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Determination of the ecological water quality in the Orienco stream using benthic macroinvertebrates in the Northern Ecuadorian Amazon

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EDITOR’S NOTE:
This article is part of the special series “Diversity of Knowledge for a Sustainable Future in Latin America” and highlights timely research presented at the virtual SETAC Latin America 14th Biennial Meeting (2021). These articles reflect the urgent need to combine different knowledge sources and expertise to face current environmental challenges, decision making, and problem solving. Risk, recovery, restoration, modeling, regulations, anthropic impact, and human health are some of the global environmental issues covered in this special series.

Abstract
In recent years, pollution of watercourses in nearby protected ecosystems has increased due to urbanization. Standard physiochemical methods and probes are one way to monitor watercourses for quality. However, they often do not provide the full ecological status of the body of water. In this work, we set out to assess the ecological water quality of an urban stream by using benthic macroinvertebrates as bioindicators. We conducted the work on the Orienco stream in Lago Agrio in the province of Sucumbíos in the Northern Ecuadorian Amazon (NEA). The stream has become a sink of raw domestic sanitary wastewater from rural and urban areas. A total of 4511 macroinvertebrates from 10 families were identified across 17 sampling points. We compared our results from the biotic indices derived from the macroinvertebrates to standard water-quality parameters (temperature, conductivity, dissolved oxygen, biochemical oxygen demand, chemical oxygen demand, total suspended solids, ammonia–nitrogen, and pH) simultaneously sampled in the stream. The standard parameter results indicated that the water-quality levels of the stream met the Ecuadorian water-quality criteria most of the time. However, the results from the biotic indices classified the stream water as poor or very poor water quality. The results from the Biological Monitoring Working Party, Average Score per Taxon, and Family Biotic Indices had overall scores of heavily polluted waters of 45, 4.5, and 8.74, respectively. Furthermore, these results were consistent with reduced richness and evenness, and overall lower Shannon diversity and relatively higher Simpson Dominance indices of 0.71 and 2.56, respectively. We conclude that the macroinvertebrates were better indicators of the ecological water quality of the Orienco stream than the water-quality parameters from standard methods and probes alone. Our findings highlight the need for more integrated ecological assessments, which can provide critical information to the management and conservation strategies of urban watercourses in the NEA region.

KEYWORDS: Aquatic macroinvertebrates; Bioindicators; Ecological index assessment; Ecological water quality; Ecuadorian Amazon

INTRODUCTION
The Amazon Rainforest is one of the largest natural biomes remaining on Earth. Its tropical forests provide global ecosystem services, including carbon sequestration, climate and water cycle regulation, and habitat for biodiversity (Usma-Oviedo et al., 2016). The Ecuadorian Amazon constitutes 45% of the country’s territory and has a 1.5% share of the Amazon River basin. Its area includes six provinces...
named Sucumbíos, Orellana, Napo, Pastaza, Morona Santiago, and Zamora Chinchipe (López et al., 2013). The Northern Ecuadorian Amazon (NEA) is one of the richest ecosystems in South America and includes the province of Sucumbíos (0.110000; –76.877667), which borders the Southeastern Colombian Amazon (Usma-Oviedo et al., 2016). The NEA region is home to endemic plants (e.g., orchids and bromeliads) and animals (e.g., the Amazonian manatee and Amazon River dolphins), which are supported by the abundance, richness, and diversity of species, including invertebrates (López et al., 2013). This region is not only home to wildlife, but also is part of the ancestral territories of indigenous groups, including the Achuar, Andoa, Cofán, Kichwa, Secoya, Siona, Shiwiar, Shuar, Waorani, and Sapara (López et al., 2013). Moreover, the NEA region is also home to the Reserva de Producción Faunística Cuyabeno (Cuyabeno Reserve), which represents one of the most impressive ecosystems of biological and cultural diversity in the world (Usma-Oviedo et al., 2016).

Despite the ecosystem services and cultural heritage associated with the Amazon, the world’s largest and most biodiverse tropical wilderness faces human pressures from habitat loss, forest loss, land use conversion, uncontrolled pesticide use, mining, urbanization, and agricultural development (Barbieri et al., 2009; Furley et al., 2018; Rivera-Sepúlveda et al., 2020; Zalles et al., 2021). Lago Agrio is in the province of Sucumbíos in the NEA region and is the largest Amazonian city with a population of 176,472 inhabitants (Instituto Nacional de Estadísticas y Censos [INEC], 2015). Oil companies have built roads in and around the city to lay pipelines to extract and pump oil across the Andes for export over the last 30 years (PetroAmazonas, 2021). As a result, oil extraction and rapid urbanization have caused ecosystem degradation and deforestation in the NEA region (Barbieri et al., 2009; Zalles et al., 2021). These anthropogenic activities have not only adversely impaired the ecosystem health of the region, but also have impacted access to clean natural resources for the human population (Barbieri et al., 2009; Rivera-Sepúlveda et al., 2020; Zalles et al., 2021). Particularly, the water resources are under constant threats due to the limited coverage of sewage system and wastewater treatment plants for urban and rural areas (Empresa Pública Municipal de Agua Potable y Alcantarillado de Lago Agrio EP [Emapala], 2021; Gobierno Autónomo Descentralizado Municipal del cantón Lago Agrio [Gadmla], 2021). In Lago Agrio, 44% and 10% of rural and urban areas, respectively, release their raw domestic sanitary wastewater into watercourses within their neighborhoods due to the lack of wastewater treatment systems (INEC, 2015). In Ecuador and other parts of the world, water-quality parameters are often measured using physicochemical methods and probes, which can analyze water samples in the lab or in the field (Ministerio del Ambiente Agua y Transición Ecológica del Ecuador [MAATE], 2015; US Environmental Protection Agency [USEPA], 1986). This methodology has been used to ensure compliance with health and nuisance-related standards at the national level, and to protect the aquatic systems, including streams, rivers, lakes, and estuaries (MAATE, 2015; USEPA, 1986). However, most of the time the Ecuadorian Ministry of the Environment (MAATE) and local governments fail to comply with water-quality monitoring programs in isolated and rural places such as the NEA region (Barbieri et al., 2009; INEC, 2015; Varela, 2016). Furthermore, the water-quality parameters only provide a snapshot of the ecological water quality of an aquatic system due to their fluctuations in measurements, which can impair the management and use of water (Bega et al., 2021; Wan Abdul Ghani et al., 2018).

To address water quality in a more comprehensive and ecological way, over the last decades research has been conducted to develop several biotic indices based on macroinvertebrates to monitor the ecological water quality of aquatic systems (Herman & Nejadhashemi, 2015; Nestlerode et al., 2020; Wan Abdul Ghani et al., 2018). For example, the EPT (Ephemeroptera, Plecoptera, and Trichoptera) index was developed as a biological indicator of pristine rivers (Herman & Nejadhashemi, 2015). In contrast, the Biological Monitoring Working Party (BMWP) index was developed to determine the degree of pollution in aquatic systems (Herman & Nejadhashemi, 2015; Roldán, 2003). The BMWP index relates the presence of pollution-tolerant macroinvertebrates, identified at the family level, to the water-quality parameters, and organic or inorganic pollutants (Herman & Nejadhashemi, 2015; Roldán, 2003; Wan Abdul Ghani et al., 2018). Examples of organic pollutants associated with degraded aquatic environments as determined by tolerant macroinvertebrates include hospital sewage, domestic and animal waste (e.g., tilapia farms), agriculture residues, and algae detritus (Cabrera et al., 2021; Cota et al., 2002; Roche et al., 2010). The BMWP index has been adapted for use in different countries to assess the environmental effects of water from many geographic locations, including South America (Herman & Nejadhashemi, 2015; Roche et al., 2010; Roldán, 2003; Wan Abdul Ghani et al., 2018). Furthermore, additional measures have been developed, including the associated Average Score Per Taxon (ASPT), which is determined by dividing the total BMWP score by the number of taxa present (Friberg et al., 2009; Roche et al., 2010). These biotic indices use metrics (i.e., species abundance and trophic composition) and score values (i.e., 1 more degradation to 10 less degradation) to describe the ecological status of the aquatic system (Herman & Nejadhashemi, 2015; Roldán, 2003), which can be easily interpreted by decision-makers and stakeholders.

In this study, we assessed the ecological water quality of the Orienco stream, which is a major urban watercourse in Lago Agrio. The stream has been reported to receive raw domestic sanitary wastewater from both rural and urban areas (EMAPALA, 2021; GADMLA, 2021; Varela, 2016). The overall goal of the present study was to provide a first demonstration of the effectiveness of the use of benthic macroinvertebrates as both feasible and sensitive bioindicators to better characterize the ecological water quality of the Orienco stream in the NEA compared to the standard water-quality parameters alone.
MATERIALS AND METHODS

The study area in the Orienco stream

The Orienco stream is an urban stream that crosses Lago Agrio from the west (286535; 10009098) to east (292823; 10011197) in Sucumbios (Supporting Information: Figure S1). Sucumbios is tropical and humid with an annual average precipitation of 2800–4500 mm (Harris et al., 2020; Instituto Nacional de Meteorología e Hidrología del Ecuador [INAMHI], 2020). Rainfall is present year-round, with March to May and October to November having higher rainfall (INAMHI, 2020). The relative humidity is between 80 and 90% throughout the year, with annual temperatures between 24°C and 26.5°C (Figure S2A,B) (Harris et al., 2020; INAMHI, 2020). The Orienco stream is the result of watersheds in the south and the stream drains its waters to the Teteye River in the north, which eventually meets the Aguarico River (Varela, 2016). The area of study in the stream included a length of 10.22 km with an elevation of 293–308 m above sea level and an area of 25.55 km². Benthic macroinvertebrates and water-quality parameters were sampled across 17 sampling points (SPs) of the stream to cover primary and secondary vegetation and urban areas in December 2019.

Benthic macroinvertebrates and biotic indices

The macroinvertebrates were sampled using a standardized Surber sampler frame (0.15 m x 0.15 m) with a net size of 500 µm attached to a 1.50 m handle. The sampling was conducted for 10 min over a stretch of 5 m around each SP; it covered a variety of habitats, such as bed substrate, litters, macrophytes, and terrestrial vegetation immersed in the stream. In the field, macroinvertebrates were separated from the sediment and debris prior to preservation in 70% ethanol. Macroinvertebrate samples in each SP were combined into a single population group to have representative taxa in each sampling location. In the laboratory, macroinvertebrates were analyzed with a stereoscope and identified into families according to taxonomic references (Dominguez & Fernández, 2009; Roldán, 2003). The details on the determination of the BMWP, ASPT, and Family-Level Biotic Index (FBI) indices, and Pielou, diversity, and dominance indices can be found in the Supporting Information.

Water sampling and analysis

Water-quality parameters on surface water were measured at each SP where macroinvertebrates were collected. The procedure used in the analysis followed standard procedures reported elsewhere (American Public Health Association, 2017; USEPA, 1986). The parameters included water temperature (WT), water conductivity (WC), dissolved oxygen (DO), biochemical oxygen demand (BOD), chemical oxygen demand (COD), total suspended solids (TSS), ammonia–nitrogen (NH₃–N), and pH. Procedure details are available in the Supporting Information.

Data analysis

The first analysis was a two-sided comparison using a Student’s test to determine whether statistically significant differences existed between the two independent samples (measured and reference levels). The second analysis was a one-way analysis of variance (ANOVA) to determine whether statistically significant differences existed among the measured parameter levels across all SPs. The ANOVA analysis was conducted using a generalized linear model in which the fixed effects were the parameter-measured levels. Post-hoc analysis using Tukey’s test was conducted where statistically significant differences were found. A significant level of α = 0.05 was used for both the Student’s test and one-way ANOVA. Both analyses were performed by R software (Version 4.2.0) using the tidyverse, ggplot2, and mulcompView packages (RStudio Team, 2020). The third analysis was a principal component analysis (PCA) using the measured water-quality parameters from each SP to identify the components that can explain the relationship between standard water-quality parameters and the sampling points. In this analysis, the principal components that explained >60% of the relationship in the PCA were maintained in the analysis. The fourth analysis was a constrained Canonical Component Analysis (CCA) to determine the relationship between measured water-quality parameters, geographic coordinates of sampling points, and their taxa composition. For this analysis, the taxa data were log10 (x + 1) transformed to approach the assumption of normality and homoscedasticity of the data prior to CCA. Both PCA and CCA were performed by R software using the FactoMiner and Vegan packages, respectively (RStudio Team, 2020).

RESULTS

Stream characterization by standard water-quality parameters

In total, eight major water-quality parameters were measured across 17 SPs in the Orienco stream (Supporting Information: Table S1). Results from the measurements were then compared to the parameter reference levels of the water-quality criteria established by the Ecuadorian Ministry of the Environment for freshwater systems (MAATE, 2015) using the Student’s test, and second, measurements were compared across all SPs to determine significant differences among each parameter category using one-way ANOVA. For the first parameter, the measured WT ranged from 23.53 ± 0.02 to 26.83 ± 0.02°C. Student’s test results showed no statistical differences (t < 0.5, df = 4, p > 0.60) between the reference WT of 25.00 ± 0.00°C and measured WT in SP2, 7, 9, 12, 13, 15, and 16. However, slightly significant (t > 3, df = 4, p < 0.02) higher temperatures were found in SP1, 3–6, 8, SP10, 11, and 14. The lowest temperature at 23.53 ± 0.02°C was registered in SP17, which might have been influenced by the waters from the Teteye river (GADMLA, 2021; Varela, 2016). Our ANOVA results showed that there was a highly significant difference among
the measured WC levels \( (F_{16,34} = 9.17, \ p = 4.05 \times 10^{-08}) \). The results from the Tukey multiple comparison of means showed highly significant differences \( (p < 0.0016) \) among the lowest measured temperature of SP17 \( (23.53 \pm 0.02 \degree C) \) and temperatures of SP1, 3–8, 11, and 14, and significant differences \( (p < 0.026) \) among the highest temperature of SP4 \( (26.83 \pm 0.02 \degree C) \), and of SP2, 9, 12, 13, and 15–17 (Supporting Information: Table S1).

The measured pH parameter from the Orienco stream ranged from 6.74 ± 0.22 to 7.46 ± 0.27. The Student’s test results showed no significant differences \( (t < 2, df = 4, \ p > 0.05) \) found between the reference pH of 7.00 ± 0.0 and the measured pH levels of SP1–17 (Supporting Information: Table S1). These results demonstrated little to no variability of measured WT levels compared to the reference levels, and an overall neutral pH of the water stream during the rainy season of sampling. These results were confirmed by ANOVA analysis, which showed no significant differences \( (p > 0.05) \) among the measured pH levels among all SPs.

The measured DO levels ranged from 11.00 ± 0.29 mg/L and levels of SP3, 5, and 7–17 (Supporting Information: Table S1). Likewise, highly significant differences \( (p < 0.000015) \) were found among the highest measured DO level of SP5 \( 25.00 \pm 0.17 \text{ mg/L} \) and levels of SP1–4 and 6–17 (Supporting Information: Table S1).

In the case of the COD levels, the Student’s test results indicated that measured levels were significantly lower \( (t > 10, df = 4, \ p < 0.00005) \) than the reference COD of 40.00 ± 00 mg/L across all 17 SPs, except SP5 with no significant differences \( (t < 1, df = 4, \ p > 1.00) \) (Supporting Information: Table S1). Interestingly, the lower BOD and COD levels indicated reduced aerobic and oxidation activities in watercourses (Cota et al., 2002; Wan Abdul Ghani et al., 2018), which could be considered indicative of good water quality. The ANOVA results among all SPs showed highly significant differences among the measured COD levels \( (F_{16,34} = 68.75, \ p < 2.20 \times 10^{-16}) \). The Tukey results confirmed the significant differences \( (p < 0.023) \) among the lower measured levels of SP1 \( 18.00 \pm 0.58 \text{ mg/L} \) and (SP2) \( 18.00 \pm 0.45 \text{ mg/L} \) compared to the levels of SP3 and 5–17 (Supporting Information: Table S1). Likewise, highly significant differences \( (p < 0.000008) \) were found among the highest measured COD level of SP5 \( 40.00 \pm 1.42 \text{ mg/L} \) and levels of SP1-4 and 6–17 (Supporting Information: Table S1).

The Student’s test results from the measured WC levels also showed signifi cantly lower levels \( (t > 47, df = 4, \ p < 1.16 \times 10^{-14}) \) than their reference WC of 450.00 ± 00 \text{ μS/cm} \) across all 17 SPs (Supporting Information: Table S1). The lower WC levels were expected as no sources of dissolved ions have been identified near the stream (EMAPALA, 2021; Varela, 2016). Our ANOVA results showed highly significant differences among the measured WC levels \( (F_{16,34} = 748.10, \ p < 2.20 \times 10^{-16}) \). Further, Tukey’s test indicated significant differences \( (p < 0.001) \) among the lower measured levels of SP2 (170.90 ± 0.59 mg/L), SP7 (154.70 ± 1.00 mg/L), SP9 (157.17 ± 1.31 mg/L), and SP11 (148.73 ± 1.37 mg/L), and the higher levels of SP1, 3, 5, 6, 7, 8, 10, and 12–17 (Supporting Information: Table S1).

The measured TSS levels were consistently at 30.00 ± 0.00 mg/L across all 17 SPs (Supporting Information: Table S1). These levels were significantly higher than the reference TSS of 10.00 ± 00 mg/L \( (p < 0.001) \). Likewise, ANOVA results showed no significant differences \( (p > 0.05) \) among the measured TSS levels. This result is consistent with the turbidity and brown color of the water as a result of the rainwater and its constant dissolution of organic matter, vegetation, and soil into the stream (EMAPALA, 2021; Varela, 2016). Finally, the measured NH3– N levels ranged from 0.50 ± 0.06 mg/L to 4.00 ± 0.29 mg/L (Supporting Information: Table S1). The Student’s test results indicated that the level in SP1–11 was significantly lower \( (t > 4, df = 4, \ p < 0.01) \) than the reference NH3–N of 3.37 ± 00 mg/L. The results also showed significantly higher measured NH3–N levels of SP12, 15, and 17 \( (t > 5, df = 4, \ p < 0.005) \) compared to the reference levels. These results suggest a gradient of nitrogen-based residues along the stream as shown by
the fluctuations of NH$_3$–N levels. Moreover, our ANOVA results showed highly significant differences among the measured NH$_3$–N levels ($F_{16,34} = 32.14$, $p < 6.73 \times 10^{-16}$). The results from Tukey’s test confirmed the highly significant differences ($p < 0.00053$) among the lowest measured NH$_3$–N level of SP1 (0.50 ± 0.06 mg/L) and the levels of SP2–17 (Supporting Information: Table S1). Likewise, highly significant differences ($p < 0.000029$) were found among the lower measured NH$_3$–N levels of SP2–11 (2.00 ± 0.06 mg/L to 2.00 ± 0.36 mg/L) and the higher levels of SP12–17 (4.00 ± 0.06 mg/L to 4.00 ± 0.29 mg/L).

**Ecological macroinvertebrate composition and distribution**

A total abundance of 4511 macroinvertebrates from 10 families were identified across the 17 sampling points of the Orienco stream (Figure 1A,B and Supporting Information: Table S2). The taxa analysis showed a family composition of 76% Tubificidae, 19% Chironomidae, 4% Physidae, and 1% represented by the sum of Glossiphoniidae, Hyalellidae, Hydropsychidae, Nauocoridae, Corydalidae, Culidae, and Hydrophilidae (Figure 1B). The results demonstrated that Tubificidae and Physidae were the first dominant taxa in 15 and 13 out of the 17 SPs, respectively. Moreover, Chironomidae and Glossiphoniidae were the second dominant taxa in eight out of the 17 SPs, respectively. The results also indicated small occurrences of Nauocoridae taxa in two out of 17 SPs, and taxa from Hydropsychidae, Hyalellidae, Corydalidae, Culidae, and Hydrophilidae in one out of 17 SPs for the families. The analysis of the composition and distribution of taxa of the 17 SPs demonstrated a high occurrence of very pollution-tolerant taxa in the Orienco stream (Supporting Information: Table S2).

In addition to the composition and distribution of taxa, the change in species diversity of the Orienco stream was also quantified using Shannon’s diversity index ($H'$) (Morris et al., 2014). Table 1 indicates diversity values ranging from 0.026 to 1.100 across all 17 SPs, and an overall $H'$ value of 0.71 as shown in Supporting Information: Table S3. These lower values showed lower taxa diversity in the stream. The taxa richness ($S$) was also lower with one or up to four families per SP (Table 1) and varying taxa abundance across the SPs (Supporting Information: Figure S3). For example, SP1–3 and 11 resulted in a reduced number of families compared to SP10, 12, and 17 (Supporting Information: Table S2). Furthermore, the taxa dominance was quantified using Simpson’s dominance index ($D_2$) (Morris et al., 2014). The results ranged from 0.473 to 0.992, and an overall $D_2$ value of 2.56, which indicated dominant taxa and reduced diversity in the stream (Table 1 and Supporting Information: Table S3). Likewise, Figure 1A,B indicates Tubificidae (3409) > Chironomidae (874) > Physidae (181) as the dominant macroinvertebrates. Additionally, dominant families across all 17 SPs are shown in Supporting Information: Figure S4, where families are reported as an (%) absolute frequency based on their occurrence per SP. These results demonstrate that the dominance of pollution-tolerant families and lower taxa diversity in the Orienco stream is indicative of a polluted aquatic environment.

Furthermore, each taxon registered was classified by its ecological functional role and trophic group based on major functional groups of aquatic macroinvertebrates (Wallace & Webster, 1996). Our results showed that representatives of almost every trophic group were found in the Orienco stream (Supporting Information: Table S4). The most abundant group was the shredders represented by Tubificidae,
Hyalellidae and Hydropsychidae, and Hydrophilidae, followed by collectors and scrappers, represented by Chironomidae, and Physidae, Glossiphoniidae, respectively. The presence of predators represented by Naucoridae, Corydalidae, and Culidae was minimal. The distribution of functional and trophic groups indicates that the dominant habitats of taxa have been associated with the reported accumulation of organic detritus and deposits in the Orienco stream (EMAPALA, 2021; GADMLA, 2021; Varela, 2016).

**Determination of ecological water quality using biological indices**

The sensitivity of the aquatic macroinvertebrates to pollution in the Orienco stream was estimated using the BMWP index (Herman & Nejadhashemi, 2015; Roche et al., 2010; Roldán, 2003; Wan Abdul Ghani et al., 2018). The grade values (I–VI) of the water quality using the BMWP index for the geography of Latin America have been reported previously (Roche et al., 2010; Roldán, 2003). The BMWP scores and their corresponding water-quality grades across all 17 SPs are shown in Figure 2. The results indicated that six out of the 17 SPs were polluted sites with poor water quality (Grade V), while the remaining 11 SPs were highly polluted sites with very poor water quality (Grade VI). Furthermore, individual BMWP, ASPT, and FBI scores ranged from 3 to 16, 8.97 to 6.50, and from 1.50 to 4.66, respectively (Table 1), while the overall scores were 45, 4.5, and 8.74 for BMWP, ASPT, and FBI, respectively (Supporting Information: Table S3). These lower values have been associated with the scores of heavily polluted streams (Cota et al., 2002; Herman & Nejadhashemi, 2015; Hilsenhoff, 1988; Roche et al., 2010). The index results provide a more sensitive and consistent metric to assess the ecological water quality of the Orienco stream.

**Association between biotic and parameter variables across the stream**

The PCA was conducted to determine the association between the water-quality parameters and sampling points. Supporting Information: Figure S5 shows a biplot with PC1 (42.80%) and PC2 (22.89%) explaining 65.53% of the variation in the data. The PC1 had a positive association with higher levels of NH\textsubscript{3}–N, COD, BOD, and DO for SP12–17. These SPs were located on the eastern side of the Orienco stream, which reflects all the domestic inflows coming from the western side of the stream (Supporting Information: Figure S1). Moreover, slightly higher WT levels had a strong association with SP3, 6, and 8, where some of the shallowest parts of the stream are present. In the case of PC2, a positive association was found between higher WC levels and SP3, 5, 6, 8, and 14, while higher DO and pH levels had a strong association with SP4, 7, 9, 11, and 15. The rapid flow of the stream toward the eastern side may explain the higher DO levels on these SPs (Varela, 2016).

### Table 1

**Biotic and diversity indices of the benthic macroinvertebrates sampled in Lago Agrio, Ecuador, during 2019**

<table>
<thead>
<tr>
<th>SP1</th>
<th>SP2</th>
<th>SP3</th>
<th>SP4</th>
<th>SP5</th>
<th>SP6</th>
<th>SP7</th>
<th>SP8</th>
<th>SP9</th>
<th>SP10</th>
<th>SP11</th>
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<th>SP13</th>
<th>SP14</th>
<th>SP15</th>
<th>SP16</th>
<th>SP17</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abundance</td>
<td>14</td>
<td>3</td>
<td>2</td>
<td>6</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<tr>
<td>Richness (S)(^a)</td>
<td>223</td>
<td>232</td>
<td>243</td>
<td>242</td>
<td>245</td>
<td>223</td>
<td>224</td>
<td>-</td>
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<td>-</td>
<td>-</td>
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</tr>
<tr>
<td>Pei(l)ou (J(^b))</td>
<td>0.985</td>
<td>0.976</td>
<td>0.960</td>
<td>0.954</td>
<td>0.946</td>
<td>0.960</td>
<td>0.946</td>
<td>0.935</td>
<td>0.927</td>
<td>0.916</td>
<td>0.905</td>
<td>0.900</td>
<td>0.896</td>
<td>0.892</td>
<td>0.888</td>
<td>0.884</td>
</tr>
<tr>
<td>Shannon’s diversity (H(^c))</td>
<td>0.473</td>
<td>-</td>
<td>0.473</td>
<td>-</td>
<td>0.473</td>
<td>-</td>
<td>0.473</td>
<td>-</td>
<td>0.473</td>
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<td>0.473</td>
<td>-</td>
<td>0.473</td>
<td>-</td>
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<td>-</td>
</tr>
<tr>
<td>Simpson’s dominance (D(^d))</td>
<td>0.473</td>
<td>0.473</td>
<td>0.473</td>
<td>0.473</td>
<td>0.473</td>
<td>0.473</td>
<td>0.473</td>
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<td>0.473</td>
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<td>0.473</td>
</tr>
<tr>
<td>BMWP(^e)</td>
<td>5</td>
<td>1</td>
<td>1</td>
<td>4</td>
<td>9</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
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Abbreviations: ASPT, Average Score Per Taxon; BMWP, Biological Monitoring Working Party; FBI, Family-Level Biotic Index; SP, sampling point.

\(^a\)Morris et al. (2014).

\(^b\)Jost (2010).

\(^c\)Roldán (2003).

\(^d\)Roche et al. (2010).

\(^e\)Hilsenhoff (1988).
sampling points, an analysis of CCA was performed. Supporting information: Figure S6 shows a triplot with CCA axes and the stream variables. The CCA results were not statistically significant ($p=0.74$) for the groups of variables analyzed here. The CCA1 and 2 from the triplot only explained 54% of the data variation. However, some preliminary associations could be drawn from the triplot. For example, CC1 shows a positive trend between Hydropsychiidae, Tubificidae, and Corydalidae, and NH$_3$, WC, DO, BOD, and COD levels in the SP10, 16, and 17, while pH levels were negatively associated with Corydalidae in the SP4–6, 8, 14, and 15. The CC2 indicated a positive trend between Hydropsychiidae, Hyalellidae, Chironomidae, and NH$_3$–N levels in the SP7, 12, and 13, while WT levels were negatively associated with Physidae, Glossiphoniidae, and Culicidae mainly in the SP11. Although the overall CCA was not statistically significant for the association among taxa abundances, standard water-quality parameters, and sampling points, the triplot suggests preliminary trends that could be relevant with more data from a more continuous sampling program of the stream.

**DISCUSSION**

Macroinvertebrate communities represented by taxa, distribution, and structure create a more realistic ecological status of an ecosystem. Thus, the present study was the first attempt, that we are aware of, to employ bioindicators to assess the ecological water quality of the Orienco stream and compare it to standard water-quality parameters. Our results derived from biotic indices, starting with the BMWP scores, graded 11 out of the 17 SPs (64.70% of the stream) as very poor water quality, while the remaining six SPs (35.30% of the stream) were graded as poor water quality (Figure 2). Moreover, the ASPT and FBI indices for the Orienco stream scored overall values of 4.50 and 8.75, which suggest high levels of pollution across watercourses (Cota et al., 2002; Herman & Nejadhashemi, 2015; Hilsenhoff, 1988; Roche et al., 2010; Roldán, 2003). The dominant families identified in the stream included Tubificidae, Chironomidae, and Physidae (Figure 1A,B). These families are dominant in aquatic systems, which are affected by pollution (Aston, 1973; Pinder, 1986). Tubificids were the most abundant bioindicators reported in this study. They are aquatic annelids widely distributed in marine, estuarine, and freshwater habitats (Aston, 1973). Because their body can adapt to different body positions in habitats containing decomposing organic material (e.g., low DO levels), they can be found in higher densities compared to other taxa (Aston, 1973). This was the case in a study assessing the effects of urbanization on stream benthic invertebrate communities in the Amazon, where the authors reported a 32% increase of Oligochaeta (i.e., Tubificidae) and Psychodidae in streams impacted by urbanization over seven years (Martins et al., 2017). Our results showed that Tubificids represent 76% of the sampled macroinvertebrates in the Orienco stream (Figure 1A,B), which is also a stream impacted by urbanization (EMAPLA, 2021; GADMLA, 2021; Varela, 2016).

The second most abundant bioindicators reported here were Chironomids. They were dominant in SP11, 12, and 13 with population percentages of 78%, 85%, and 98%, respectively (Supporting Information: Table S2). Chironomids are the most widely distributed, and frequently the most abundant group of insects in freshwater habitats (Pinder, 1986). They can be found in both undisturbed and disturbed streams (Couceiro et al., 2012). Because of this, it is recommended that their presence should be contextualized in light of Chironomidae morphotypes or another pollutant-tolerant taxon, for example, Tubificids from the Oligochaeta taxon (Kleine & Trivinho-Strixino, 2005). To this end, in a study investigating the Chironomidae and Oligochaeta assemblages in response to the organic pollution
gradient of a river in southeastern Brazil, the authors reported that 74.32% of Chironomidae and 25.68% of Oligochaeta families associated with urban areas influenced by the discharge of domestic effluents (Rosa et al., 2014). The higher percentage of Chironomidae was associated with the higher population of the genus Chironomus larvae, which is found in areas with organic pollution (Rosa et al., 2014). In the present study, we did not report taxa at the genus level, but our results indicated that the Chironomid population represents 25% of the Tubificid population (Figure 1A,B). This suggests that Chironomids are part of the pollution-tolerant taxa in the Orienco stream because they were found together with Tubificids but at a much lower population percentage.

The third most abundant bioindicators were the Physids. Their ability to reproduce continually through self-reproduction has allowed them to colonize a wide variety of freshwater habitats worldwide (Strong et al., 2007). The densities and occurrence of Physids can be influenced by environmental conditions as well (Mena-Rivera et al., 2018). For example, Physids demonstrated a positive correlation with the temperature and BOD in a freshwater river polluted with wastewater discharges in Costa Rica (Mena-Rivera et al., 2018). Our results demonstrated that Physids were dominant in SP1 and 9 with population distribution percentages of 43% and 64%, respectively (Supporting Information: Tables S1 and S2). Interestingly, both SP1 and 9 had BOD at 11.00 ± 0.00 and 16.00 ± 0.00 mg/L, respectively, which were values significantly lower than the reference BOD. These parameter results would suggest that the water quality of SP1 and 9 were within the recommended levels by the Ministry of the Environment, although the presence of Physid population might be indicating lower water quality. To this end, Physids have been associated with poor to moderate water quality in other Ecuadorian aquatic systems (Holguin-Gonzalez et al., 2013). For example, in a work by Holguin-Gonzalez et al. (2013) integrating a generic framework for decision support in water management developed for the River Cuenca in Ecuador, the authors reported a significant association between Physidae and fecal coliforms in untreated wastewater discharges (Holguin-Gonzalez et al., 2013). Here, we also reported the presence of Physids across 14 SPs as a taxon associated with a stream habitat impacted by organic pollution and urbanization.

The abundance of pollution-tolerant families in the Orienco stream implies that environmental conditions are not favorable to support a richer, more diverse macrofauna community in the stream. This finding is supported by an overall lower Shannon’s diversity index (H’) of 0.71. Furthermore, the Simpson’s Dominance Index ranged from 0.473 to 0.992 for SP1 and 17, respectively, indicating that the pollutant tolerant taxa were dominant. In contrast, fewer varieties, and an abundance of more intolerant-pollution individuals (e.g., EPT taxa) also resulted in a significantly reduced richness (S) and evenness in the total population of 4511 sampled in the stream (Supporting Information: Table S3). The poor water quality of the Orienco stream can be linked to water degradation due to the lack of urban sewage systems and wastewater treatment plants (Varela, 2016). The population of Lago Agrio has increased by 26.90% in the last decade, resulting in higher untreated domestic and industrial wastewater discharged into the stream waters (INEC, 2015). The ecological roles of the taxa reported here are presented mainly by shredders, collectors, scrapers, and predators (Supporting Information: Table S4). The functional feeding behavior of these groups influences the structure and biotic interactions, such as competition and health of stream ecosystems (Aston, 1973; Mena-Rivera et al., 2018; Rosa et al., 2014). Thus, the distribution and dominance of Tubificids, Chironomids, and Physids across all 17 SPs reflect the lower diversity and richness, and poor ecological water quality of the Orienco stream.

Recently, there has been a great interest in refining water-quality criteria in tropical regions because regions such as the NEA are prone to daily variation in weather, including high precipitation (Bega et al., 2021; Briciu et al., 2020; Nobre et al., 2020). Precipitation in the NEA region can range from 2800–4500 mm/year (INAMHI, 2020). Considering this and the relatively small scale (25.55 km²) and length of the stream (10.22 km), we hypothesized that this could have contributed to the fluctuations of the standard water-quality parameter measurements (Supporting Information: Table S1). In this regard, in a study investigating the effects of landscape properties, precipitation patterns, and land use on the water quality of tropical aquatic systems in northeast Brazil (Nobre et al., 2020), the authors reported that not only landscape properties but also a combination of precipitation patterns influenced the water quality of Brazilian lakes by increasing runoff events in aquatic systems, even if the events were temporary (Nobre et al., 2020). In the Orienco stream, the TSS levels were consistently higher than the reference levels across all 17 SPs (Supporting Information: Table S1). Moreover, our findings from PCA indicated that the parameters with the greatest association included NH₃–N, COD, BOD, and DO for the SP12–17 (Supporting Information: Figure S5). The fluctuations of the parameter measurements and fewer associations, which could have been influenced by the regional climate conditions, did not fully explain the overall ecological water quality of the stream. Ultimately, further evaluation is necessary to understand the influence of the NEA climate conditions on standard water-quality measurements.

In this study, the levels of water-quality parameters measured in the Orienco stream were compared to the reference levels of the water-quality criteria by the Ecuadorian Ministry of the Environment to determine whether the stream met the national standard criteria for freshwater systems (MAATE, 2015). The standard water-quality parameter levels from the SPs did not always meet the reference levels, resulting in an unclear classification of the water quality of the stream. Moreover, results from further comparisons of sampling points showed significant differences.
among the measured levels of each parameter category (WT, pH, WC, NH₃–N, COD, BOD, DO, and TSS), except in the cases of pH and TSS, in which measured levels showed little to no variation across all SPs (Supporting Information: Table S1). Our results of WT (23.53–26.83 °C), DO (4.00–12.00 mg/L), and WC (148.73–296.67 µS/cm) were closely related to ranges of the water-quality levels of WT (20.10–24.20 °C), DO (8.00–9.00 mg/L), and WC (126–902 µS/cm) reported by Cabrera et al. (2021) in a study focused on the composition and distribution of macroinvertebrate community of the Aguarico and Coca River Basins in the Ecuadorian Amazon. However, the Ammonium-N was in general much lower (0.02–0.12 mg/L) than our results (0.50–4.00 mg/L). This could be the result of sampling rivers with higher flow velocity and larger areas (Coca: 5705 km²; Aguarico: 10,290 km²) compared to a much slower flow and smaller area (25.55 km²) watercourse such as the Orienco stream. The authors also reported little to no variation in measured pH levels (7.60–8.40) across all the 15 sampling sites of their work (Cabrera et al., 2021).

We used CCA to further understand whether the standard parameters could explain the abundance and distribution of pollution-tolerant taxa, and therefore, the overall ecological water quality of the stream. The CCA results could not support a direct association when considering the suite of variables in analyses (Supporting Information: Figure S6). This could be improved in future studies in the NEA region by increasing the sampling throughout the year to include both the rainy and dry seasons. The need for better estimates of the water quality of watercourses has been recently reported (Bega et al., 2021; Briciu et al., 2020). For example, in a study assessing the variation in water quality variables in tropical first-order urban streams in Brazil, the authors reported significant variation in water-quality parameters throughout the day, resulting in distinct water-quality classifications for the same stream (Bega et al., 2021). Further, previous studies have also reported the influence of seasonal water on surface water quality in rivers (Briciu et al., 2020). Although the aquatic macroinvertebrate community and structure can also be influenced by environmental conditions, including precipitation, their life histories and ecological roles can be used to assess the ecosystem’s health (Álvarez-Cabria et al., 2010; Menezes et al., 2010). It is also important to mention that in freshwater environments, the importance of structure and function of aquatic communities of bottom-up processes (e.g., organic matter decomposers) mediated by benthic organisms is well established (Aston, 1973; Couceiro et al., 2012; Pinder, 1986; Strong et al., 2007). This scenario is consistent with observations made in a study focused on major energy supporting macroinvertebrate communities in a floodplain lake of the Bolivian Amazon (Molina et al., 2011). Here, the authors identified aquatic food chains (e.g., consumers, primary and secondary predators) in which larger specimens, such as snail populations, exhibited a strong dependence on bottom sediments, from decomposers and shredders, as an energy source (Molina et al., 2011). Therefore, short- and long-term changes in abundance, richness, and diversity of benthic macroinvertebrates might adversely impact the productivity of the trophic levels of food webs in the ecosystem. Not only are macroinvertebrates essential to sustain ecosystem productivity but our research also demonstrated how they could provide a more comprehensive estimation of the ecological water quality of urban streams in places, such as the NEA, where standard water-quality parameters might be variant.

CONCLUSIONS

To conclude, our results demonstrated that the ecological water quality of the Orienco stream was better predicted by the bioindicators represented by the macroinvertebrate families and structure than the standard water-quality parameters from physicochemical methods and probes alone. The parameters were not always consistent in classifying the water quality of the stream across all sampling points. Alternatively, the BMWP, ASPT, and FBI indices in the sampling points were more influential in determining the overall ecological water quality of the stream, which was characterized as poor and very poor water quality. Moreover, our findings bear some implications for stream assessment and conservation. First, stream bioassessment should be given attention in tropical areas where climatic conditions might heavily influence the in-situ measurements by physicochemical methods and probes, which would affect the water-quality classification. We also recommend measuring biological contaminants, such as fecal coliform, algae toxins, and pathogenic viruses, as part of the regular monitoring campaign in these watercourses. One of the limitations of the present study was sampling only during the rainy season of 2019; therefore, we also recommend continuous monitoring throughout the year to compare season water quality. Second, research efforts should focus on the characterization of local macroinvertebrate communities because their life histories are mainly regulated by regional environmental conditions, providing a more comprehensive ecological assessment of watercourses. Third, there is an urgent need for the dissemination and training on cost-effective tools such as bioindicators among the public and indigenous communities in isolated and impoverished locations such as the NEA region, especially when there are budget constraints for the implementation of standard water-quality methods or equipment. Finally, the local population, including indigenous communities, must be involved in the environmental management of stream ecosystems as part of the efforts to monitor and reestablish water quality in watercourses to ensure clean water resources for the residents and ecological equilibrium in nearby protected ecosystems.

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**DISCLAIMER**

The authors declare no conflicts of interest. Mention of trade names, products, or services does not imply endorsement by the authors. The peer review for this article was managed by the Editorial Board without the involvement of Federico Sinche.

**DATA AVAILABILITY STATEMENT**

The data sets included in the present study are available upon request from corresponding author Federico Sinche (fsinche@luc.edu).

**SUPPORTING INFORMATION**

Supporting information contains procedure details, supplementary tables, and figures that complement the content of the article.

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