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# Using the benthic macroinvertebrates as indicators of the water quality in the "Cachoeira do Paraíso" waterfall (Itinguçu State Park, Peruíbe, SP, Brazil)

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#### **ABSTRACT**

Rivers protection is fundamental for the socioecological systems, and the benthic macroinvertebrates communities can be used to assess the quality of river waters. This study aimed to assess the water quality at Cachoeira do Paraíso waterfall, Peruíbe, SP, based on the benthic macrofauna. Sampling took place at 3 sampling sites, under different levels of anthropic influence. Water physical-chemical and sedimentological parameters were evaluated, as well as macrobenthic organisms. Samplings were done by kick sampling technique. The organisms were identified at the lowest possible taxonomic level and points were attributed to each group according to their respective sensitivity and tolerance to pollutants. Then, a biological index was calculated to indicate the water quality. Ecological indices were calculated and compared using ANOVA. The water physical-chemical parameters showed to be within the legal standards. 13 taxonomic groups of benthic macroinvertebrates were identified, with the Shannon diversity indices indicating similar taxonomic diversity. The score given to each *taxa* generated biological indices for each sample, ranging from regular to excellent. Diptera (with head) I was the most representative taxon, and sensitive taxonomic groups, were found in all the sampling sites, suggesting a good quality of the water body during the period of study.

Keywords: mosaic of Jureia-Itatins, environmental quality, macrofauna, coastal creeks.

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Uso de macroinvertebrados bentônicos como indicadores de qualidade da água na Cachoeira do Paraíso (Parque Estadual do Itingucu, Peruíbe, SP, Brasil)

#### RESUMO

A proteção dos rios é vital para os sistemas sócio-ecológicos e as comunidades de macroinvertebrados bentônicos podem ser utilizados para avaliar a qualidade de suas águas. Este estudo avaliou a qualidade da água na Cachoeira do Paraíso, Peruíbe, SP, a partir da análise da macrofauna bentônica. A amostragem ocorreu em 3 pontos de coleta, sob diferentes graus de influência humana. Foram medidos parâmetros físico-químicos da água, análises sedimentológicas da comunidade macrobentônica. As coletas foram realizadas pela técnica "kick sampling". Os organismos foram identificados ao menor nível taxonômico e foram atribuídos pontos de acordo com sua sensibilidade e tolerância aos poluentes. Então, foi calculado um índice biológico para indicação da qualidade de água. Índices ecológicos foram calculados e comparados por meio de ANOVA. Os parâmetros estiveram dentro dos limites legais aceitáveis. Foram identificados 13 grupos de macroinvertebrados bentônicos, com os índices de diversidade de Shannon indicando diversidade taxonômica similar. A pontuação dada a cada táxon gerou índices biológicos, variando de regular à excelente. O grupo Diptera (com cabeça) I foi o mais representativo, e grupos taxonômicos sensíveis, foram encontrados nos diferentes pontos amostrais, sugerindo uma boa qualidade do corpo hídrico no período.

Palavras-chave: mosaico da Juréia-Itatins, qualidade ambiental, macrofauna, riachos costeiros

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## INTRODUCTION

Rivers are streams which flow from a higher region towards a place with lower relief, whose mouth can occur in seas, lakes or larger rivers. The waters of the fluvial flow can come from distinct sources, such as melting glaciers, rainfall and groundwater (DUNN, 1989). The unidirectional flow of waters favors the establishment of different habitats, which dwell different species of fauna and flora (BUSS, 2008). These water courses have several ecological functions, such as regulation of erosion and deposition processes, sediment transport, maintenance of the hydrological cycle, and others (ANA, 2008). Furthermore, they play a fundamental role for the human survival, as they provide several essential environmental services.

To keep the constant supply of environmental goods and services by the ecosystem processes, a good quality of water is required. However, 53% of Brazilian cities show a reduction in the quality and quantity of water resources, 38% of which are affected by water pollution (CAMPANILI et al., 2010). In developing countries, such as Brazil, domestic, industrial effluents or agricultural runoff are frequently discharged into water bodies without effective treatment (SNIS, 2017). The massive presence of contaminants in the water causes the degradation of the riverine ecosystems; besides, the decomposition of organic matter from sewage and industrial effluents leads to a decrease in the dissolved oxygen levels, affecting aquatic species (CETESB, 2019). Furthermore, fertilizers, which have a large amount of phosphorus in their composition, contribute to induce or increase the eutrophication process and the disordered growth of algae, including those capable of producing and releasing toxic substances into the water (BARRETO et al., 2013). The disposal of other chemical pollutants, such as drugs, pesticides, and pharmaceutical and personal care products (PPCPs), associated with sewage, industrial wastes, and agricultural systems, aggravates the contamination of waters (Khan, 1977; Liu et al., 2013). In addition, the natural flow of rivers favors the transport of pollutants over long distances, reaching environments far from the source (ABESSA et al., 2018).

In this context, the protection of aquatic ecosystems, especially rivers, is of paramount importance to meet the needs of populations and maintain the integrity of the environment (STOLTON; DUDLEY:, 2003). There are tools for the protection of riverside regions and water sources, such as, for example, the legal protection provided by riparian forests, which in Brazil is regulated by the Forest Code (Brasil, 2012), or by the creation of protected areas, which aim to maintain the quality of water resources, protect biodiversity, ecological processes, forest remnants and environmental services (MAGINI; ABESSA:, 2017).

The growing establishment of protected areas around the world is often driven by the need to protect habitats and/or species that are threatened or in critical condition (BORRINI-FEYERABEND et al., 2017), but the water conservation is often overlooked. However, in any situation, estimating the carrying capacity of the protected area is essential for the conservation of the ecosystem in question (JALLIAN et al., 2012) and, therefore, should be conducted and monitored.

Studies show the environmental impact generated by tourism in rivers inserted in protected areas, such as the Formoso River, located in the Formoso River Ecological Park, in Bonito, and the Prata River, present in the Private Reserve of Natural Heritage (RPPN) Fazenda Cabeceira do Prata (ESCARPINATI et al., 2011; COELHO et al., 2011), both in the state of Mato Grosso do Sul (MS), central-west region of Brazil, and important touristic destinations.

The Brazilian System of Protected Areas (which acronym is SNUC) includes, within its objectives, the development of means and incentives for scientific research and environmental monitoring (BRASIL, 2000). One of the tools that can be used to achieve these objectives is the biomonitoring, which consists of assessing bioindicator organisms or communities, i.e., identifying the presence of organisms that have known tolerance ranges to pollutants, according to the literature

(PHILLIPS; RAINBOW:, 2013). A commonly used form of biomonitoring is the analysis of the benthic macrofauna, through the taxonomic identification of macroinvertebrates to assess water quality, in view of the responses to the environmental variations provided by these organisms (PIEDRAS et al, 2006). Macroinvertebrates are organisms of high ecological importance and represent important links within the food chain. Because they are found associated with the substrate, submerged vegetation and/or water column, and are sensitive to environmental changes, they are commonly used to monitor the quality of water bodies around the world (FRANÇA; CALLISTO:, 2012). There is a range of protocols available for biomonitoring of watersheds through microbenthic communities (MORSE et al., 2007; BUSS, 2008).

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In developing countries, as is the case of Brazil, protected areas frequently do not have their respective management plan, and due to this reason research becomes even more urgent and necessary to generate knowledge and support the management of these areas. The Jureia-Itatins Mosaic of Protected Areas is an important example where the management plan is lacking and scientific information is required. The region is inserted in the Atlantic Forest biome, which is a biodiversity hotspot, consisting of a priority site for conservation, because it is one of largest fragments of preserved Atlantic tropical rainforest in the country and has been losing a large area due to the establishment of cities and agricultural activities at its vicinities (CERATI; ZANINI:, 2009).

The Itinguçu State Park (PEIT) is one of the protected areas comprised in the mosaic, and includes the Cachoeira do Paraíso waterfall, which it is formed along the flow of the Itinguçu river. So far, the biota of the region includes 29 species of fish, divided into 12 families, in addition to insects, lizards, squirrels, snakes, sloths and others (MAGINI; ABESSA:, 2017). The waterfall is the main tourist attraction in the region, having the highest peak of visitation in the summer. In 2008, for example, the site received 45,217 visitors (SMA, 2009), which led the authorities to reduce the visitation by the establishment of limits for the daily number of visitors, until further studies could assist the park in defining the maximum number of visitors (MAGINI and ABESSA, 2017).

The creation of rules for disciplining the use of the site to avoid adverse impacts is a necessary action, considering that tourism, even if classified as ecotourism can still constitute a potentially impacting activity (RUSCHMANN, 1993). In this context, contamination of water bodies by tourists can occur from the use of products such as sunscreens, repellents, moisturizing creams, solid waste, silting and others (RUSCHMANN, 1993). The present study aims to assess water quality at Cachoeira do Paraíso waterfall (Peruíbe, SP, Brazil), based on the biomonitoring of benthic macrofauna.

## MATERIALS AND METHODS

## Characterization of the study area

The Itinguçu State Park (PEIt) has an area of 5,040 ha and is located between the cities of Peruíbe and Iguape, in the state of São Paulo (SÃO PAULO, 2013). It is classified as a whole protection area (i.e. no-take area), which allows public visitation for recreational purposes, if possible encompassing some forms of environmental education. The territory of PEIt is made up of short rivers that extend from the mountainous region to the beaches, such as the Guaraú and Itinguçu rivers, and the beaches of Guarauzinho, Arpoador, Parnapoã, Brava and Juquiazinho (MAGINI; ABESSA:, 2017). The park has two main sections, Arpoador and Itinguçu, both presenting Atlantic Dense Rainforests; the Arpoador section includes also mangroves and restinga forests.



Figure 1: Map of sample points from Cachoeira do Paraíso, PEIt.

The PEIt has several trails, used for visitation, monitoring, circulation, etc.; including that used to access the Cachoeira do Paraíso waterfall (Figure 1), as previously mentioned (SMA, 2009; MAGINI and ABESSA, 2017). The waterfall is located between the Itatins Mountain Ridge and the coastal plain, with an altitude of 35m, and it does not have a vertical drop, but a 17-meter step, called Paraíso toboggan. Its access path is reasonably preserved; however, the existence of trails and visitation can generate impacts, such as habitat fragmentation, erosion, silting, soil compaction, soil and water pollution (SMA, 2009).

In this context, the Normative Ordinance 144/2010 of Fundação Florestal establishes the daily limit of visitors at Cachoeira do Paraíso waterfall, which is currently 180 for independent visitors and 90 for organized groups. This restriction was imposed to reduce potential impacts on the preserved area and guarantee the public use of the waterfall as an instrument of environmental education. The presence of restaurants in the vicinity of the visitor center (MAGINI; ABESSA:, 2017), despite contributing as a source of income for the local population, can also generate impacts, such as water pollution due to incorrect waste disposal, in addition to increase the river silting up, which leads to potential impacts to the biota.

# Sampling method

The sampling was carried out on October 19, 2020, in a dry period; i.e., without rain along the 48 hours prior to the collection, to avoid the occurrence of waterspouts, a phenomenon in which rainfall at the head of the river causes a sudden increase of the water flow. This was required to avoid risks to the researchers involved in the sampling, as well as to avoid interferences on the investigation caused by possible displacement of organisms (BUSS, 2008). Samples were taken at three collection sites (Figure 1): (P1) located above the areas of public use (reference); (P2) the second, in the main bathing site; and P3 in the medium bathing site.

Water samples were taken to validate parameters such as pH, dissolved oxygen, conductivity and temperature, using a multimeter probe. Sediment samples were also collected and stored at 4°C in the laboratory for further analysis of organic matter (OM) and calcium carbonate (CaCO3) contents, and grain size distribution. Initially, the sediment samples were dried at 60 °C for 48 hours (CARMO; SILVA:, 2012). The quantification of OM was performed using Goldin's gravimetric method (1987), in

which 5g aliquots were distributed in porcelain crucibles and weighed before and after conditioning in the muffle for 4 hours at 550 °C. The OM content was indicated by the loss of mass of the incinerated waste. The calcium carbonate content was calculated using gravimetry by hydrochloric acid (HCl) digestion, which reacts with CaCO3, releases a volume of carbon dioxide and solubilizing the Ca, resulting in sample weight loss (GROSS, 1971; LORING; RENTALA:, 1992). The grain size distribution was carried out through the dry-sieving method. Basically, the 60g of dry sediment were sieved in a set of meshes ( $\varphi$  scale), and mechanically agitated for 15 minutes; subsequently, the amount present in each mesh was weighed on a high precision balance to determine the amounts of each class of particle size of the sample. The granulometric classification method was based on the Wentworth (1922, 1933) (mm) and Krumbein (1934) (Fi) scale.

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The sampling method used the protocol proposed by Buss (2008), with the adaptations proposed by CETESB (2019), which indicates that sampling should consider the existence of different mesohabitats, such as, fine substrate (fine sands and muds), thicker materials (coarser sands), and also gravel and pebbles and loose rocks, and others. In the case of Cachoeira do Paraíso waterfall, the environment was characterized as a mesohabitat of thick substrate, which is expected to present less biodiversity in comparison to the others (BUSS, 2008). Each sampling site was sampled in three different sections, within the same mesohabitat, using a sieve with 05-0.6 mm mesh (BUSS, 2008), by the kick sampling technique, where the collection was made on the substrate (BUSS, 2008; FEELEY et al., 2011; CETESB, 2019). The collected organisms were trialed under a stereo's microscope, separated from the sediment and fixed in 40% alcohol. Then, they were examined and their taxonomic classification was made, as further described.

## **Macroinvertebrates**

Macroinvertebrates are those organisms that are retained on the 1 mm mesh and can be observed with the naked eye, without the aid of equipment such as microscopes. Benthic macrofauna can be composed of a variety of taxonomic groups, such as insects, mollusks and crustaceans, among others.

Biomonitoring uses data on the distribution of organisms in each habitat to diagnose the environment. Thus, the sampled organisms were identified at the lowest possible taxonomic level with the aid of identification keys. The method proposed by CETESB (2019) proposes the attribution of points to each organism, according to their sensitivity and tolerance to pollutants, being (5) sensitive species, (4) indifferent, (3) tolerant, (2) second order opportunists and (1) first order opportunists (BORJA et al., 2000; BORJA; MUXIKA:, 2005). A biological index is then created from the sum of the values to indicate the water quality, ranging from very poor, poor, fair, good and excellent quality (BUSS, 2008; CETESB, 2019). The determination of the indices allows the establishment of qualitative comparisons between the sampling sites.

In addition, ecological indices were calculated to assess the responses to environmental variations presented by the macroinvertebrate community, using the Primer® 6.0 software, such as specific richness, evenness and Shannon-Wiener diversity (BORJA et al., 2000; BORJA; MUXIKA:, 2005). The ecological indices were also compared using Analysis of Variance (ANOVA). To assess the community structure, cluster analysis was performed with the objects and descriptors, using Simpson's index and comparison by paired groups.

## **RESULTS**

# Physicochemical parameters of water samples

The physical-chemical parameters of the water of the Itinguçu River are within the acceptable ranges for freshwater bodies established by CONAMA Resolution 357/05 (BRASIL, 2005). Slightly acidic waters were observed, with high concentrations of dissolved oxygen and low turbidity (Table 1). Temperatures ranged between 22.7 and 23.1 °C.

Table 1. Physicochemical parameters of water samples

	P1	P2	Р3
Dissolved Oxigen (%)	88.8	84.0	81.4
Conductivity (mS/cm)	0.117	0.057	0.074
pН	5.57	6.23	6.64
Temperature (°C)	22.7	23.0	23.1
Turbidity (NTU)	0	0	0

# **Sedimentological properties**

The sediment textural data obtained for the samples collected at the Cachoeira do Paraíso waterfall showed predominance of coarse and very coarse sands (Table 2); and the standard deviation suggested a poor selection of grains, because the sediment also presented both pebbles and very fine sands. Such results indicate that the sediments are close to their source area (MCCAVE; SYVITSKI:, 1991). Besides, the predominance of coarser grains is an indicative of a high energy environment (determined by the water flow), typical from waterfalls. In general, these sediments occurred at low depth regarding the water depth (BUJAN et al., 2019).

Table 2. Properties of sedimentological samples

	•	G	P1	P2	Р3
		Average Diameter	-0.22	0.27	0.11
Cranulamatria Dia	stribution Statistics	Standard Deviation	0.77	0.75	0.84
Granulometric Dis	stribution Statistics	Asymmetry	0.32	0.84	0.07
		Kurtosis	2.96	3.98	2.61
	Gravel	Pebbles	0	0	0
		Granules	26.24	6.3	16.78
	Sand	Very Coarse	40.24	34.62	30.8
Granulometric		Coarse	27.31	43.89	37.77
Range		Medium	5.73	13.08	13.69
		Fine	0.47	1.9	0.95
		Very Fine	0	0.21	0.02
	Clay	Silt	0	0	0
Organic Matter (%)			4.51	6.28	4.74
Calcium Carbonate (%)			0.845	0.199	0.099

The levels of CaCO<sub>3</sub> found in each sediment sample were low, ranging between 0.099 and 0.845 (Table 2), evidencing to the lower presence of bioclastic structures such as shells. These low levels are also explained by the high energy of the river flow, which quickly removes the fragments of CaCO<sub>3</sub>, which are lighter and easily carried downstream. The levels of OM were low (4.51; 6.28; 4.74, respectively), also due to the high water flow (TEIXEIRA et al., 2000), which hinder the deposition of organic matter.

## **Benthic macroinvertebrates**

21 taxonomic groups were identified considering the 3 sampling sites (Table 3), until the taxonomic level of order and family. Beghelli et al (2012) showed that the identification of benthic macroinvertebrates to species level is only possible when there is the collection and analysis of the phases of nymph, pupa and larva, which would be impossible at the time. Additionally, Silveira (2004) showed that biomonitoring of microbenthic communities can be successfully achieved when the organisms are identified to main taxonomic groups, such as class.

Table 3. Macroinvertebrates taxonomic groups per sample

	P1A	P1B	P1C	P2A	P2B	P2C	P3A	РЗВ	P3C
Diptera (with head) I	4	7	3	8	3	6	4	3	11
Diptera (with head) II	4	5	1	2					2
Diptera (with head) III	1	1		1			1		
Diptera (with head) IV					4			6	
Diptera (with head) V					1		2		
Diptera (without head)	1	3	5		3	7	3		3
Ceratopogonidae I	2								
Ceratopogonidae II	1			2	2	3			1
Trichoptera (with house) I	1								
Trichoptera (with house) II							1	2	
Trichoptera (without	1	2					1		2
house)			1						
Plecoptera I			1						
Plecoptera II						1			
Ephemeroptera I	1		1						1
Ephemeroptera II									1
Odonata			1						
Nematoda				1	1	2			
Turbellaria				1	1	2	2		
Polichaeta					1				
Oligochaeta						1			
Hirudinea			3	2			1		
Total	16	18	15	17	16	22	15	11	21

Diptera (with head - I) was the most frequent, being present in all sites, followed by Diptera (with head - II), Diptera (without head) and Ceratopogonidae - II. In addition, the presence of groups such as Plecoptera and Trichoptera, which are considered sensitive to pollution and stressors, indicates good water quality in the Itinguçu River, in the region of Cachoeira do Paraíso waterfall. These taxonomic groups have a higher score in the scope of the calculation of the biological index proposed in the CETESB's biomonitoring manual (CETESB, 2019). Plecoptera and Trichoptera were found in the replicates P1C, P2C and P1A, P3A, P3B, respectively.

In a preliminary analysis made by replicate, P3C and P2C had the greater number of individuals sampled; however, the replicate P1A showed a greater number of taxonomic groups (10), in contrast to P3B, which presented only 3 groups. The values found for Pielou's equitability (J) at each sampling site indicated that a high percentage (above 77%) of the maximum diversity was found in each sample and little variation between them. The Shannon diversity index showed variations between replicates, but the mean values calculated for each sample corresponded to 2.13; 2.16 and 2.12, respectively (Table 4). This evidences that the assemblies seem to be homogeneous between the 3 sampling sites. Considering only the replicates, the Margaleff richness values ranged from 0.83 (at P3B) to 3.11 at (P1A). The ANOVA indicated that the ecological indexes (diversity, richness and evenness) were similar among samples. The high diversity, richness and equitability suggest a good water quality in the Itinguçu River, particularly at the Cachoeira do Paraíso waterfall.

Table 4. Ecological Indexes for the sampling sites P1, P2 and P3

Sample	Richness	Evenness	Shannon Diversity Index	Simpson Diversity Index
P1A	3.11	0.92	2.12	0.91
P1B	1.38	0.89	1.43	0.77
P1C	2.22	0.89	1.73	0.85
P2A	2.12	0.83	1.61	0.77
P2B	2.53	0.93	1.93	0.89
P2C	1.94	0.88	1.71	0.82
P3A	2.59	0.93	1.93	0.90
P3B	0.83	0.91	0.99	0.65
P3C	2.27	0.78	1.62	0.74

Biological indices were calculated according to CETESB (2019), and showed the presence of sensitive organisms, which have the maximum score (5), such as Trichoptera (with house) and Plecoptera (see Table 5). Tolerant organisms (1) such as Diptera, Nematoda, Hirudinea, Turbellaria, Polychaeta and Oligochaeta were also sampled, as shown in Table 4. The sum of the points attributed to the organisms found at each sampling site made it possible to calculate the ecological index for each replicate and sampling site. Most of replicates showed were ranked as excellent or good quality, and only two replicates (P2A and P3B) were classified as regular quality. Furthermore, when the replicates were grouped to determine the water quality in each sampling site, the three locations presented were classified as presenting excellent water quality according to the index.

Table 5. Taxonomic groups and punctuation for calculating the biological index, according to the manual proposed by CETESB (2019)

Taxonomic Group	Punctuation
Diptera (with head) I	1
Diptera (with head) II	1
Diptera (with head) III	1
Diptera (with head) IV	1
Diptera (with head) V	1
Diptera (without head)	3
Ceratopogonidae I	2
Ceratopogonidae II	2
Trichoptera (with house) I	5
Trichoptera (with house) II	5
Trichoptera (without house)	2
Plecoptera I	5
Plecoptera II	5
Ephemeroptera I	3
Ephemeroptera II	3
Odonata	2
Nematoda	1
Turbellaria	1
Polichaeta	1
Oligochaeta	1
Hirudinea	1

The cluster analysis showed that the sampling sites present similarities equal to or greater than 50%, based on the taxonomic composition of the respective sampling replicates (Figure 2). In addition, two main groups were formed: one included all the replicates of P2 and two from P3 (P3A, P3B), but subdivided by sampling site; and a second group formed by the replicates from P1 and P3C. This analysis allows us to perceive a greater similarity between the assemblies from P1 and P3. On the other hand, the cluster analysis made with the species did not show very clear associations possibly due to the large number of "double-zeros", since most groups occurred in a few replicates, with the exception of Diptera with head I and II, and Diptera without head.

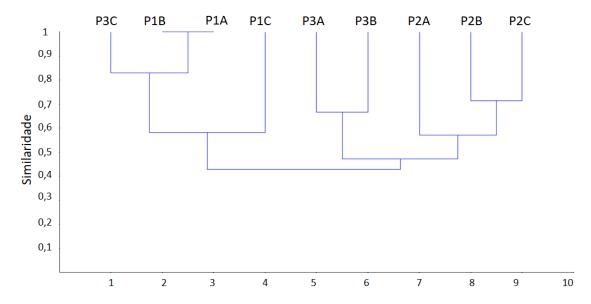


Figure 2. Cluster analysis of the replicates to access the relation of faunistic composition.

#### DISCUSSION

There is a scarcity of research in the context of the benthic ecology of rivers and creeks along the Atlantic coast of Brazil, especially within protected areas, such as the Jureia-Itatins Mosaic, where the PEIt is located. Thus, the present study is extremely relevant in terms of providing new information regarding water quality and the composition, diversity and status of benthic macroinvertebrates at the Cachoeira do Paraíso waterfall.

The water quality depends on its physical, chemical and biological variables. The physical-chemical parameters are important but measurements made punctually indicate instantaneous conditions that can be or not indicative of the general conditions of water quality. On the other hand, resident organisms may monitor water quality over time (GORNI; ALVES:, 2012). The physical-chemical parameters of the Itinguçu River were within the normal ranges determined by the federal standards for protected areas (Class 1 waters), and were also comparable to those reported in the literature for environments with good water quality (LUTZ, 1968; YANOVIAK 1999<sup>a</sup>; HROVAT et al., 2009; MONTEIRO et al., 2008; TANIWAKI; SMITH:, 2011; TSZYDEL et al., 2015). In addition, these values were similar to those found in the rivers located in the Intervales State Park, SP (ALVES et al., 2008; MELO, 2009).

The sedimentological data did not show any divergence between the sampling sites, indicating that differences in the distribution of the taxa along the sampling sites were due to the sedimentological factors. Instead, the similarity between sites may help to explain the similarities of the benthic assemblies in the sampling sites. The amount of organic matter found in the Cachoeira do Paraíso sediment was similar to the reported by Cunha and Calijuri (2008) in a study of lotic environments located close to our study site in Iguape, SP. Similarly to our study, the authors attributed the low rate of organic matter as a result of various factors, including the high energy (CUNHA; CAJURI;, 2008). There is a need to address that river regime in the Cachoeira do Paraíso waterfall is highly dependent of the rains, and that intense rainstorms strongly modify the conditions, making this river a very unstable environment. Under the influence of rains and thunderstorms, the benthic organisms may be easily displaced and transported downstream, justifying the characteristics of the samples.

The Diptera (with head) taxonomic groups were the most abundant sampled organisms, in total 80 organisms were sampled. First order opportunistic organisms have a high tolerance to contaminants, changes of physical-chemical patterns, and climatic variations. Diptera, Nematoda, Hirudinea, Turbellaria, Polychaeta and Oligochaeta are a few examples of taxonomic groups that fit in this category. Furthermore, some group representatives have their distribution related to the type of substrate and the

water flow of the environment (POE; STEFAN:, 1974; GORNI; ALVES:, 2012). These freshwater organisms tend to remain closer to the sediment surface and play a crucial role in the decomposition of organic matter in the environment. For example, Oligochaeta individuals actively participate in water purification, are primary consumers or decomposers (UZUNOV et al., 1988; ABEBE et al., 2006). Other representatives of this category, such as Turbellaria and Hirudinea, have low dispersion in the environment, and may be typical of the type of environment studied (KNAKIEVICZ, 2014; METCALFE et al., 1984).

The order Diptera includes several organisms, which can be found in contaminated water bodies, such as those that receive input from sewers, standing water in urban environments, and others (FAGUNDES; SHIMIZU:, 1997). Other studies show a higher density of Diptera in degraded environments, due to the high rate of organic matter present (OLIVEIRA et al., 2010; MACHADO et al., 2015). However, the dominance of first order opportunistic organisms in the Itinguçu River probably is due to natural factors, such as the wide variations of river flow, then to anthropic factors.

In their turn, second order opportunistic organisms include the family Ceratopogonidae, which is part of the order Diptera, and also the Odonada order. Studies show greater density and richness of larval groups of Ceratopogonidae in forested environments, when compared to places with a greater deforested area, since these end up preventing the deposition of eggs due to the high exposure (YANOVIAK 1999a, 2001; NGAI et al. 2008). Thus, the presence of these organisms in the samples collected can be justified by the good level of preservation of the environment in the Cachoeira do Paraíso waterfall and its vicinities.

Both Ceratopoginidae and Odonata may be influenced by periods of rain and dry, and by the water physical-chemical parameters, such as conductivity, temperature, pH and turbidity (BULÁNKOVÁ, 1997; YANOVIAK 1999<sup>a</sup>). The larval development of many Odonata species is related to temperature; Lutz (1968) suggested that 20 °C would be limiting temperature in temperate environments, but this value is unknown for tropical and subtropical rivers. In this study, Odonata were observed only in P1.

The tolerant group is composed in this study by the Ephemeroptera order, which has a long life cycle and presents some sensitivity to changes in the physical-chemical parameters of the aquatic environment, such as conductivity, nitrate and dissolved oxygen (TIMM, 1997; HROVAT et al., 2009). Hrovat et al. (2009) showed that the depth of water body has an influence on the distribution of these organisms. Ephemeroptera individuals are also commonly found in lotic environments, where they may reach high density, but spatial changes have a determining role in their distribution, as they tend to occur at the stream banks (HROVAT et al., 2009). In the Cachoeira do Paraíso waterfall, they were observed in P1 and P3, at low densities.

As previously mentioned, sensitive organisms were collected in all the sampling sites. The order Plecoptera has a high score in the calculation of the biological index due to its high sensitivity to pollution and low levels of dissolved oxygen (TIMM, 1997). In studies carried out in Estonia, Plecoptera exhibited sensitivity to physical-chemical water parameters, such as pH and temperature (TIMM, 1997; HROVAT et al., 2009). The order Trichoptera encompasses several species distributed all around the world, which tend to be more common in cold and flowing waters, such as waterfalls and springs. Their larval forms are exclusively aquatic. These group has been considered as excellent biological indicator, especially some species that are more sensitive to pollutants (HOLZENTHAL et al., 2015). These group includes species that build structures for protection, predation or to facilitate the filtration of organic matter, which can be formed by grains of sand, small rocks, leaves and others (HOLZENTHAL et al., 2015), and also other species of free life which do not build structures (HOLZENTHAL et al., 2015).

Despite the homogeneity of the benthic macrofauna assemblies among the 3 sampling sites, some minor aspects were explicit in the cluster analysis. First, in general there was a good similarity between replicates; and second, the sampling sites tended to exhibit subtle differences, as shown in Figure 2. The causes of such differences are unknown and can be related with natural factors (such as hydrogeochemical characteristics) or even anthropic factors (pollution or trampling).

The collection of organisms in mesohabitats proved to be adequate considering that these zones favor the establishment and presence of benthic macroinvertebrates, providing, for example, food source, breeding place and physical substrate. But variation of these factors may influence the distribution of organisms throughout the different locations of mesohabitats (GORNI; ALVES:, 2012).

In addition, these organisms are good spatial representatives of habitat disturbances, considering their low mobility in the environment (BEGHELLI et al., 2012).

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Within the scope of ecological indices, the biological diversity reported in this research indicates high values, when compared to studies carried out in areas influenced by anthropic activities. At those regions, the most common organisms were Diptera and Oligochaeta, which are highly tolerant to contaminants (BEGHELLI et al., 2012; TANIWAKI; SMITH:, 2011). The values obtained for the Shannon diversity index in the 3 sampling sites were similar to those reported by Rosa et al., (2011) to the Poço D'Anta,a biological reserve in the state of Minas Gerais. The richness values were lower to those reported in muddy sediments. As already mentioned, the water regime in the Itinguçu river and the texture of the sediment do not favor the development of macrobenthic communities, in comparison with places containing fine sand and muddy sediment (HYNES, 1970, ROSA et al., 2011) and more stable conditions. Besides, the high equitability suggests a good quality of the water body, as these values tend to decrease in places with lower quality. In this sense, the data obtained in this research collaborate to the conclusion that the Itinguçu River has good quality in the region of Cachoeira do Paraíso waterfall (SILVEIRA, 2004). This good water quality was also supported by the biological indices calculated (CETESB, 2019), since all three sampling sites were ranked as presenting excellent quality. However, since the sampling was conducted in 2020, during the COVID-19 pandemic, when restrictions for visitors were imposed, our results should be considered with some caution. The continuous biomonitoring of the river should be made, in order to verify if the conditions will keep unaltered after the return of the visitors, especially in P2 and P3 (GOUVEIA, 2014). Such studies would help to determine which factors effectively influence the structure of the benthic macrofauna community in the Cachoeira do Paraíso waterfall.

Biomonitoring from observation and calculation of biological indexes based on the presence of macroinvertebrates is a technique that has proved to be efficient, thus this method can be an alternative to standard measurements of water quality such as chemical and microbiological analyzes (ESCARPINATI et al, 2011).

## **CONCLUSION**

The present study obtained the first data regarding the composition of the macroinvertebrates community of the Cachoeira do Paraíso waterfall, formed by the Itinguçu River (PEIt, SP, Brazil), and the quality of this water body. The physical-chemical parameters were suitable for rivers located in restrictive protected areas, and were similar in the all the sampling sites. Sediments were sandy and presented low amounts of organic matter and CaCO<sub>4</sub>. Classical ecological indices, exploratory analysis and a biomonitoring index consistently indicated the good to excellent quality of waters in the Cachoeira do Paraíso waterfall. Finally, the biomonitoring method based on the evaluation of the benthic macroinvertebrates proved to be suitable for the streams situated in the protected areas of the Atlantic coast of Brazil.

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The author's contribution statement declare the contributions to this research were divided as equals between the authors, such as field effort, data sampling, data analyses, writing and reviewing. The authors declare no conflict of interests.

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