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## Human pressures degrade the ecological condition of the Upper Graipu River

*Pressões humanas degradam a condição ecológica do Alto Ribeirão Graipu*

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### ABSTRACT

Environmental degradation from human pressures includes the conversion of native vegetation cover into pastures and cropland, as well as riparian deforestation, leading to river siltation, biotic homogenization, and loss of ecosystem services. The objective of our study was to evaluate water quality and benthic macroinvertebrate assemblage structure in response to changes in land use at local and buffer spatial extents. We assumed that human disturbances negatively affect water quality and macroinvertebrate assemblage condition. Greater human influence was observed at the local extent (Local Disturbance Index – LDI) than at the buffer (Buffer Disturbance Index – BDI) extent. Likewise, biological metric responses were stronger relative to the LDI than to the BDI or to the Integrated Disturbance Index (IDI). These results support establishing a biomonitoring program for assessing water body quality in the Doce River basin to facilitate conserving aquatic biodiversity and ecosystem services in the upper Graipu River.

**Keywords:** Degradation; Bioindicators; Water quality; Benthic macroinvertebrates.

### RESUMO

A degradação ambiental causada por pressões antrópicas inclui a conversão de cobertura vegetal nativa em pastagens e terras agrícolas, bem como o desmatamento de zonas ripárias, levando ao assoreamento de rios, à homogeneização biótica e à perda de serviços ecossistêmicos. O objetivo do nosso estudo foi avaliar a qualidade de água e a estrutura de assembleia de macroinvertebrados bentônicos em resposta a mudanças no uso da terra em extensões espaciais locais e de microbacia (*buffer*). Para tanto, assumimos que os distúrbios humanos afetam negativamente a qualidade de água e a assembleia de macroinvertebrados bentônicos. Maior influência humana foi observada na escala local (Índice de Distúrbio Local – LDI) do que na escala de *buffer* (Índice de Distúrbio Buffer – BDI). Da mesma forma, as respostas das métricas biológicas foram mais correlacionadas ao LDI do que ao BDI ou o Índice Integrado de Distúrbio (IDI). Esses resultados apoiam o estabelecimento de um programa de biomonitoramento para avaliar a qualidade de corpos hídricos na bacia do rio Doce, fomentando a conservação de biodiversidade aquática e serviços ecossistêmicos no alto ribeirão Graipu.

**Palavras-chave:** Degradação; Bioindicadores; Qualidade de água; Macroinvertebrados bentônicos.



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## INTRODUCTION

The conservation of freshwater ecosystems and their biodiversity is essential to guarantee the maintenance of ecosystem goods and services, including water supply for multiple uses and climate regulation (Dudgeon et al., 2006). One way to assess the ecological quality of aquatic ecosystems is by using benthic macroinvertebrates as bioindicators (Callisto et al., 2019a; Linares et al., 2019; Macedo et al., 2022).

Benthic macroinvertebrates inhabit the bottom substrate (sediment, macrophytes, filamentous algae, twigs) of freshwater ecosystems during at least part of their life cycles (Rosenberg & Resh, 1993; Mugnai et al., 2010). These assemblages can indicate anthropogenic disturbances of ecosystems and their catchments through their taxonomic composition, presence, abundance, functional traits, and distribution. If studies are designed and analyzed appropriately, benthic macroinvertebrates can distinguish anthropogenic from natural disturbances (Cao & Wang, 2023; Holt & Miller, 2010; Moya et al., 2011; Silva et al., 2017). These organism responses to multiple anthropogenic pressures enable assessing rapid causal link responses between environmental stressors and aquatic biota (Rosenberg & Resh, 1993; Barbour et al., 1999; França & Callisto, 2019). Furthermore, benthic macroinvertebrates play key roles in food chains, serving as food for other invertebrates and vertebrates, including fish, amphibians, reptiles, and birds (Agouridis et al., 2015; Rosa et al., 2023; Simeone, 2023). Benthic macroinvertebrates are commonly used in biomonitoring programs for diagnosing and assessing biotic condition in river basins in response to anthropogenic pressures (Karr, 1999; Callisto et al., 2019a; Feio et al., 2022).

In freshwater monitoring programs, biological responses are, in general, preferable to physical or chemical parameters because they provide a more comprehensive assessment of aquatic ecosystem condition (Callisto et al., 2019b; Thompson et al., 2008). Martins et al. (2020) recommended the application of multimetric indices as a tool for diagnosing and monitoring river basins under intense human activity. These indices integrate, in a single measure, several assemblage components (such as richness, dominance, taxonomic composition, trophic groups, mobility, tolerance, etc.) in response to natural environmental variations and anthropogenic pressures (Macedo et al., 2016; Silva et al., 2017; Terra et al., 2013). The application of multimetric indices is a reality globally and recommended as a practical tool for biomonitoring ecosystem condition (Feio et al., 2022; Vadas et al., 2022).

Local riparian zone condition is an important factor affecting the structure and function of lotic ecosystems (Kaufmann et al., 2022). The riparian zone acts as a filter of anthropogenic disturbances; therefore, riparian deforestation is reflected in macroinvertebrate assemblages (Castro et al., 2018; Martins et al., 2021). At regional and catchment extents, anthropogenic activities generally are comprised of land use changes, which affect lotic ecosystem structure and function even long after these uses cease (Linares et al., 2023). Both recent and historical catchment deforestation can degrade aquatic assemblages as a result of complex interactions among direct and indirect pathways and latent effects, but because of their indirect nature, these effects tend to be more subtle than those at local extents (Betts et al., 2022; Leitão et al., 2018; Alvarenga et al., 2021). Among the many methodologies developed to characterize

anthropogenic disturbances, the Integrated Disturbance approach, that uses Local, Buffer and Integrated Indices, has proven to be a powerful tool in biomonitoring efforts (Ligeiro et al., 2013).

Thus, our aim was to evaluate the responses of benthic macroinvertebrate assemblages to diffuse anthropogenic pressures from land use and cover. For that we tested the hypothesis that benthic macroinvertebrate assemblages would respond more readily to local anthropogenic disturbances. We predicted that the Local Disturbance Index (LDI) would have more biological metrics with significant negative correlations than the Buffer Disturbance Index (BDI) or the Integrated Disturbance Index (IDI).

## MATERIAL AND METHODS

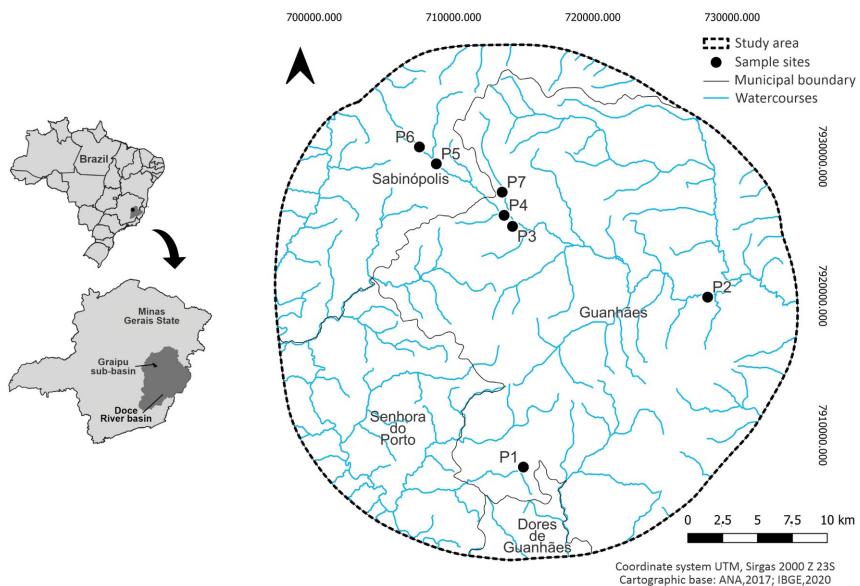
### Study area

The Graipu River basin is located between the Guanhães and Sabinópolis municipalities, in the Vale do Rio Doce, central-northeastern Minas Gerais state, Brazil. The mean altitude of the region is 852 m and its human population is 34,818 (Instituto Brasileiro de Geografia e Estatística, 2022). The Graipu River basin covers an area of 266 km<sup>2</sup>, and is a tributary of the Corrente Grande River, which flows into the Doce River (Instituto Brasileiro de Geografia e Estatística, 2022) (Figure 1).

The climate is predominantly tropical rainy savannah (Aw), with dry winters and abundant summer rainfall (Tonello et al., 2009). The basin is in the Atlantic Forest biome (Instituto Brasileiro de Geografia e Estatística, 2022), with an annual average precipitation of 1,212 mm and average temperature of 22.2 °C (Souza et al., 2006). This biome is considered an important biodiversity hotspot because of its high richness and abundance of endemic and endangered species of fauna (mammals, arthropods, etc.) and flora (Myers et al., 2000). Previously it was dominated by semideciduous seasonal forest, which in recent years has been mostly converted into pasture (Tonello et al., 2009).

We sampled seven sites on headwater streams in the upper basin. Five of these sites (P3, P4, P5, P6 and P7) were selected because of their importance to the water supply of the Ganhães municipality. The other two (P1 and P2) were in conservation areas in the same river basin, to serve as reference sites. To separate natural from anthropogenic factors, the selected streams were all uniformly shallow (~30 cm depth), narrow (<1m wide), and had sandy bottom substrate. Because site locations may result in spatial autocorrelation, we performed a Moran's I test for spatial autocorrelation (Lecocq et al., 2019; Smeraldo et al., 2020) as an *a priori* test, for richness and BMWP. Because we found no spatial autocorrelation we proceeded with our analyses (Supplementary Material S1).

Site P1 is located inside the Serra da Candonga State Park in the Guanhães River basin (Figure 1). Site P2 is on the Graipu River in the Legal Reserve area of the Forestal Company Celulose Nipo Brasileira (CENIBRA S.A.) in the middle basin. Site P3 is on the Graipu River, downstream from the dam that collects raw water for the municipality of Guanhães. Site P4 is on the Graipu River in a pasture area downstream of Santa Cruz Stream upstream of the dam. Site P5, on the Graipu River, is located near the entrance of the Minas Mineração Guanhães Company, which is affected by heavy vehicle traffic, and mining and domestic/cattle wastewaters.



**Figure 1.** Distribution of the seven sampling sites.

**Table 1.** Land use in the 1-km buffers of the seven sampling sites (%).

| Land use       | P1 | P2  | P3 | P4 | P5  | P6 | P7 |
|----------------|----|-----|----|----|-----|----|----|
| Natural Forest | 71 | 27  | 27 | 26 | 29  | 37 | 4  |
| Forestry       | 0  | 6   | 0  | 8  | 0   | 7  | 9  |
| Pasture        | 26 | 57  | 62 | 60 | 52  | 48 | 44 |
| Agriculture    | 3  | 7   | 11 | 7  | 4   | 7  | 7  |
| Urban Area     | 0  | 0.4 | 0  | 0  | 15  | 0  | 0  |
| Rocky Outcrop  | 0  | 3   | 0  | 0  | 0   | 0  | 0  |
| Water course   | 0  | 0   | 0  | 0  | 0.4 | 0  | 0  |

Site P6 is in a rural area close to the Graipu River. Site P7 is located upstream of the Santa Cruz Stream and the dam (Figure 1). The land use around each stream was dominated by pasture, except for P1, which was dominated by natural forest (Table 1).

### Benthic macroinvertebrate sampling

In each of the seven 25-m long sites, benthic macroinvertebrates were collected from three randomly selected stations with a minimum distance of 5 m from each other for a total area of 0.27 m<sup>2</sup> sampled per site, following an adapted version of Linares et al. (2019). Each station was sampled for 3 minutes (1 minute per sub-sample) by using a D-frame kicknet (250 µm mesh) attached to a 1.5 m metal pole. The three sub-samples were then pooled together for the site analyses.

All the collected material was transferred to plastic bags identified by site and fixed with ethanol 70%. The samples then were taken for processing at the Benthic Ecology Laboratory of the Universidade Federal de Minas Gerais. The samples were washed over a 0.50 mm mesh sieve and then transferred to a clear glass tray superimposed on a light box. Benthic macroinvertebrates were sorted and identified to family level for Insecta, and subclass for Mollusca, Annelida, and Acari through use of taxonomic keys (Mugnai et al., 2010; Hamada et al., 2014).

### Physical and chemical water quality

We measured water temperature (°C), pH, electrical conductivity (µS/cm), turbidity (NTU), and total dissolved solids (TDS; ppm) *in situ* at each site via portable Digimed probes. We measured dissolved oxygen (mg/L and % saturation) using the Winkler method (American Public Health Association, 2005). We collected water samples in polypropylene bottles for subsequent analysis of total nitrogen (TN) and total phosphorus (TP) in the Geomorphology and Water Resources Laboratory of the Universidade Federal de Minas Gerais. We analyzed biochemical oxygen demand (BOD) samples at the Autonomous Water and Sewage Service (SAAE) laboratory in Guanhães. All protocols carefully followed American Public Health Association (2005). The results obtained for each parameter were compared to the maximum value allowed (MVA) by the governments of Brazil (CONAMA 357/2005; Brasil, 2005) and the states of Minas Gerais [ND COPAM/CERH-MG N°8, 11/2022; Minas Gerais (2022)] and São Paulo [CETESB; Companhia Ambiental do Estado de São Paulo (2009)].

### Local, buffer, and integrated disturbance indices

To evaluate land use and cover, we used local and intermediate extent data (*Shapefile*) available on the MapBiomas (2020) digital

platforms, and the Instituto Brasileiro de Geografia e Estatística (Instituto Brasileiro de Geografia e Estatística, 2022). We obtained data on landscape structure, forest formation, watercourses, pasture areas, agriculture, and urbanization for the basin. The maps and all operations of the geographic information system were performed using the Qgis Software (3.22.10 version).

The local disturbance index (LDI) was calculated using the Callisto et al. (2002) protocol, which evaluates the land-use types on the sample site margins, presence of erosion, anthropogenic alterations, vegetation cover, and physical habitat complexity of the sampled site. The degree of disturbance is scored from 00 to 100. To calculate the buffer disturbance index (BDI) the percentages of pasture, agriculture, and urbanization inside a 1-km radius buffer was calculated as follows:

$$(BDI) = 4 \times \% \text{ urban areas} + 2 \times \% \text{ agricultural areas} + \% \text{ pasture areas} \quad (1)$$

After calculating the LDI and BDI, we calculated the integrated disturbance index (IDI; Ligeiro et al., 2013). The IDI was calculated as the Euclidian distance between the position of the site in the disturbance plane based on the values obtained by the BDI and LDI through the Pythagorean theorem (Ligeiro et al., 2013). For each index, the greater the score, the greater the anthropogenic disturbances of the site.

## Biotic indicators

To evaluate the responses of benthic macroinvertebrate assemblages to anthropogenic disturbance we calculated individual biological metrics and multimetric indices. Metrics included taxonomic richness, abundance of individuals, % EPT, and taxa richness of resistant organisms (Callisto et al., 2022; Carrera & Fierro, 2018; Junqueira et al., 2000; Merritt et al., 2014) plus BMWP and BMWP-ASPT (Monteiro et al., 2008; Junqueira et al., 2018). We also used three multimetric indices (MMIs): Ferreira et al., 2011, Macedo et al., 2016; Silva et al., 2017). The Biological Monitoring Working Party (BMWP) and the Average Score per Taxon (BMWP-ASPT) evaluate the presence of families of benthic macroinvertebrates considering their degree of tolerance to organic pollution. Values from 1 to 10 are assigned according to the degree of tolerance or sensitivity of each family, with 1 for the most tolerant organisms and 10 for the most sensitive organisms (Monteiro et al., 2008; Junqueira et al., 2018). In the BMWP, five scores are assigned to define water quality: > 81 indicates “excellent” water quality; 80-61 indicate “good” water quality; 60-41 “fair”; 40-26 “bad”; and scores < 25 indicate “terrible” water quality. For the BMWP-ASPT index, score classifications are: > 6 “very good” water quality; 5.0-6.0 “good”; 3.9-4.9 “fair”; 2.5-3.8 “bad”; < 2.5 “terrible” (Monteiro et al., 2008; Junqueira et al., 2018). The MMIs are calculated from a set of biological metrics (e.g., taxonomic richness, functional structure, species composition) that classify the ecological quality of aquatic ecosystems by comparing the results reflecting anthropogenic disturbances (Silva et al., 2017) with those of locations considered as being in reference condition (Baptista, 2008; Vadas et al., 2022).

## Data analyses

To test how anthropogenic impacts at local and buffer extents influenced the condition of benthic macroinvertebrate assemblages, we ran Generalized Linear Models (GLMs) with the disturbance indices (IDI, LDI and BDI) as predictor variables and the biological indicators (Richness, Abundance, BMWP, BMWP/ASPT, % EPT, Resistant taxa richness, and MMIs as response “variables”. To fit the models to the response variables, we used quasipoisson for Abundance, Richness, BMWP, Resistant Taxa Richness, and the MMIs, and Gaussian for the remaining biological metrics. The models were then tested with a deviance analysis (F test). All tests were carried out using R 4.2.1 (R Core Team, 2015).

## RESULTS AND DISCUSSION

The results of water quality conditions were in compliance with the limits established by the Brazilian National water standards (CONAMA Resolution 357/2005, Class 2; Brasil, 2005), with few exceptions (Table 2). Turbidity, pH, total dissolved solids, dissolved oxygen, and total nitrogen were within the limits established by the National CONAMA 357/2005 Resolution for class 2 waters (Brasil, 2005) and Minas Gerais [ND 08 COPAM/CERH 11/2022; Minas Gerais (2022)]. The exception was site P5, where 4.59 mg/L of dissolved oxygen was recorded. According to Paula et al. (2018), characteristics such as absence and/or replacement of riparian vegetation, sewage effluents, and low discharge contribute to reduced dissolved oxygen levels in aquatic ecosystems. This site borders the ore disposal road and is exposed to diffuse sources of sediment and dust related to mining activities, as well as domestic wastewaters.

Total phosphorus (TP) and BOD results were within the standards established by the Brazilian environmental legislation, but site P2 exceeded the TP standard and site P5 exceeded the BOD standard. These exceedances are likely associated with domestic sewage and livestock excrement (Von Sperling, 2005). In summary, the water quality standards only indicated meaningful violation at site P5, the only one directly affected by heavy vehicle traffic and mining, livestock, and domestic wastewaters.

The LDI, BDI, and IDI scores showed different patterns (Table 3). The local disturbance index (LDI) values indicated the greatest anthropogenic disturbances for sites P6 and P7, and the least for sites P1 and P2. However, the buffer disturbance index (BDI) showed the greatest anthropogenic disturbances at sites P3 and P5, and the least at site P1. On the other hand, the Integrated Disturbance Index (IDI), which reflects the combination of the LDI and the BDI, indicated that sites P5 and P7 experienced the greatest pressure from human activities, and sites P1 and P2 the least (Table 3). Unlike the water quality results, the landscape indices indicated moderate disturbance at sites P5, P6 and P7—not only P5. Others have also found landscape condition indicators useful and important for predicting and understanding water body condition (Allan, 2004; Herlihy et al., 2020; Hughes et al., 2006). Geodynamic factors, such as land use, influence geomorphological processes, physical habitat structure, and aquatic assemblages at small, medium, and large spatial extents (local, buffer, and integrated disturbance) (Allan, 2004; Macedo et al., 2014). Furthermore,

these factors govern energy inputs into freshwater ecosystems, further influencing network connectivity, river channel shape, and local habitat structure (Allan, 2004; Callisto et al., 2019a; Goldstein et al., 2007).

In total, we collected 1441 benthic macroinvertebrates, classified into 41 different taxa (Appendix A). Chironomidae (Diptera) were the most abundant in all sites (3 to 269, at P7 and P4, respectively). These organisms are very common in sand-bottom streams, but increase with increased human pressures, reaching about 50% of the total abundance of benthic macroinvertebrates (Callisto et al., 2007; Moretti et al., 2007). The highest family richness values were observed in sites P2 (20 taxa), and P3 (19 taxa). Based on the BMWP and BMWP/ASPT scores (Table 4), sites P4, P5, P6 and P7 were classified as having fair water quality; and sites P1, P2 and P3 had good water quality. Sites P5, P6, and P7 receive

domestic sewage and the BMWP and BMWP/ASPT are sensitive to organic pollution.

Unlike the water quality results, the biotic indicators revealed moderate to high disturbance at sites P4, P5, P6 and P7—not only P5. The % EPT metric and resistant organism richness showed good environmental conditions at sites P1, P2, P3 and P4, whereas the opposite occurred at sites P5, P6, and P7. EPT taxa richness or % EPT were reported to be excellent indicators of river and stream biological condition across the conterminous USA (Stoddard et al., 2008), and also in Brazil (Callisto et al., 2022, 2023). Both Macedo et al. (2016) and Silva et al. (2017) included Ephemeroptera richness in their MMIs.

The Macedo and Silva MMIs classified sites P4, P5 and P6 as poor (high human activities pressures) and sites P1 and P2 as good. Based on 190 Cerrado stream sites, Silva et al. (2017)

**Table 2.** Water quality in the seven sites. Bold = legal standard violation.

| Parameters                      | Standard limits           | Sites  |        |        |        |             |        |        |
|---------------------------------|---------------------------|--------|--------|--------|--------|-------------|--------|--------|
|                                 |                           | P1     | P2     | P3     | P4     | P5          | P7     |        |
| Temperature (°C)                | -                         | 21.00  | 20.50  | 22.50  | 22.50  | 21.80       | 25.00  | 28.00  |
| pH                              | 6.0 to 9.0 <sup>1,2</sup> | 5.12   | 8.06   | 8.43   | 8.83   | 5.52        | 8.85   | 8.41   |
| Turbidity (NTU)                 | ≤ 100 <sup>1,2</sup>      | 4.00   | 1.00   | 12.00  | 13.00  | 19.00       | 14.00  | 16.00  |
| Total Dissolved Solids (ppm)    | 500 <sup>1</sup>          | 69.30  | 70.10  | 162.0  | 36.90  | 26.00       | 38.00  | 10.88  |
| Dissolved oxygen (mg/L)         | ≥ 5.0 <sup>1,2</sup>      | 8.70   | 5.19   | 7.49   | 7.42   | <b>4.59</b> | 7.83   | 7.62   |
| Dissolved oxygen (% saturation) | -                         | 80.77  | 47.31  | 70.89  | 70.25  | 42.60       | 78.39  | 80.46  |
| Electrical conductivity (µS/cm) | 100 <sup>3</sup>          | 147.10 | 451.00 | 326.00 | 104.00 | 49.80       | 71.80  | 28.90  |
| Water redox potential (mV)      | -                         | 136.00 | 58.00  | 81.00  | 61.00  | 21.00       | 62.00  | 30.00  |
| Sediment redox potential (mV)   | -                         | 179.00 | 64.00  | 113.00 | -90.00 | -170.00     | -100.0 | -96.00 |
| Total Nitrogen (mg/L)           | 10 <sup>1</sup>           | 1.30   | 1.75   | 1.40   | 1.64   | 1.59        | 1.31   | 2.34   |
| Total Phosphorus (mg/L)         | 0.03 <sup>1,2</sup>       | 16     | 0.00   | 23     | 38     | 23          | 61     | 36     |
| BOD (mg/L)                      | ≤ 5 <sup>1,2</sup>        | 0.42   | 1.08   | 1.46   | 0.52   | <b>5.66</b> | 0.79   | 1.35   |
| Discharge (m <sup>3</sup> /s)   | -                         | 3.25   | 0.33   | 0.31   | 0.24   | 0.81        | 0.18   | 0.17   |

<sup>1</sup>Resolution CONAMA 357/05 standards - Fresh waters - class II (Brasil, 2005). <sup>2</sup>ND COPAM/CERH-MG N° 8, 11/2022 standards - Fresh waters - class II (Minas Gerais, 2022). <sup>3</sup>CETESB standards (Companhia Ambiental do Estado de São Paulo, 2009).

**Table 3.** Local disturbance (LDI), buffer disturbance (BDI) and integrated disturbance (IDI) index scores of the seven sites. Bold = moderately disturbed.

|     | P1    | P2    | P3           | P4    | P5           | P6           | P7           |
|-----|-------|-------|--------------|-------|--------------|--------------|--------------|
| LDI | 0.067 | 0.067 | 0.333        | 0.200 | <b>0.400</b> | <b>0.467</b> | <b>0.533</b> |
| BDI | 0.108 | 0.241 | 0.280        | 0.247 | <b>0.398</b> | 0.208        | 0.194        |
| IDI | 0.127 | 0.250 | <b>0.435</b> | 0.318 | <b>0.564</b> | <b>0.511</b> | <b>0.567</b> |

**Table 4.** Biotic and disturbance index scores at the seven stream sites.

| Site | Richness | Abundance | Density | BMWP | ASPT | EPT% | Resistant | MMIF | MMIM | MMIS | BDI  | LDI  | IDI  |
|------|----------|-----------|---------|------|------|------|-----------|------|------|------|------|------|------|
| P1   | 17       | 226       | 2511    | 113  | 6    | 15   | 3         | 28   | 65   | 69   | 0.11 | 0.07 | 0.13 |
| P2   | 20       | 96        | 1066    | 119  | 6    | 31   | 5         | 28   | 63   | 65   | 0.24 | 0.07 | 0.25 |
| P3   | 19       | 240       | 2667    | 95   | 5    | 44   | 7         | 28   | 60   | 51   | 0.28 | 0.34 | 0.44 |
| P4   | 16       | 398       | 4422    | 60   | 4.   | 17   | 9         | 28   | 29   | 17   | 0.25 | 0.2  | 0.32 |
| P5   | 12       | 218       | 2422    | 55   | 5    | 0    | 8         | 22   | 33   | 16   | 0.40 | 0.4  | 0.56 |
| P6   | 12       | 234       | 2600    | 51   | 4    | 2    | 8         | 20   | 21   | 43   | 0.21 | 0.47 | 0.51 |
| P7   | 5        | 29        | 322     | 21   | 4    | 0    | 3         | 18   | 52   | 40   | 0.19 | 0.53 | 0.57 |

EPT %: % Ephemeroptera, Plecoptera, Trichoptera; MMIF: Macroinvertebrate Multimetric Index (Ferreira et al., 2011); MMIM: Macroinvertebrate Multimetric Index (Macedo et al., 2016); MMIS: Macroinvertebrate Multimetric Index (Silva et al., 2017); BDI: Buffer Disturbance Index; LDI: Local Disturbance Index; IDI: Integrated Disturbance Index.

**Table 5.** Benthic macroinvertebrate indicator responses to disturbances: local disturbance (LDI), buffer disturbance (BDI) and integrated disturbance (IDI), Minas Gerais, Brazil. Bold font indicates  $p < 0.05$ .

|                             | LDI         |              | BDI  |       | IDI         |             |
|-----------------------------|-------------|--------------|------|-------|-------------|-------------|
|                             | P           | F            | P    | F     | P           | F           |
| Richness                    | <b>0.04</b> | 6.77         | 0.99 | <0.01 | 0.10        | 4.00        |
| Abundance                   | 0.60        | 0.31         | 0.76 | 0.10  | 0.62        | 0.28        |
| BMWP                        | <b>0.02</b> | <b>12.07</b> | 0.63 | 0.26  | <b>0.04</b> | <b>7.67</b> |
| BMWP/ASPT                   | <b>0.03</b> | <b>9.78</b>  | 0.42 | 0.76  | <b>0.03</b> | <b>9.37</b> |
| % EPT                       | 0.22        | 1.96         | 0.99 | <0.01 | 0.30        | 1.35        |
| Resistant taxa richness     | 0.70        | 0.17         | 0.15 | 2.86  | 0.48        | 0.59        |
| MMI (Ferreira et al., 2011) | <b>0.01</b> | <b>13.85</b> | 0.82 | 0.06  | <b>0.04</b> | <b>8.13</b> |
| MMI (Macedo et al., 2016)   | 0.25        | 1.62         | 0.40 | 0.83  | 0.23        | 1.83        |
| MMI (Silva et al., 2017)    | 0.25        | 1.70         | 0.10 | 3.93  | 0.14        | 3.01        |

p: Means the p value; F: means the F value; %EPT: % Ephemeroptera, Plecoptera, Trichoptera; MMI: Macroinvertebrate Multimetric Index (Ferreira et al., 2011); MMI: Macroinvertebrate Multimetric Index (Macedo et al., 2016); MMI: Macroinvertebrate Multimetric Index (Silva et al., 2017).

classified 38%, 35%, and 27% of the total stream length as being in good, fair, and poor condition, respectively. In summary, we found that family taxa richness, % EPT, and the Macedo or Silva MMIs indicated good conditions in sites P1, P2, and P3; and % EPT, the MMIs, and BMWP indicated poor or fair conditions in sites P4, P5, P6, and P7. Those results indicate that the biota are markedly more sensitive to disturbance than the water quality standards, as has been reported by others (e.g., Ohio Environmental Protection Agency, 1990; Vadas et al., 2022).

Our hypothesis was corroborated because we found that benthic macroinvertebrate assemblage structure was negatively affected by anthropogenic disturbances at both local and buffer extents. Several biological metrics and indices were negatively correlated with anthropogenic disturbances at both extents. Significant correlations (Table 5) occurred between taxonomic richness, abundance of individuals, BMWP, BMWP/ASPT, and the Ferreira et al. (2011) MMI versus LDI scores. BMWP and BMWP/ASPT, and the Ferreira et al. (2011) MMI were significantly correlated with the IDI. On the other hand, no biological variable showed significant correlation with the BDI. These results indicate bioindicator responses to anthropogenic disturbances were more strongly associated with local-extent disturbances, like what was reported in other Cerrado studies (Macedo et al., 2016; Martins et al., 2020).

Responses of aquatic biological indicators on a global scale (Feio et al., 2022), show knowledge gaps in South America and the applicability of biological approaches in watershed assessments (Callisto et al., 2022). On the other hand, multi-year biomonitoring studies have shown the resilience of urban ecosystems subjected to disturbances from domestic sewage spills (Linares et al., 2021) and the positive effects after stream rehabilitation in large cities (Macedo et al., 2022; Golgher et al., 2023).

Our results support biomonitoring by providing the basis for future efforts, showing which biological metrics respond to anthropogenic disturbances and at which spatial extents they work better. Therefore, our results can be used as an approach for establishing improved biomonitoring programs in the Rio Doce basin. The DN COPAM ND 08 COPAM/CERH 11/2022 (Minas Gerais, 2022) establishes the need for biomonitoring in Minas Gerais. We demonstrated that benthic bioindicators are more efficient and accurate tools for monitoring water resource condition than

simple, limited numbers of water quality variables in river basins. Also, recent rapid bioassessments performed in protected areas in the Doce River basin, including Serra do Gandarela National Park (Callisto et al., 2023) and Santuário do Caraça (Fernandes et al., 2022) show the importance of conserving freshwater biodiversity in reference sites in the basin. Furthermore, training public school and university students to conduct rapid biomonitoring and bioassessment approaches builds capacity and academic research as investments for the future of sustainable use of freshwaters in the Doce River basin (França et al., 2019).

## CONCLUSIONS

Human activities in the Graipu River basin are responsible for water quality and biological deterioration, but minimally disturbed reference sites had a greater abundance of groups sensitive to pollution and land use disturbances. The opposite occurred at degraded sites, which supported benthic bioindicators resistant to pollution and reduced water quality. The biological indicators were clearly more sensitive indicators than either water quality or land use. In fact, our results show how woefully inadequate such abiotic variables as used by CONAMA are for reflecting water body condition. Whereas the biotic variables responded to local scale disturbances, the water quality variables responded only in the direst of the cases. These results are of fundamental importance for implementing biomonitoring programs in this basin and elsewhere, as well as for sustainable management of water resources for cities, agriculture, and aquatic biodiversity. Because our results are based on a rapid bioassessment that could be easily and quickly employed by citizen scientists, we suggest that future studies in the area apply more thorough assessments that require additional biological assemblages and more quantitative habitat structure assessments.

Our results also suggest that it is important to mitigate anthropogenic pressures by limiting the amounts of agricultural development, mining, and road building at both the catchment- and riparian-extents. In addition, we recommend that the SAAE's water abstraction and wastewater treatment (and water use for agricultural and industrial activities) be improved to minimize impairments of aquatic ecosystem condition and to keep river reaches that are currently in reference conditions minimally altered by human disturbances.

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**APPENDIX A. MACROINVERTEBRATE TAXONOMIC COMPOSITION IN THE SEVEN SAMPLING SITES, MINAS GERAIS, BRAZIL.**

| Order             | Family            | Sampling sites |    |     |     |     |     |    |
|-------------------|-------------------|----------------|----|-----|-----|-----|-----|----|
|                   |                   | P1             | P2 | P3  | P4  | P5  | P6  | P7 |
| Ephemeroptera     | Leptophlebiidae   | 7              | 11 | 10  | 0   | 0   | 0   | 0  |
|                   | Leptocephidae     | 4              | 0  | 0   | 0   | 0   | 0   | 0  |
|                   | Baetidae          | 1              | 1  | 81  | 0   | 0   | 0   | 0  |
|                   | Caenidae          | 0              | 0  | 0   | 1   | 0   | 3   | 0  |
| Plecoptera        | Gripopterygidae   | 1              | 0  | 0   | 0   | 0   | 0   | 0  |
|                   | Perlidae          | 2              | 1  | 0   | 0   | 0   | 0   | 0  |
| Trichoptera       | Helichopsychidae  | 3              | 0  | 0   | 0   | 0   | 0   | 0  |
|                   | Hydropsychidae    | 4              | 13 | 8   | 66  | 0   | 1   | 0  |
|                   | Odontoceridae     | 2              | 0  | 2   | 0   | 0   | 0   | 0  |
|                   | Hydroptilidae     | 0              | 0  | 1   | 0   | 0   | 0   | 0  |
|                   | Calamoceratidae   | 0              | 1  | 0   | 0   | 0   | 0   | 0  |
|                   | Leptoceridae      | 10             | 1  | 4   | 1   | 0   | 0   | 0  |
|                   | Polycentropodidae | 0              | 2  | 0   | 0   | 0   | 0   | 0  |
|                   | Corduliidae       | 0              | 0  | 0   | 0   | 2   | 0   | 0  |
| Odonata           | Gomphidae         | 10             | 0  | 1   | 0   | 0   | 0   | 1  |
|                   | Libellulidae      | 0              | 1  | 3   | 0   | 0   | 0   | 0  |
|                   | Calopterygidae    | 1              | 0  | 0   | 0   | 0   | 0   | 0  |
|                   | Perilestidae      | 0              | 0  | 0   | 0   | 0   | 1   | 0  |
|                   | Aeshnidae         | 0              | 1  | 1   | 0   | 0   | 0   | 1  |
|                   | Veliidae          | 0              | 2  | 0   | 0   | 0   | 0   | 0  |
| Heteroptera       | Naucoridae        | 7              | 0  | 0   | 3   | 0   | 0   | 0  |
|                   | Mesoveliidae      | 0              | 0  | 15  | 0   | 0   | 0   | 0  |
|                   | Helotrophidae     | 0              | 0  | 0   | 1   | 0   | 0   | 0  |
|                   | Gerridae          | 0              | 0  | 1   | 0   | 0   | 0   | 0  |
|                   | Veliidae          | 0              | 2  | 0   | 0   | 0   | 0   | 0  |
| Coleoptera        | Hydrophilidae     | 0              | 0  | 0   | 0   | 19  | 2   | 0  |
|                   | Elmidae           | 16             | 4  | 1   | 6   | 0   | 0   | 0  |
|                   | Hydrophiloidea    | 0              | 5  | 0   | 0   | 0   | 0   | 0  |
| Megaloptera       | Corydalidae       | 1              | 1  | 0   | 0   | 0   | 0   | 0  |
|                   | Diptera           | Chaoboridae    | 0  | 0   | 3   | 4   | 0   | 0  |
|                   | Tipulidae         | 0              | 12 | 0   | 10  | 0   | 2   | 0  |
|                   | Chironomidae      | 152            | 10 | 80  | 269 | 135 | 158 | 3  |
|                   | Empididae         | 0              | 0  | 0   | 6   | 7   | 0   | 0  |
| Oligochaeta       | Ceratopogonidae   | 4              | 1  | 4   | 4   | 10  | 2   | 0  |
|                   | Psychodidae       | 0              | 0  | 0   | 1   | 1   | 0   | 0  |
|                   | Simuliidae        | 0              | 2  | 2   | 19  | 6   | 2   | 0  |
| Gastropoda        | Planorbidae       | 0              | 22 | 13  | 1   | 14  | 17  | 13 |
|                   | Bivalvia          | 1              | 0  | 0   | 0   | 0   | 5   | 0  |
| Hirudinida        | Hyriidae          | 0              | 0  | 1   | 0   | 9   | 3   | 0  |
|                   | Glossiphonidae    | 0              | 0  | 9   | 5   | 13  | 38  | 11 |
| Acari Hydracarina |                   | 0              | 1  | 0   | 1   | 1   | 0   | 0  |
|                   | Lepidoptera       | Pyralidae      | 0  | 4   | 0   | 0   | 1   | 0  |
| Total abundance   |                   | 226            | 96 | 240 | 398 | 218 | 234 | 29 |

## SUPPLEMENTARY MATERIAL

Supplementary material accompanies this paper.

Supplementary Material S1.

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