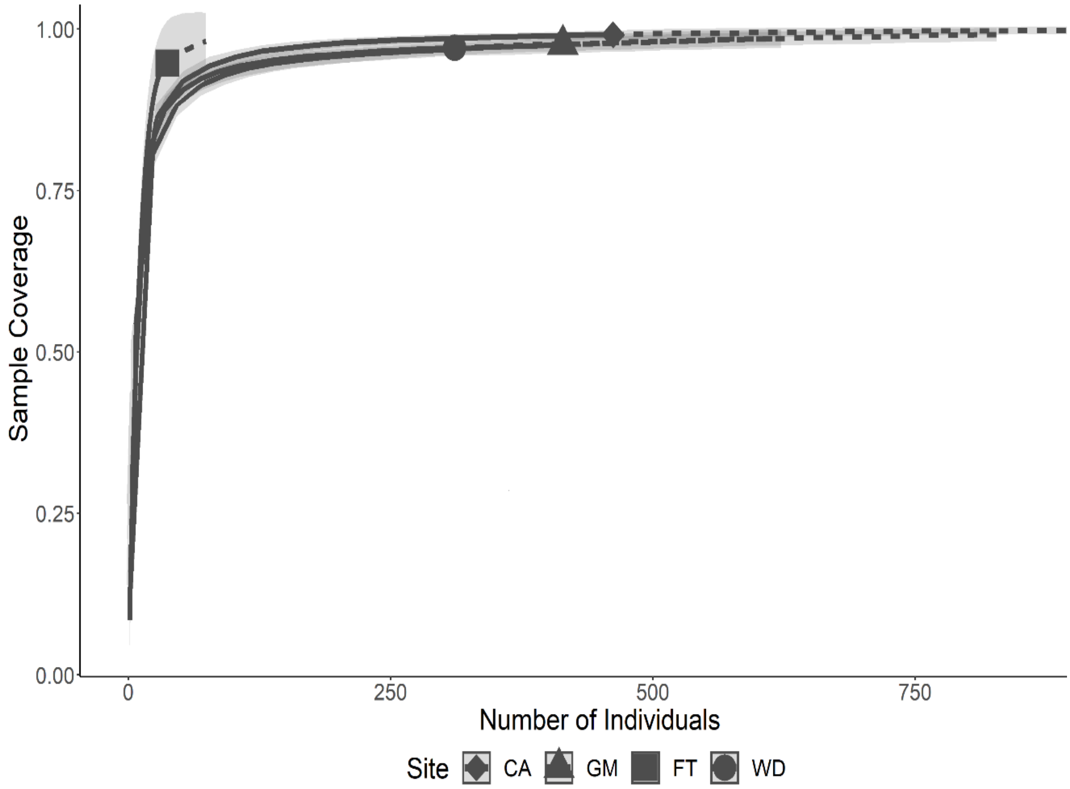

Figure 2. Plot of sample coverage: the reference samples (solid line) and the extrapolated sample (dashed line) as a function of sample size of invertebrates in four anthropic gradients.
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The lowest sample coverage value was for the GM areas (with 0.942); therefore, for macroinvertebrate communities to be comparable and to estimate the diversity of all zones, a much lower value (from 0.9) was used. In Figure 3, error bars are "95% confidence interval" which assumes uncertainly arises from the process of randomly sampling fixed number of individuals in the standardized sample. The GM zones present large error bars, which means that these sites confidence interval is very wide. Nevertheless, Alpha diversity indicators (Figure3); Richness, Shannon and Simpson were significant between all the zones ($p>0.05$). For q0 it can be seen that the species richness (15 families) and abundance were higher for FT (0.976) and have significant differences compared to the rest of the sites (WD, GM, and CA) with 11, 9, and 8 families respectively. It can also be noted that WD and CA do not have significant differences from GM but between them, WD (11 families) presents greater diversity than CA (8 families). For the diversity of the orders q1 (common species) and q2(dominant species), it can be noted that there are no significant differences between GM (q1= 8 and q2=7) and the rest of the impacted sites. Then, FT sites (q1=10 and q2= 8) remain predominant with significant differences in relation to WD (q1=5 and q2=3) and CA (q1=3 and q2=2), which means that these zones (FT) have greater richness and diversity of families than the rest (with a p-value less than 0.05).

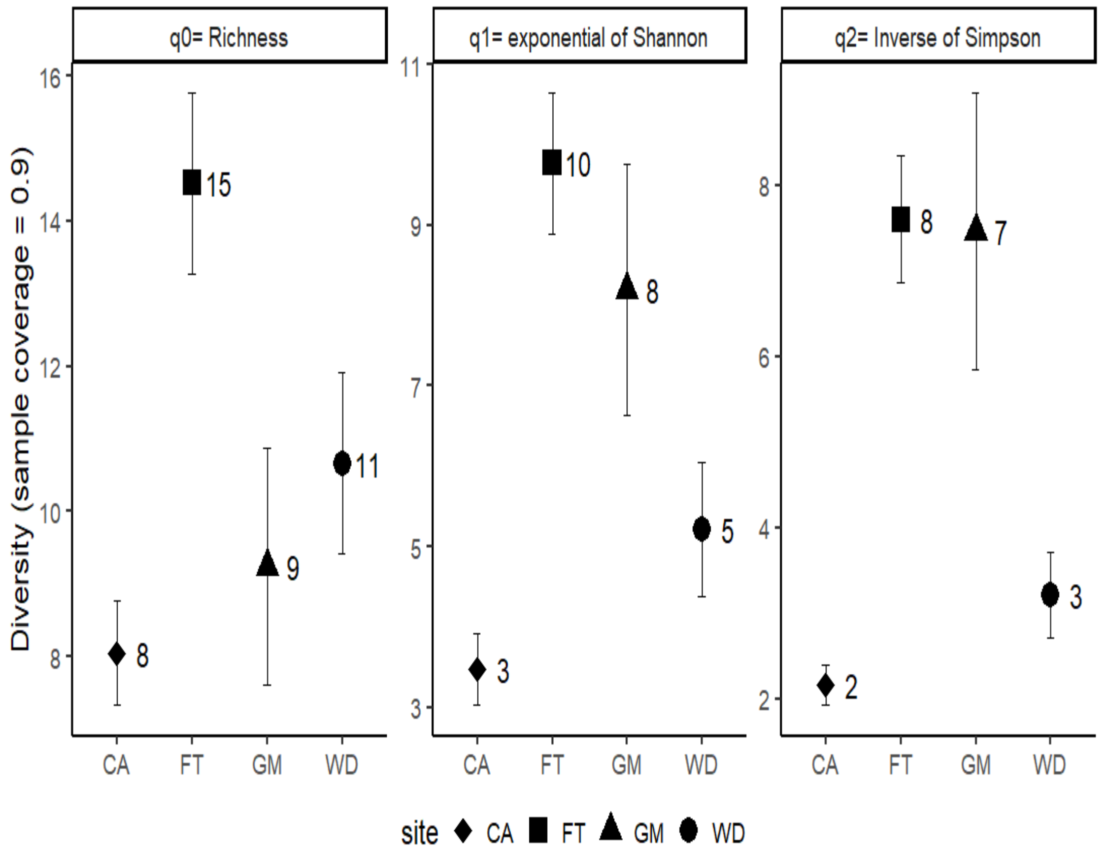

Figure 3. Alpha diversity analysis: q0 (Richness), q1 (Exponential of Shannon), and q2 (Inverse of Simpson).
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There were significant differences in species composition and relative abundance between the sites (Table 1, Figure 4). At Crop or Aquaculture (CA) zones, 460 macroinvertebrates were recorded belonging to ten Orders and 19 Families. The rank abundance curve in CA zones present a steep slope indicating low evenness; as the Diptera order have much higher abundance than the other orders. Diptera has more dominance (quantify of individuals) but this quantify was distributed in only one family. Then the curve presents a low slope, that indicates high evenness as the abundances of the rest orders are similar. The order Ephemeroptera (Baetidae, Euthyplociidae, Caenidae, Leptohyphidae, and Leptophlebiidae) presented more diversity (number of Families) with individuals evenly distributed, but in small quantities. In this zone, Chironomidae was the most abundant Family (303), followed by Hydropsychidae although this Family only has 35 individuals (Figure 4).

33 individuals (into ten Families) were found in Gold Mining (GM) areas, Ephemeroptera and *Plecoptera* were the most dominant and diverse orders; nevertheless, it does not have difference between the number of individuals present in the other five orders (see Table 1). Leptophlebiidae and Perlidae were the most abundant families (Figure 4). Few Threats (FT) sites had 414 individuals within thirty Families (Table 1). The FT curve presents a shallow slope that indicates high evenness as the abundances of different orders are similar. Diptera (Ceratopogonidae, Chironomidae, Empididae, Culicidae, and Simulidae) had the most individuals (148), and together with Ephemeroptera (Leptophlebiidae, Leptohyphidae, Euthyplociidae, Caenidae, and Baetidae) were the Orders with more diversity (number of Families) than the others (see Table 1). Chironomidae and Elmidae were the most abundant families in this site followed by Simuliidae, because present 80, 75 and 59 individuals respectively (Figure 4).

At Wasterwater Discharge (WD) sites, 311 individuals were recorded. The curve in WD sites, presents a steep slope that indicates low evenness as the Diptera order have higher abundance than the other orders (163 individuals); then the curve presents a shallow slope, that indicates evenness in the abundances of the rest orders (Figure 4). Diptera (Chironomidae, Culicidae, Ceratopogonidae, and Empididae) had more dominance of individuals, while Neotaeniglossa was the second order in abundance of individuals (41), just in one Family (Thiaridae) (see Table 1). Chironomidae followed by Thiaridae were the most abundant families in these sites, 160 and 41 respectively.

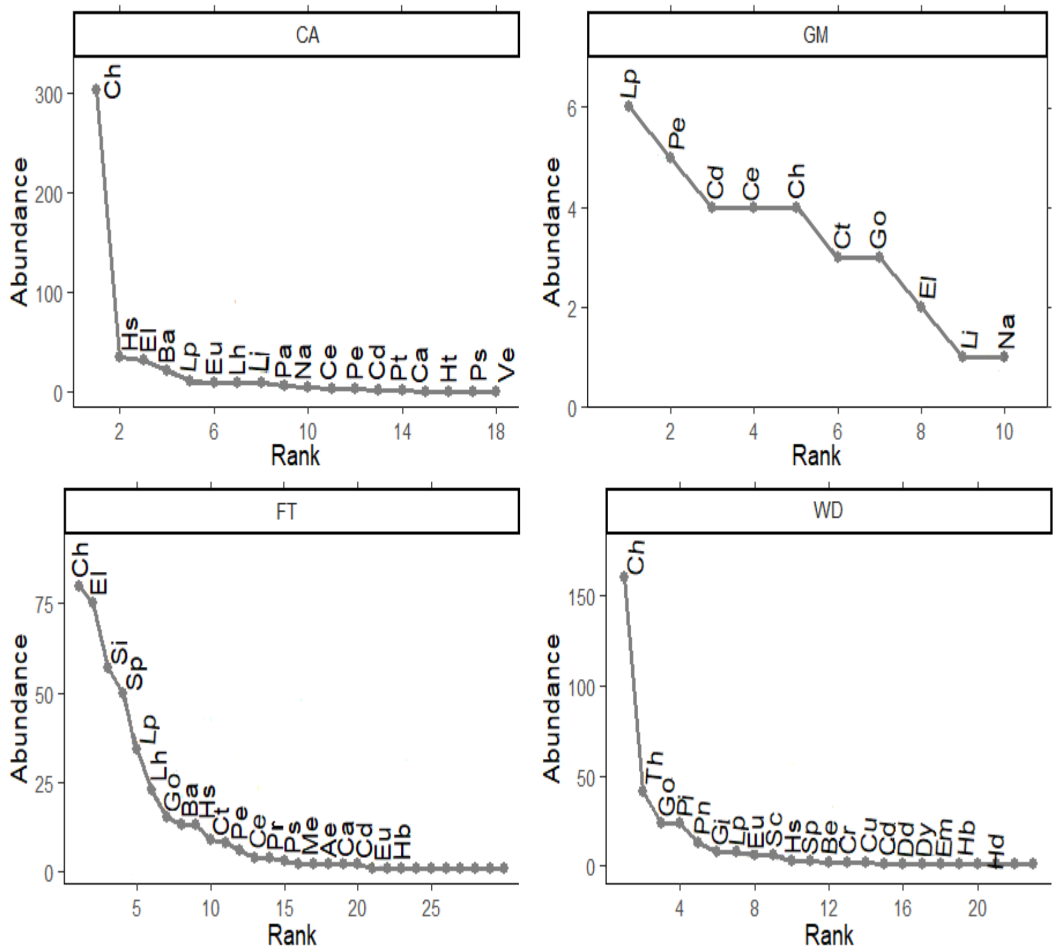

Figure 4. Abundance range curves of the taxa capture in Crop or Aquaculture (CA), Gold Mining (GM), Wastewater Discharge (WD), and in Few Threats (FT) zones.
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3.2. Ecological Water quality

According to the AAMBI index (Figure 5a), all the sites are in a range of quality “Moderate” and “Very Bad”. FT zones obtained the higher score of 84, which describes a “Moderate” ecological quality of water. Then, CA zones were described with “Bad” quality as it shows a score of 25; and finally, WD and GM sites obtained a score of 23 and 13 respectively and were rated as “Very Bad” water quality sites. Regarding the ecological quality quotient for the AAMBI index (CCE-A); the FT (control) sites were significantly different form the rest (Figure 5c) because were described with a “Good” ecological water quality. This index also shows that the ecological quality level for CA and GM was “Moderate”; while WD had an ecological quality index of “Poor”.

On the other hand, for the BMWP-Col index (Figure 5b), water quality ranges vary between “Moderate” and “Very Bad”. FT sites have obtained a rating of 87 and establishing in a range of “Moderate” water quality. While CA and WD zones with scores of 26 and 23 were located in the “Bad” category, and then, GM areas obtained 12 and are in the “Very bad” quality range. Finally, the ecological quality quotient (CCE-B) calculated from the tolerance levels of invertebrates to BMWP-Col; the FT (control) sites were not significantly different (Figure 5d) since had a “Good” ecological water quality level. In the CA zone, the ecological quality level was “Moderate”; while GM and WD has a “Poor” ecological quality.

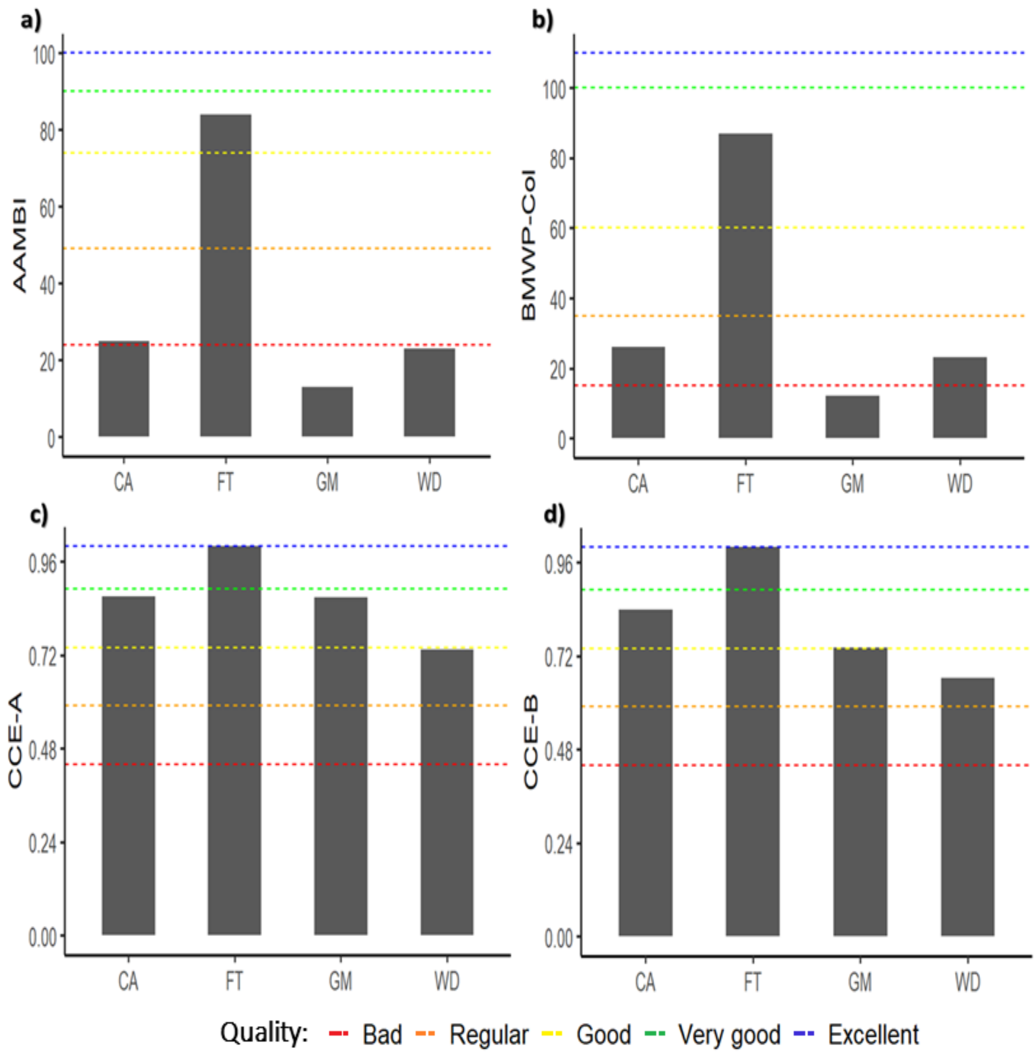

Figure 5. Quality results: a) Andean-Amazon Biotic Index, b) Biological Monitoring Working Party, c) CCE-A (index of the Ecological Quality Quotient derived from AAMBI), d) CCE-B (index of the Ecological Quality Quotient derived from BMWP-Col).
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3.3. Multivariable Analysis

PCA ordination of 36 samples in the first two dimensions is shown in Figure 6. The first eigenvalue (PCA 1) explains 33.3% of the total variance, and second eigenvalue (PCA 2) further 26.1%, Therefore, about 59.4% of the variation is explained by the first two eigenvalues. The result of the PCA analysis on the macroinvertebrate diversity data and abiotic parameters show that the highest biotic water quality indexes (AAMBI and BMWP-Col) and upper pH and DO% values had a positive correlation among them; in addition, according to the clusters both them are also characteristic parameters of FT. However, these parameters had a negative correlation with high TDS, turbidity, and temperatures whose are related with GM areas. Then, high macroinvertebrate diversity indexes (Species richness, Shannon diversity, and Simpson diversity) had a positive correlation with TDS; thus, specifically the variable "species richness" had a negative correlation with Temperature and Turbidity. Finally, WD and CA zones don´t have variables that highlight its, since they are related to all the parameters described.

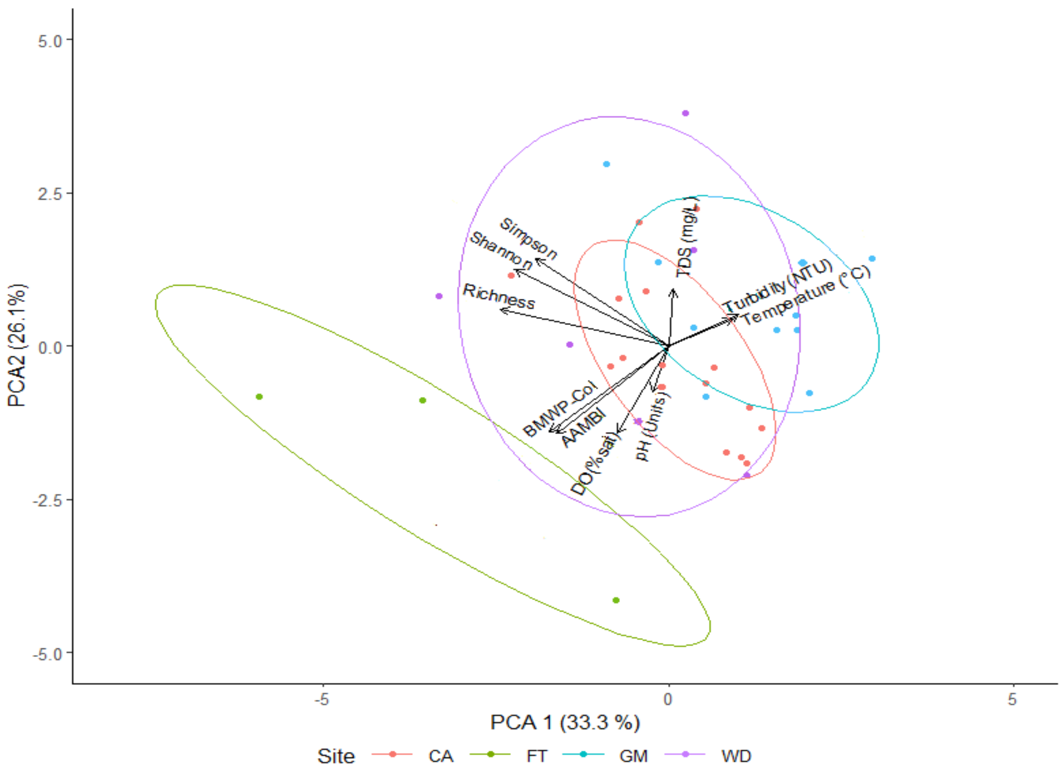

Figure 6. Principal component analysis (PCA) clustering for the correlations between abiotic parameters and macroinvertebrates information (structure and ecological water quality indexes).

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4. DISCUSSION

The aquatic environments of the Ecuadorian Amazon basin region lack in-depth knowledge of both macroinvertebrates taxonomic and functional diversity, complementary to the physical and chemical data; this shortfall impedes the development of useful mechanisms and tools for stakeholders [26] to have a better assessment of the water quality using an integrative approach trough multiples lines of evidence, as have been previously shown on studies made in many of these study sites [40,44,47]. This study provides an overview of the situation regarding the ecological quality of waterways affected by some human activities, and ascertains the impacts about the structure the aquatic communities that develop in freshwater ecosystems on the Napo River Basin in Ecuador. The differences might be explained by the fact of that many streams in the Upper NRB are influenced by industrial effluent, crop or aquaculture activities, and gold mining industries. These activities are related to the removal of riparian vegetation, which has a negative effect on the input of organic matter (source of energy in small stream ecosystem); this can affect the habitat, the water quality, and consequently the biota (e.g. the diversity and abundance of macroinvertebrates) [66–68].

Macroinvertebrates are ideal indicator organisms as various taxa are associated with different levels of water quality [69] and tend to be sensitive to certain types of human activities. Changes in water quality could modify species composition, particularly those that are sensitive to pollution; while several morphospecies grow in any environmental state or anthropogenic disturbance [67], a pattern that was determined in all samples in the NRB.

Crop or Aquaculture (CA) had a largest abundance of individuals (406) than the other sites but has a low number of families. These sites indicated low evenness in the species distribution, because presented a great quantity of individuals distributed in only one family (Chironomidae). Chironomidae is frequently one of the most abundant families in all types of aquatic ecosystems, fresh or brackish water, stagnant or flowing; and it is also adapted to live in water with all kinds of organic and inorganic contaminants [70,71]. As is the case of *Chironomus sp.* which has hemoglobin in its hemolymph, this respiratory pigment allows it to capture oxygen in conditions of almost total lack of dissolved oxygen in an hypoxic aquatic environment [72]. Even though in these sites also were found a diversity of insect families highly sensitive to their habitat changes (some families of Ephemeroptera [27]) evenly distributed, these families were in small quantities (Figure 4-CA).

Gold Mining (GM) present the lowest sample coverage value (0.941). These sites had absence (0 to 7 individuals per site; see Richness in Table T5) or low abundance of macroinvertebrate families (10 families), with the exception of Leptophlebiidae and Perlidae. Although, it should be emphasized that the family with more individuals presents only 6 of them, meaning no significant difference between the number of individuals present in other families (Figure 4-GM). However, the low diversity of taxa at this site (10 families) could be evidence of the negative effects of pollution on the structure of the macroinvertebrate community since undisturbed habitats are characterized by a high diversity and number of different families [73,74]. In addition, predatory and omnivorous macroinvertebrates (as Perlidae) bioaccumulate high Hg concentrations compared to other functional feeding groups; Perlidae rely mainly on dietary inputs from periphyton, and this food source tends to have high Hg concentrations [75,76]. Then, Leptophlebiidae is a family with several generalist genera widespread in aquatic ecosystems and related to changes in habitat quality that has been categorized as sensitive to environmental alterations of its habitat; the abundance decrease in many species (specially shredders) of this taxa can probably disturb nutrient cycling [66,77]. Its presence could be an indicator of recovery to the previous conditions of the ecosystem in GM areas, which could become favorable for the habitat conditions of the tributaries to improve the habitat quality in the coming years; for example, if the progressive formation of a riparian forest is allowed [13]. Nevertheless, it must be taken into account that the GM zones present large error bars (Figure 3), which could be because in some of these sites no individuals were found (Table T5). Therefore, to increase reliably, one should think about increasing the number of study sites in each one of the anthropic gradient areas thus having a more accurate notion about the changes in diversity compositions.

Wasterwater Discharge (WD) sites, had the third in abundance of individuals (311), although these are mostly distributed within two families (Table 1). These sites presented low evenness in the individual's distribution because the Diptera had higher family's abundance than the other orders; although, these sites also indicated evenness in the abundances than the other orders (Figure 4-WD). Diptera (Chironomidae, Culicidae, and Empididae) had more dominance of individuals (163), while Noetaeniglossa was the second order with most individuals (41), with the only family (Thiaridae) (Table 1). Thus, Chironomidae followed by Thiaridae were the most abundant families in these sites. It is known that Chironomidae can colonize varied environments with different trophic conditions [78]. But, talking about human sewage and depending on its genera the species of this Family can cause problems by generating allergic responses in humans; although can be pests or pest controllers, also are well known for being transmitters of diseases to other organisms [19,49]. Thiaridae, on the other hand, is a family of freshwater gastropods introduced from Malaysia, which can colonize varied environments with different trophic conditions: from oligotrophic to intensely eutrophic, lotic or lentic, and even brackish systems; its high rate of reproduction due to its impressive ecological plasticity, represents a great threat to native species by displacing them from their niches; besides they are transmitters of trematodes that can parasitize fish of commercial importance [23,79].

Few Threats (FT) presented the highest abundance and the family´s richness (30 families) and a greater diversity of common (q1=10) and dominant families (q2=8) than the rest of the sites (Table 1 and Figure 3). These sites can be categorized as aquatic environments that are not very affected since it presents equity in the diversity and abundance of macroinvertebrates sensitive to habitat disturbances, and contains common families that can almost adapt to any environmental conditions (e.g. Chironomidae) [78]. Chironomidae and Elmidae were the most abundant families in these sites. Elmidae is a family of the Coleoptera Order, that comprise the largest group of insects in terms of species richness are functionally important by being involved in a range of ecosystem processes and trophic interaction networks such as nutrient cycling and processing, and act as a good indicator of water quality [19,49,80]. In a general way, Diptera had the major quantity of families and equal to Ephemeroptera presented more diversity and richness at these sites. Ephemeroptera lives in freshwater environments in its larval stages, from flowing to stagnant waters, and in virtually all available microhabitats [19]. A large number of families in this Order are good indicators of ecosystem quality and are generally highly sensitive to acidic conditions [27].

Regarding the biotic environmental assessment of the families analyzed using the AAMBI, BMWP-Col, and CCE indices. The BMWP-Col water quality varied between moderate and very bad, and the sites were ranked in this order: FT>CA>WD>GM. The application of BMWP-Col can be extended to applied research because it is already covered by the current regulations in many countries, where the identification of upstream and downstream impacts on anthropogenic works with effects on aquatic systems are a priority [81]. Then, the results showed that the ecological water quality of sampling zones ranged from moderate and very bad for AAMBI index, and the zones were ranked as FT>CA>WD>GM. The AAMBI has been recently adapted (2019) for the Andean-Amazonic ecosystems of Ecuador [23]. However, in studies such as Galarza et al.,[40] and Capparelli et al.,[44] AAMBI was the biotic index which better detected anthropic effects on water quality compared with other water indicators. Then, in the main index of the integrity of aquatic ecosystem (CCE) that was used as response variable; the control values (FT) were significantly different from the rest, but the sites with anthropogenic activities (CA, GM, and WD) did not present significant differences between them. In this index, in both cases (CCE-A and CCE-B) the water quality varied between good and poor, categorized as FT>CA>GM>WD, as compared to AAMBI and BMWP-Col by themselves.

Overall, these indices require less rigorous levels of identification. The BMWP-Col, however, appear to be more useful than the AAMBI and CCE scores. In this case, the first must be recommended as was sensitive to the whole range of water quality in this study and appears to have wider geographical applicability [17]. In addition, the original BMWP values indicates that midges (Chironomidae) are considered as one of the most tolerant taxa, with BMWP-Col values of 2 [81]. Chironomidae was found in all sites (Table 1 and Figure 4), no matter the level of the biological water quality (including very bad status). Agree with the revision conducted by Mao et al.,[82], the prevalence of this family can be prominent reason for their low BMWP-Col values.

As can be seen, in all these indices the sites that presented the best biological water quality were FT (Figure 5). Nevertheless, the specific ecological water quality of each anthropic gradient was described as:

In Crop or Aquaculture (CA) sites, the biological indices describe a regular ecological integrity of the aquatic ecosystem with bad water quality, but perhaps with a good water cleansing. The latter could be because in these sites the common use of pesticides highly toxic and illegal has already been described, giving the possibility of negatively influencing the biological water quality, because this activity apparently causes an increase in turbidity [25]; and although in this study, mostly the physicochemical water quality parameters were within the limits for environmental safety set by TULSMA and the WHO, the water quality results showed some indicators of eutrophication. Crop or

Aquaculture practices cause nutrients input (phosphorus, nitrates) that can leads to eutrophication and depletion of DO concentrations, and causes imbalances at the base food webs that impair ecosystem function and community structure [40,83]. Was founded indications that in these sites the water quality might be influenced by pesticide use [25], but more research is necessary to prove this correlation. Equally important as the above listed research needs are studies that will enable producers to minimize potential pesticide pollution while continuing to generate economic profits [84].

Gold Mining (GM): the biological indices describe good ecological integrity of the aquatic ecosystems and reported average water quality and cleansing. The latter could explain the positive relation founded between GM and the high turbidity and temperatures in the multivariable analysis. In addition to the fact that the quality indexes of these sites were among the lowest compared to the rest of the sites. In these same areas Capparelli et al.,[44] reported the alteration of physicochemical parameters TSS, DO, turbidity, and color; which means a constant load of potentially metal (V, B and Cr) contaminated sediments into the rivers; also Ag, Al, As, Cd, Cu, Fe, Mn, Pb, and Zn in water were detected above quality standards at sampled sites; in addition, was reported that an associated impact of gold mining is the erosion of the riverbanks and intense modifications to the landscape. Thus, this would be one more reason why mining activities and their methods of disposal of toxic by-products are considered one of the main reasons for the deterioration of environmental health [30]. So, given the importance of the Amazon region for biodiversity preservation and ecosystem service provision, is required further control of the gold mining expansion and its continued environmental monitoring. Emphasizing, that enforcing compliance with environmental policies in the gold mining sector in the Amazon depends on community participation in decision-making, coordination between government institutions, and control of mining activities.

In Wastewater Discharge (WD): the biological indices describe a bad ecological integrity of the aquatic ecosystem, with bad water quality and an average water cleansing. These kinds of sites were previously reported such as zones with bad water quality because had high EC (Emergent Contaminants) values, which are likely due to the high load of ions from the leachate water that is discharged within the water bodies [40]. According to Capparelli et al.,[41] the absence of waste management in Amazonian cities may result in pervasive contamination of freshwater ecosystems and potential adverse effects on aquatic biota and human health. Then, these areas are urgently needed proper sewage treatment and management actions toward local sewage treatment.

Few Threats (FT): the biological indices describe a very good ecological integrity of the aquatic ecosystem, with moderate water quality and high cleansing. FT showed the highest ecological water quality indexes and were related to upper DO% percentages.

Variability of macroinvertebrate groups composition is usually linked with physicochemical interactions and site-specific conditions of which the impact is hard to be unscrambled by biotic indices [26]. Multiple physicochemical (e.g. high temperature, TDS, and turbidity values) parameters were identified as variables related to the anthropic treatments, but specially in the GM zones (Figure 6). While upper DO% and pH values were the characteristics variables in the FT sites on the NRB. In the future, more parameters of environmental conditions (e.g. nutrients, conductivity, elevation, velocity, depth, width, etc.) must be considered as influential variables, since the longitudinal gradient of the river is affected by upstream locations and surrounding land use[85]. Moreover, similar biomonitoring techniques is recommended to be done during both, dry and rainy seasons to be able to assess possible seasonal differences in the ecological water quality [51], and the data collected in this study can be used as base line in order to detect possible changes that the water quality could have with future anthropic pressures over this important river basin.

5. CONCLUSION

The bioassessment tools developed in this research clearly indicate that the anthropic gradient declines water quality and biodiversity in all the tributary's locations, while in few threats' sites the quality and the diversity had better conditions. This research also gives stakeholders scientifically defensible rationales for measuring the evolution of the ecology in the streams, either to monitor a type of anthropogenic disturbance or to evaluate the feasibility of a restoration project. Specially in gold mining zones, where previous research proved that there are levels of contamination that could be considered alarming which explains the low ecological water quality values; as well as the loss of diversity and richness of the macroinvertebrate families, with the dominance of communities associated with environmental degraded conditions. In this sense, sanitation steps should be taken to gradually reduce water contamination, and monitor how the macroinvertebrate community evolves to the application of these measures. Then, the development of farming systems that minimize the pesticide discharge in runoff is a high priority research needed. Thus, the challenge of responsible pesticide use requires research investments for a better understanding of the environmental fate and behavior of pesticides, and for the development of production techniques that reduces the probability of pesticide pollution in the water bodies lands while maintaining productivity.

On the other hand, the present study provided baseline information about the macroinvertebrate community composition in the NRB (Napo, Ecuador). Using this information as a future prospect is very important because is possible to document the location of correctly identified species with their ecological georeferenced data, as well as to monitor seasonal trends and the correlation with various environmental parameters. Biodiversity is threatened and the biogeographical distribution is changing rapidly, because of climate change and human interventions. Thus, is necessary to continue doing basic and applied environmental works (as the present research) about macroinvertebrates in this important biogeographic region to enable better protection of its aquatic biodiversity.

Lastly, in the future is possible to use this research to adjust the values of biotic indexes to different sections of the rivers. In addition, there is still a possibility that the low diversity values that were reported in this study could be due to the number of sites where the monitoring was carried out. Therefore, one should think about increasing the number of study sites in these areas for having a clearer notion of changes in diversity compositions and also to have a quasi-experimental design.

Credit authorship contribution statement

Daniela Alvear-S: Conceptualization, Formal analysisis, Writing-original draft, Writing-review & editing. **Daning Montaño:** Formal analysis, conceptualization and review. **Rodrigo Espinoza:** Review & editing. **Mariana V. Caparelli:** Resources & review.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationship that could have appeared to influence the work reported in this paper.

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7. SUPPLEMENTARY MATERIALS

The estimated sample coverage (Figure 3) was high for all samples; S1 with 0.987, followed by S3 (0.977), S4 (0.969), and S2 (0.885) indicating that sampling was almost complete for all studies. The species accumulation curve for all studies (S1 to S4) approached the asymptote (1.00), showing that our richness estimate derived from samples was an accurate making studies comparable to each other.

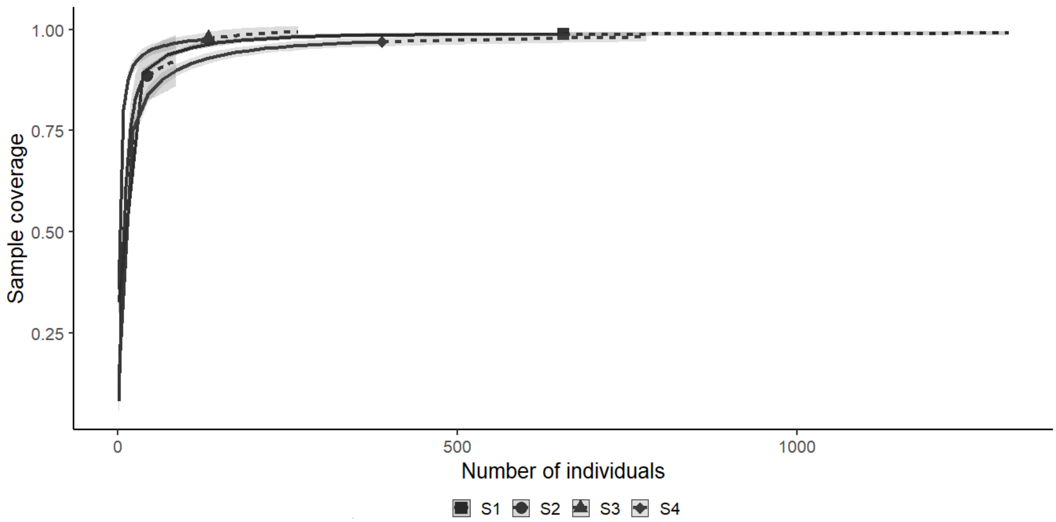

Figure 7. Species accumulation curves, for the reference sample (solid line "interpolated") and the extrapolated sample (dashed line) on the macroinvertebrates captured in four different studies.
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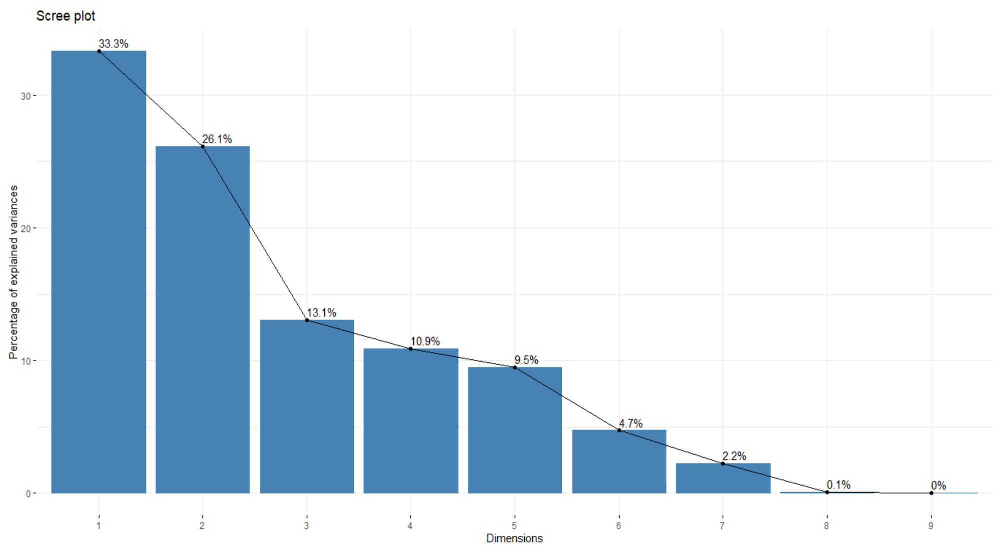

Figure 8. Scree plot of eigenvalues ordered from largest to the smallest.
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Figure 9. Chironomidae larva: habitus lateral view.
By: Daniela Alvear-S, 2022

Figure 10. Elmidae larvae and adults, habitus.
By: Daniela Alvear-S, 2022

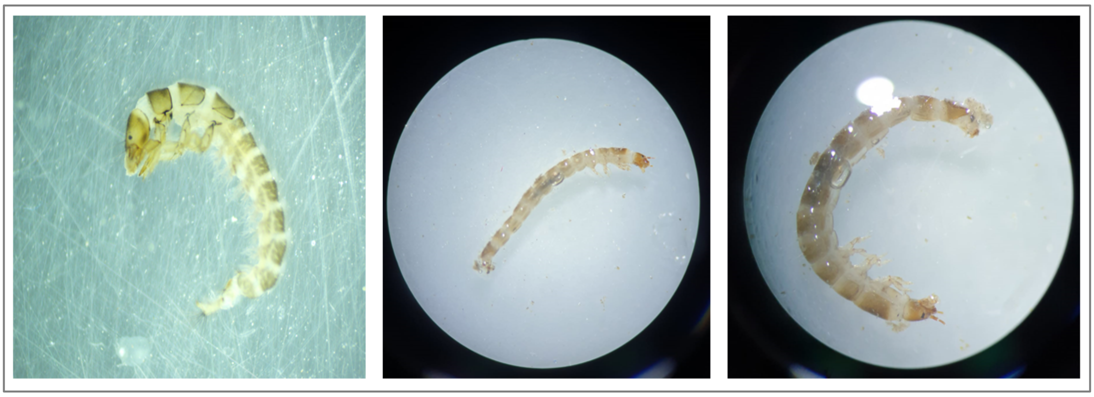

Figure 11. Hyropsychidae, lateral view of the larva.
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Figure 12. Leptophlebiidae, dorsal view.
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Table T1. Sampling sites of Figure 1. Estate of water ecosystem and description of the surrounding human activities of the study:

Sampling code	Information	Surronding activities	Estate of water ecosystem	UTM (18S)	
				X	Y
S1	Via Ahuano	Crop: Maize	Crop or Aquaculture	212168	9883964
	Canuayaca / Ahuano	Crop: Maize, Banana, Cacao, Yuca, Sugarcane	Crop or Aquaculture	200008	9883394
	Ahuano / San Pedro	Crop: Maize, Platano, Rice, Cacao	Crop or Aquaculture	212786	9879281
	Chontapunta	Crop: Rice, Cacao	Crop or Aquaculture	232449	9895107
	Chontapunta	Crop: Platano, Cacao	Crop or Aquaculture	230953	9892469
	Chontapunta	Crop: Banana, Cacao	Crop or Aquaculture	229652	9890805
	Comunidad Chambiro (vía Muyuna)	Crop: Maize, Banana, Cacao, Orillo	Crop or Aquaculture	183954	9890763
	Puerto Napo	Crop: Maize, Banana, Yuca, Cacao	Crop or Aquaculture	190730	9881909
	Pashimbi	Crop: Orillo	Crop or Aquaculture	181174	9895042
	Hatun Sumaku	Crop: Naranjilla	Crop or Aquaculture	210983	9926019
	Sumaco Pucuno	Crop: Naranjilla	Crop or Aquaculture	210932	9921730
	Marchangara	Crop: Naranjilla	Crop or Aquaculture	199941	9919766
	Cotundo (Sardina river)	Crop: Naranjilla	Crop or Aquaculture	189784	9916567
	Arosemena Tola	Crop: Platano, Cacao, Coffea, Guayaba, Lemon, Naranja, Mandarina	Crop or Aquaculture	181437	9869740
	Arosemena Tola	Crop: Cacao	Crop or Aquaculture	180874	9873331
S2	Tributary stream of the Napo River: located in Puerto Napo, near the Jatunyacu river	Fish Farming	Crop or Aquaculture	186419	9883804
	Without data	Gold Mining	Gold Mining	180438	9896700

	Estero Paushiyacu	Urban Areas	Wastewater Discharge	187167	9889428
	Without data	Landfill	Wastewater Discharge	186311	9896648
	Without data	With few threats: areas where no direct sources of contamination could be identified, but is close to fish farming.	Crop or Aquaculture	181172	9895033
S3	Morete Cocha	Mining area: the sampling point was within the forest area, on a creek quite affected by mining activity. Sewage from tailings pool drains is discharged into the stream; the machines and workers were working during the sampling.	Gold Mining	181793	9877381
	Estrella del Oriente	The sampling point was located upstream of an area where mining activity was replaced by tilapia pools. The vegetation on the banks is secondary.	Crop or Aquaculture	179919	9873989
	Estrella del Oriente	Mining area: sewage from tailings pool drains is discharged into the stream; the machines and workers were working during the sampling.	Gold Mining	179888	9873589
	Río Chumbiyacu	A road is over the river; the construction of the road was for	Gold Mining	180783	9876572

		the mining machines.			
	Shiguacocha	Mining area: the sampling point receives the water of mining areas; moreover, the site was an abandoned mining area, where the vegetation begins to grow on a soil affected by mining	Gold Mining	184371	9877252
	Rio Chumbiyacu	Mining area: the sampling point receives the seawage of mining areas	Gold Mining	186691	9877900
	Río Huambuno	Mining area: the sampling point is located upstream of a derelict mining area	Gold Mining	220682	9890162
	Río Huambuno	Mining area: the sampling point receives the seawage of mining areas	Gold Mining	222877	9891792
	Río Tuyano	Mining area: the sampling point was highly affected by mining; the riverebed was totally modified to fill waste pools and wash away alluvial sediments. The machines were running at the time of sampling.	Gold Mining	209735	9884928
	Río Yutzupino: near to Puerto Napo	Mining area: the sampling point receives the seawage of mining areas	Gold Mining	187088	9883802

S4	Toglo river	Native Vegetation	Sites with Few Threats	188431	9888314
	Castillo stream: Santa Rosa	Rural to urban transition area near a gas station. The possible impacts on the water body are a secondary road and the sewer system.	Sites with Few Threats	187993	9885936
	Castillo stream: Santa Rosa	The site is located near a hidway and presents direct wastewater discharges due to sewer damage	Wastewater Discharge	188155	9886152
	Toglo river: Santa Rosa	Discharge: downstream of gold mining, dredging, agricultural activity, and sewage discharges	Wastewater Discharge	188180	9885933
	Wamahurco: Aguinda Family	Natural Spring	Sites with Few Threats	189531	9892965
	Wamahurco: Centro Kichwa Tamia Yura	Agricultural activity, sewage discharges, and tourist activity are visible (near, around, and in the cave)	Wastewater Discharge	188266	9892533

Crop or Aquaculture (CA), Gold Mining (GM), Wastewater Discharge (WD), and Sites with Few Threats (FT).

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Table T1. Physicochemical parameters measured in different sampling sites (Figure 1), and that were used in the multivariable analysis (Figure 6).

Research	Samples	Physicochemical Parameters					
		Temp (°C)	**DO (% sat)**	**Turbidity (NTU)**	**TDS (mg/L)**	**pH (Units)**	**Conduct (us/cm)**
S1	FT	24	**83**	2,85	17	7,45	34
	CA	24	80	2,26	49,5	7,3	90
	CA	24,4	80	55,8	24	**4,11**	48
	CA	24,3	56,2	6,42	28	7,66	55,8
	CA	25,3	**90,4**	2,44	23	6,91	48,5
	CA	26,2	**102**	2,45	31,5	7,11	63,1
	CA	24,4	**98,6**	1,11	15,3	7,9	30,6
	CA	24,2	**105,15**	0,882	15,5	7,47	31,3
	CA	23	**106,4**	0,376	15,5	7,57	31,5
	CA	18,3	**91,15**	0,33	11,3	7,77	22,9
	CA	19,5	**94,75**	0,578	32,5	7,76	64,8
	CA	20,6	**84**	1,2	18	7,59	35,2
	CA	21,3	42,75	4,92	7	**5,43**	14,3
	CA	23,1	**88,4**	22,3	13,5	7,34	26,9
	CA	27,2	52,5	1,55	6,3	6,48	13
S2	CA	24,9	**90,1**	4,56	15,5	8,5	-
	GM	23	**95**	40,34	16,5	8,06	-
	WD	23,3	57,8	43,4	69	7,53	-
	WD	24,9	19	46,6	**2350**	8	-
	CA	22,4	**86,2**	0,326	22,4	7,8	-
S3	GM	23,3	**80,6**	765	45,5	6,91	67,6
	CA	25,6	**81,5**	10,2	16,26	6,7	25,3
	GM	30	75	277	14,95	6,55	25
	GM	28,3	76,2	24	33,2	6,67	53,8
	GM	31	78,4	246	31,2	7,17	52,1
	GM	29,2	76,6	1457	27,3	6,8	45,8
	GM	30	74,7	37,3	42,2	6,61	70,9
	GM	28,1	76,9	28,2	115	8,06	187,2
	GM	26,5	56,5	339	96,2	7,18	152,3
	GM	25,1	54,5	5026	57,85	7,37	88,6
S4	FT	22,8	**107,3**	4,46	68	7,8	130,7
	WD	24,1	**107,9**	53	37	**6,03**	72,1
	WD	24,4	**134,3**	15,7	53	7,71	104,8
	WD	25,8	**115,6**	16,3	57,5	7,77	136,9
	FT	22,8	**110,7**	9,2	84	7,93	160,5
	WD	21,9	**123,3**	3,43	17,5	7,98	221,2
CCME		22,5-27,5	>80	-	>500	6,5 - 8,5	>500
TULSMA		22-28	>80	-	>1000	6,5 - 9	>1000
US EPA		22-28	>80	-	>500	6,5 - 9	>500

***** Values highlighted in bold are above the thresholds for the Water Quality Criteria for Protection of Aquatic Life.*
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Table T2. The Biological Monitoring Working Party (BMWP-COL), and the Andean-Amazon Biotic Index (AAMBI) scores.

Family	BMWP-COL
Thiaridae, Hydrobiidae	1
Chironomidae	2
Planorbidae, Glossiphonidae	3
Dytiscidae, Scirtidae, Palaemonidae, Empididae,Culicidae,Caenidae, Piscicolidae, Sphaeriidae	4
Elmidae, Hydrophilidae, Ceratopogonidae, Simuliidae, Belostomatidae, Naucoridae, Notonectidae, Crambidae, Megapodagrionidae, Planariidae, Hydropsychidae, Dendrocoelidae	5
Corydalidae, Aeshnidae, Coenagrionidae, Libellulidae	6
Baetidae, Leptohyphidae	7
Hebridae, Gomphidae, Calamoceratidae	8
Euthyplociidae, Leptophlebiidae, Platystictidae, Hydrobiosidae	9
Psephenidae, Ptilodactylidae, Perlidae	10
Family	**AAMBI**
Thiaridae	0
Culicidae, Chironomidae	2
Hydrophilidae, Caenidae, Sphaeriidae, Planorbidae, Glossiphoniidae, Hydrobiidae	3
Dytiscidae, Scirtidae, Empididae, Baetidae, Ceratopogonidae, Belostomatidae, Notonectidae, Piscicolidae	4
Elmidae, Psephenidae, Simuliidae, Naucoridae, Crambidae, Planariidae, Hydropsychidae, Dendrocoelidae	5
Aeshnidae, Coenagrionidae, Libellulidae, Megapodagrionidae, Hydroptilidae	6
Leptohyphidae, Glossosomatidae	7
Palaemonidae, Hebridae, Veliidae, Gomphidae, Calamoceratidae	8
Euthyplociidae, Corydalidae, Hydrobiosidae	9
Ptilodactylidae, Leptophlebiidae, Platystictidae, Perlidae	10

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Table T3. Water classification according to the AAMBI, BMWP-Col, and CCE indexes.

Index	Description
AAMBI	Blue "Good (>100)"; Green "Moderate (75-90)"; Yellow "Poor (50-74)"; Orange "Bad (25-49)"; Red "Very Bad (0 -24)"
BMWP-Col	Blue "Good (≥100)"; Green "Moderate (61-100)"; Yellow "Poor (36-60)"; Orange " Bad (16-35)"; Red "Very Bad (0-15) ecological quality"
CCE	Blue "Good (0.90-1)"; Light blue "Moderate (0.75-0.89)"; Green "Poor (0.60-0.74)"; Yellow "Bad (0.45-0.59)"; Red "Very Bad (0-0.44) quality"

Classification of water and meaning according to the score of the indexes, adjusted and adopted by Galarza et al.,[40] (AAMBI), Cabrera et al.,[26] (BMWP-Col); and Espinosa et al.,[54](CCE).

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Table T4. Ecological water quality and macroinvertebrate community attributes that were used in the multivariable analysis (Figure 6).

Research	Samples	Ecological Water Quality				Community attributes		
		AAMBI	CCE-A	BMWP-Col	CCE-B	Richness	Shannon	Simpson
S1	FT	102	1,000	107	1,000	16	6,562	4,816
	CA	27	0,904	28	0,903	5	2,787	2,109
	CA	**0**	**0**	**0**	**0**	**0**	**-**	**-**
	CA	30	1,005	26	0,838	5	4,18	3,658
	CA	27	0,904	26	0,838	5	3,649	2,814
	CA	24	0,804	27	0,870	5	2,599	1,881
	CA	31	0,865	35	0,940	6	3,25	2,485
	CA	25	1,047	23	0,927	4	3,454	3,161
	CA	31	0,865	30	0,806	6	4,615	3,814
	CA	7	0,586	6	0,484	2	1,356	1,198
	CA	25	0,837	27	0,870	5	3,827	3,282
	CA	27	0,904	28	0,903	5	1,902	1,41
	CA	32	1,072	31	0,999	5	2,027	1,48
	CA	33	0,921	34	0,913	6	3,077	2,032
	CA	16	0,893	17	0,913	3	1,165	1,061
S2	CA	24	0,804	23	0,742	5	3,528	3,149
	GM	**0**	**0**	**0**	**0**	**0**	**-**	**-**
	WD	2	0,335	1	0,161	1	1	1
	WD	9	0,754	9	0,725	2	1,926	1,862
	CA	34	1,139	37	1,193	5	4,154	3,6
S3	GM	9	1,507	6	0,967	1	1	1
	CA	37	1,239	35	1,128	5	4,166	3,522
	GM	15	1,256	15	1,209	2	2	2
	GM	19	1,061	15	0,806	3	2,586	2,273
	GM	11	0,921	7	0,564	2	2	2
	GM	**0**	**0**	**0**	**0**	**0**	**-**	**-**
	GM	49	1,172	49	1,128	7	5,742	4,9
	GM	18	1,507	18	1,451	2	1,89	1,8
	GM	4	0,670	5	0,806	1	1	1
	GM	7	0,586	6	0,484	2	1,755	1,6
S4	FT	91	1,172	90	1,116	13	6,419	3,836
	WD	43	0,720	45	0,725	10	6,092	4,226
	WD	22	0,461	25	0,504	8	2,661	1,902
	WD	37	1,239	32	1,032	5	4,586	4,263
	FT	59	0,760	65	0,806	13	5,149	3,32
	WD	27	0,904	26	0,838	5	2,888	2,119

*No organisms were found at sites marked in **bold;** These values were calculated individually for each of the thirty-six study sites.*

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