

Benthic macroinvertebrates as bioindicators of environmental quality of Pará River estuary, a wetland of Eastern Amazon

Macroinvertebrados bentônicos como bioindicadores da qualidade ambiental do estuário do Rio Pará, uma área úmida da Amazônia Oriental

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ABSTRACT

The study characterized and evaluated the use of the benthic macroinvertebrate community as an indicator of impacts in different estuarine environments around the Industrial Port Complex (IPC) of Vila do Conde (Pará State, Amazon region). Fauna of beaches and rivers, in sectors of the estuary under different degrees of environmental impact (high, medium, and low), was compared in different seasonal periods. Macrofauna was composed of typically fresh-water and estuarine groups. Beaches presented sediment with a texture ranging from medium to coarse sand, with a less rich macrofauna ($\bar{X} = 4.5 \pm SE 0.3$ taxa/sample) and dense ($\bar{X} = 1,838.1 \pm 164.8$ ind./m²) of organisms when compared to rivers ($\bar{X} = 5.9 \pm 0.3$ taxa/sample, and $3,248.9 \pm 77.0$ ind./m²), which were environments more muddy. For both environments, sites in the high-impact sector were less rich ($\bar{X} = 4.7 \pm 0.3$ taxa/sample) and dense ($\bar{X} = 2,812.9 \pm 232.7$ ind./m²) when compared to those in the low-impact sector ($\bar{X} = 7.6 \pm 0.4$ taxa/sample, and $3,314.3 \pm 230.1$ ind./m²). Richness ($\bar{X} = 6.4 \pm 0.3$ taxa/sample) and density ($\bar{X} = 3,859.4 \pm 190.2$ ind./m²) were higher in the rainier season when compared to the less rainy season ($\bar{X} = 4.8 \pm 0.3$ taxa/sample, and $1,933.0 \pm 172.1$ ind./m²). However, there were no significant seasonal changes in composition. Results indicated that the structure of the benthic macroinvertebrate community surrounding the IPC responds to the loss of environmental quality, with extreme effects of a drop in abundance and diversity. Taxa that are more tolerant (*Namalycastis caetensis*, *Cirolana* sp., *Pseudosphaeroma* sp., Tubificidae, and Chironominae) and sensitive (Hydropsychidae and *Eteone* sp.) to impact conditions were identified and evaluated as potential bioindicators.

Keywords: macrofauna, environmental impact; industrial pole; Amazonian estuary.

RESUMO

O estudo caracterizou e avaliou o uso da comunidade de macroinvertebrados bentônicos como indicadora de impactos em diferentes ambientes estuarinos na área do Complexo Portuário Industrial (CPI) de Vila do Conde (Pará, região amazônica). A fauna de praias e rios, em setores do estuário sob diferentes graus de impacto ambiental (alto, médio e baixo), foi comparada em distintos períodos sazonais. A macrofauna foi composta por grupos tipicamente dulcícolas e estuarinos. As praias apresentaram sedimento com textura variando de areia média a grossa, com macrofauna menos rica ($\bar{X} = 4,5 \pm SE 0,3$ táxons/amostra) e densa ($\bar{X} = 1.838,1 \pm 164,8$ ind./m²), quando comparados aos rios ($\bar{X} = 5,9 \pm 0,3$ táxons/amostra e $3.248,9 \pm 77,0$ ind./m²), os quais foram ambientes mais lamosos. Para ambos os ambientes, locais no setor de alto impacto eram de menor riqueza ($\bar{X} = 4,7 \pm 0,3$ táxons/amostra) e densidade ($\bar{X} = 2.812,9 \pm 232,7$ ind./m²), quando comparados ao do setor de baixo impacto ($\bar{X} = 7,6 \pm 0,4$ táxons/amostra e $3.314,3 \pm 230,1$ ind./m²). A riqueza ($\bar{X} = 6,4 \pm 0,3$ táxons/amostra) e densidade ($\bar{X} = 3.859,4 \pm 190,2$ ind./m²) foram mais altas no período mais chuvoso, do que no período menos chuvoso ($\bar{X} = 4,8 \pm 0,3$ táxons/amostra e $1.933,0 \pm 172,1$ ind./m²). Contudo, não ocorreram modificações sazonais significativas na composição. Os resultados indicaram que a estrutura da comunidade de macroinvertebrados bentônicos no entorno do CPI responde à perda da qualidade ambiental, com efeitos extremos de queda na abundância e diversidade. Táxons mais tolerantes (*Namalycastis caetensis*, *Cirolana* sp., *Pseudosphaeroma* sp., Tubificidae e Chironominae) e sensíveis (Hydropsychidae e *Eteone* sp.) às condições de impactos foram identificados e avaliados como potenciais bioindicadores.

Palavras-chave: macrofauna, impacto ambiental; polo industrial; estuário amazônico.

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Introduction

Roughly 30% of the Amazon's territory is protected by humid areas, which are highlighted for providing environmental services that guarantee the maintenance of biodiversity and the sustenance of human communities (BARROS; ALBERNAZ, 2014; CARVALHO; PIMENTEL; LIMA, 2019). These areas form the basis of aquatic food chains, for they have a high primary and secondary production, as well as a great diversity of plant and animal species, including invertebrates and vertebrates, many of which are valuable fishing resources (JUNK *et al.*, 2011). Besides that, they are essential for recharging aquifers, containing floods and soil erosion, retaining nutrients, and contaminants (CAVALCANTE *et al.*, 2015).

The use of environmental indicators which assess and provide data for quality management and conservation of environments is recommended for ecosystems with a high degree of sensitivity, such as those found in humid areas (JUNQUEIRA *et al.*, 2018; CHANAMÉ-ZAPATA *et al.*, 2019; MEHROTRA; BARDHAN; RAMAMRITHAM, 2019). An organism, or assembly of organisms, is considered a bioindicator when it presents a response that can be identified in the face of differences (natural variations), or changes (anthropic impacts) in its surroundings. (LIJTEROFF; LIMA; PRIERI, 2009).

Benthic macroinvertebrates are animals that have an average size of 0.5 mm and live all or part of their lives inside or on the bottom of aquatic environments (CAMARGO, 2019). They are one of the most recommended groups in literature for diagnosis and environmental monitoring, due to some attributes, such as:

- presence in practically all aquatic systems, favoring comparative studies;
- sedentary nature and relatively short life cycle, which allows a more effective spatial and temporal analysis of the effects of impacts;
- and a large number of species present therein, offering a wide range of responses to environmental stress;
- the numerous methods in the community studied, which have been used at the level of community monitoring and individual responses (FERREIRA; PAIVA; CALLISTO, 2011; JUNQUEIRA *et al.*, 2018).

The Industrial Port Complex (IPC) of Vila do Conde, in Barcarena City, Pará State, is one of the largest industrial centers in the Amazon region. The complex was created in 1976 and has, in addition to the port area, several mineral processing outsourced companies, associated with the mineral sector and general trade (QUEIROZ *et al.*, 2019). As a result of these activities, the region had its economy modified, which caused an intense and disordered population growth (BORDALO; SILVA; SANTOS, 2012; HAZEU; COSTA; NASCIMENTO, 2019). Given this scenario, recurring accidents and environmental crimes were recorded in the region, which includes: leakage/spillage of petroleum-based fuels; spillage of ores and live cargo; overflows of waste from mineral tailings dams, and releases

of urban and mineral effluents (IEC, 2018; FAIAL *et al.*, 2009; FERREIRA, BELTRÃO, 2016; LIMA *et al.*, 2018; PINHEIRO *et al.*, 2019).

Effects of environmental impacts on aquatic biota at the Vila do Conde Industrial Pole were evaluated by some studies addressing plankton (phyto- and zooplankton) and ichthyofauna. Regarding plankton, changes in density of organisms, and a higher frequency of impacting species were observed in the areas closest to the port system (SENA *et al.*, 2015; COSTA *et al.*, 2016a; 2016b; PINHEIRO *et al.*, 2019). In the ichthyofauna, in places close to the IPC, there was a reduction in the diversity of species and food guilds (VIANA; FRÉDOU; FRÉDOU, 2012; VIANA; FRÉDOU, 2014), in addition to a higher incidence of individuals with histopathological damage (VIANA *et al.*, 2013). So far, only quick inventories have been carried out on the benthic community of the area, with a view to environmental authorizations for the implantation of port and industrial enterprises.

Given the environmental vulnerability of aquatic resources in the area, the present study aims to characterize the community of benthic macroinvertebrates in different aquatic environments and seasonal periods, assessing possible changes in their structure, as well as their use as bioindicators of environmental quality.

Materials and Methods

Study site

The industrial complex of Vila do Conde has an area of 1,316.299 km² and is located on the right bank of the Pará River estuary (CDP, 2010), in Pará State, on the Brazilian Amazon coast. The region's drainage network is formed by rivers of small orders and by the river Pará, a water body of great spatial extensions, with 30–40 km of distance between its banks, and 300 km of longitudinal extension, until its mouth in the Atlantic Ocean (PRESTES *et al.*, 2017). The study site (Figure 1) is an estuarine region with a greater influence of fresh water, classified as a tidal freshwater estuary (ELLIOTT; MCLUSKY, 2002). On the banks of the river Pará, sand strips exposed during low tides are called beaches.

Sampling network

Quarterly collections were carried out from February to November 2012, covering two samples in the rainy period (February and May) and two in the less rainy period (August and November). A total of 10 collection stations were established (Figure 1) to cover the main drainages surrounding the industrial complex. Six of the stations were located on small rivers' stretches under the influence of tides: the river Arrozal (ARZ), the river Murucupi (MUR), the river São Francisco (SF), the river Curuperê-Dende (DEN), the river Arienga (ARI), the river Arapiranga (ARA); and four on the beaches of the river Pará: Caripi Beach (CAR), Itupanema Beach (ITU), Conde Beach (CON), and Beja Beach (BEJ).

The collection stations were distributed in three sectors with different potential impacts, according to secondary data on water quality, land use, and history of environmental impacts in the area. The sectors were:

- high impact sector (DEN, MUR, CON, and ITU), in the IPC area, including the port of Vila do Conde, in addition to large companies in the mineral sector, such as Hydro Alunorte and Imerys Rio Capim; the presence of sewage discharges and industrial effluents (BRABO *et al.*, 2003; LIMA *et al.*, 2009);
- medium impact sector (ARZ, SF, CAR), downstream from the IPC, with urban occupation and close to areas of companies in the mineral sector, with events for the discharge of solid materials, and of domestic and industrial effluents (FAIAL *et al.*, 2009; LIMA *et al.*, 2009);

- low impact sector (ARA, ARI and BEJ) upstream of the IPC, with riparian forests relatively preserved compared to other drainages nearby; and good water quality (LIMA *et al.*, 2009; COSTA *et al.*, 2016a; 2016b).

Field activities and laboratory

Two collection points (about 100 m apart) were established for rivers to distribute the sampling along the impacted stretch. On beaches, only one collection point was established. At each point, three biological samples were taken with the aid of a Van Veen dredge

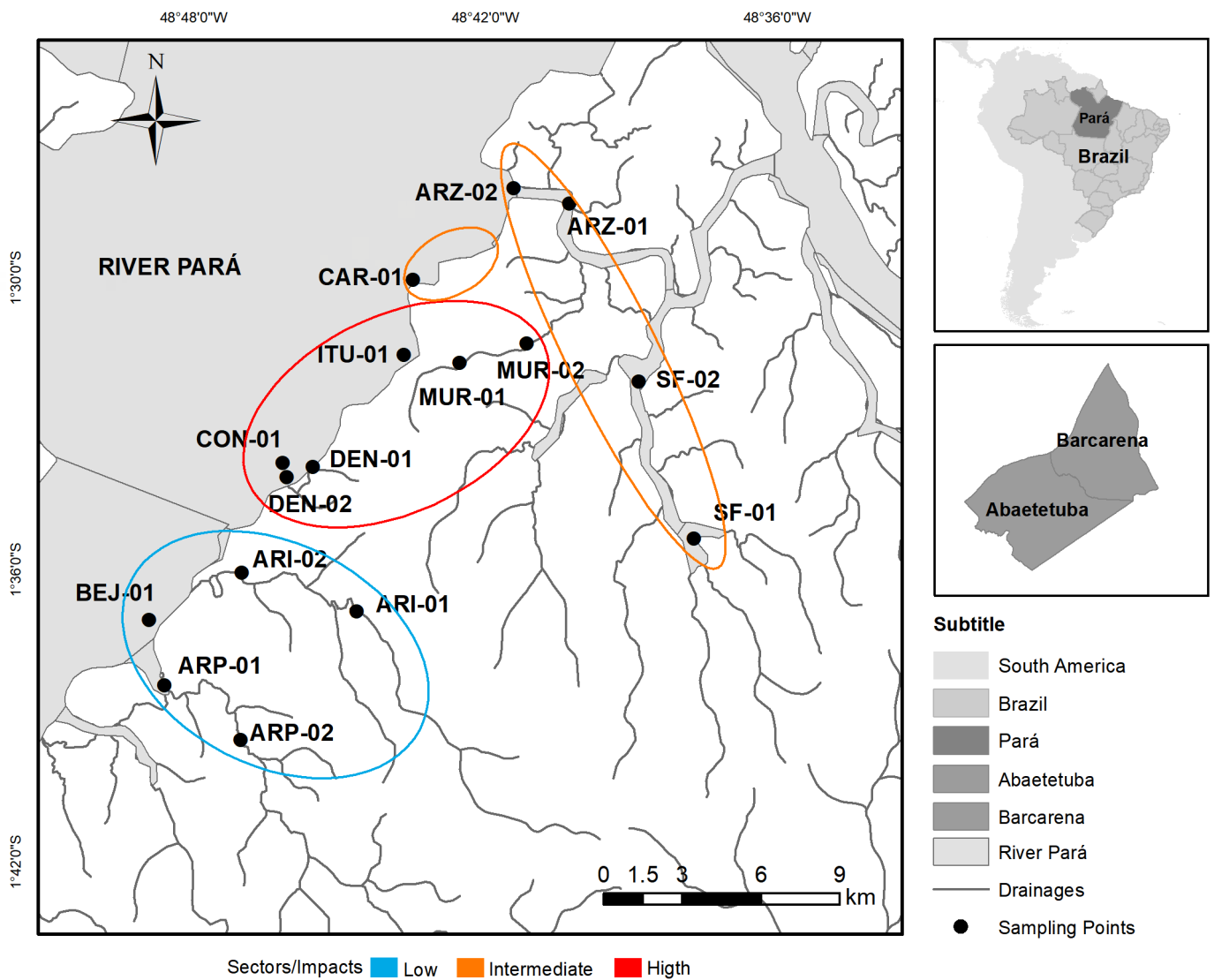


Figure 1 – Sampling network for benthic macroinvertebrates around the Industrial Port Complex of Vila do Conde (Barcarena City, Pará State, Brazil). Rivers: Arrozal (ARZ), Murucupi (MUR), São Francisco (SF), Curuperê-Dende (DEN), Arienga (ARI), Arapiranga (ARP); Estuarine beaches on the river Pará: Itupanema (ITU); Conde (CON), and Beja (BEJ).

(20 × 20 × 20 cm), at a depth of about 2 m. On each occasion and location, a sediment sample was collected for granulometric and total organic matter analysis. Biological samples were passed with a 0.3 mm opening mesh; retained material was preserved in a 5% formaldehyde solution. Sample for abiotic analysis was cooled in the field and frozen in laboratory.

In the laboratory, biological samples were screened, and organisms counted, identified at the lowest possible taxonomic level, and preserved in 70% ethyl alcohol. For sediment samples, granulometry was determined by sieving the coarse and pipetting the fines (SUGUIO, 1973). Organic matter content was determined by the muffle calcination method at 550°C for four hours (adapted from Davies, 1974). Statistical parameters (mean grain diameter, textural classification, and proportions of sand, silt, and clay) of the sediment were calculated using the equations proposed by Folk and Ward (1957).

Data analysis

Regarding sediment data, a principal component analysis (PCA) was applied to identify similarity and the most important variables for grouping locations, environments, and climatic periods. For analysis, data were first standardized and normalized; the Euclidean Distance was used to calculate similarity.

For each biological sample, biological descriptors were calculated: total abundance (number of individuals), average density (individuals per m⁻²), richness (by simply counting the number of taxa), and diversity (Shannon-Winner index). To verify spatial variations of these descriptors, the three-way ANOVA was applied for the following treatments: climatic period (less rainy and rainy), environment (river and beach), and seasons (nested to the environmental factor). ANOVA was followed by Tukey's test whenever null hypothesis was rejected. Before ANOVA, normality of data distribution (Kolmogorov-Smirnov test), and homoscedasticity of variances (Levene's test) were tested, and, when needed, transformed.

For comparison of the structure of communities in different treatments, permutational multivariate analysis of variance (PERMANOVA) was applied (ANDERSON, 2005), following the same model applied in ANOVA. Grouping analysis (CLUSTER) was used to identify spatial patterns, according to the average distance method. For both analyzes, a similarity matrix constructed from Bray Curtis similarity index was used, calculated based on data on density. Similarity Percentage (SIMPER) routine was applied to identify the most important taxa for group similarity. Finally, indicator species index (IndVal) (DUFRENE; LEGENDRE, 1997) was applied to associate the taxa with types of environment and impact sectors. IndVal ranges from 0 to 100%, in which zero is equivalent to the non-indication of species for a given environmental status, and 100 indicates that the occurrence of a given species is characteristic of that environment. In conjunction with IndVal, the Monte Carlo test

(with 1,000 randomizations) was performed to confirm the indication value significance. For all analyzes, a significance level of 5% was used.

Results

Precipitation

In general, rainfall rates for Barcarena City during the study period showed clear seasonal patterns and followed the climatological normal of the last 30 years (Figure 2). However, in the rainy season, rates much higher than those expected were recorded in March, June, and July 2012. For the less rainy period, September and October 2012, total precipitation was above and below the expected, respectively.

Sediment parameters

The sediment parameters of collection sites are shown in Table 1. Comparatively, beaches presented essentially sandy sediment, whereas rivers were predominantly muddy. For rivers, sediment ranged from fine silt (the rivers Murucupi, Curuperê-Dende, and Arienga) to medium silt (the rivers Arrozal, São Francisco, and Arapiranga), with organic matter above 5%. In both periods, the highest organic contents were observed in the rivers Arienga, Curuperê-Dende, and Murucupi. The presence of leaflets was observed predominantly in sediment samples from rivers (Table 1).

On beaches (Beja and Caripi) of relatively less impacted sectors, sediment was classified as medium sand, in both periods, and organic percentage varied from 3.5 to 5.1% (Table 1). In the sectors with the greatest impact (Itupanema and Conde), classification was of coarse (rainy season) and medium (less rainy) sand, always with a larger average grain size compared to the other beaches. Organic percentage on beaches under high impact ranged from 3.4 to 4.6% (Table 1). PCA analysis classified the environments as river and beach, according to their textural characteristics. However, concerning the periods, identifying seasonal patterns was not possible (Figure 3).

Benthic macroinvertebrates

General composition

A total of 2,024 organisms was collected, classified into 30 different taxonomic units (Supplementary material). The phylum Annelida was the most abundant, representing 54.8% of the total organisms collected, followed by Arthropoda (35.1%), Nematoda (7.3%), Mollusca (2.5%), and Platyhelminthes (0.4 %). In general, Polychaeta, Oligochaeta, and Insecta were the most abundant groups in rivers. Nonetheless, some distinctions were observed in-between seasons (Figure 4). In the high-impact sector, the river Curuperê-Dende was dominated by Nematoda, followed by Oligochaeta. On the river Mucurupi, highlights were Insecta and Oligochaeta. In the intermediate impact sector, polychaete worms (Rice) and insects (São Francisco) were the most representative. In the low impact sector, although polychaetes and oli-

gochaetes were also dominant, there was greater participation of crustaceans and mollusks (Figure 4).

On beaches, polychaete worms dominated in Beja and Caripi, low and medium impact sectors, respectively; whereas crustaceans and oligochaetes were the most representative groups in Conde and Itupanema (Figure 4). Mollusks were not very representative and presented greater abundance in the river Arienga and the beaches Beja and Caripi.

Descriptors of benthic assemblages

ANOVA indicated significant variation in density, richness, and diversity, considering the different environments, collection seasons, and seasonal periods (Table 2). In general, macrobenthic assemblages of rivers were significantly denser and more diverse (number of taxa and diversity index) than those of beaches (Figure 5), although these descriptors showed low values in some rivers. In-between seasonal periods, significant variations were identified for density and total richness, without interaction with other factors. In general, the highest values of density and richness were recorded in the rainy season.

Regarding rivers, the macrofauna observed in the river Arrozal (intermediate impact sector) had the highest average density, with values significantly different from all other locations, except for the

river Arapiranga in the rainy season (Figure 5). On the other hand, in the rivers São Francisco, Curuperê-Dendê, and Murucupi, the macrofauna was of lower density. For beaches' macrofauna, Caripi and Beja were significantly denser than Itupanema and Conde during the rainy season. In the less rainy season, assemblies on Itupanema and Conde were significantly more abundant than those on Beja.

As to richness and diversity, a similar pattern of abundance was observed; however, the highest values of the indices were observed in the rivers Arapiranga and Arienga (low impact sector), which, as well as Arrozal, differed significantly from the rivers São Francisco, Curuperê-Dendê, and Murucupi (Figure 5). In turn, among beaches, Beja and Caripi were richer than Conde and Itupanema in the rainy season. In the less rainy season, only Beja was richer than Conde beach.

Spatio-temporal variability of the macroinvertebrate community structure

PERMANOVA indicated that the structure of macrobenthic associations is dissimilar when comparing rivers and beaches, just as there are differences between locations within each type of environment (Table 3). There were no significant changes in-between seasonal periods. All rivers had a significantly dissimilar fauna structure, except for

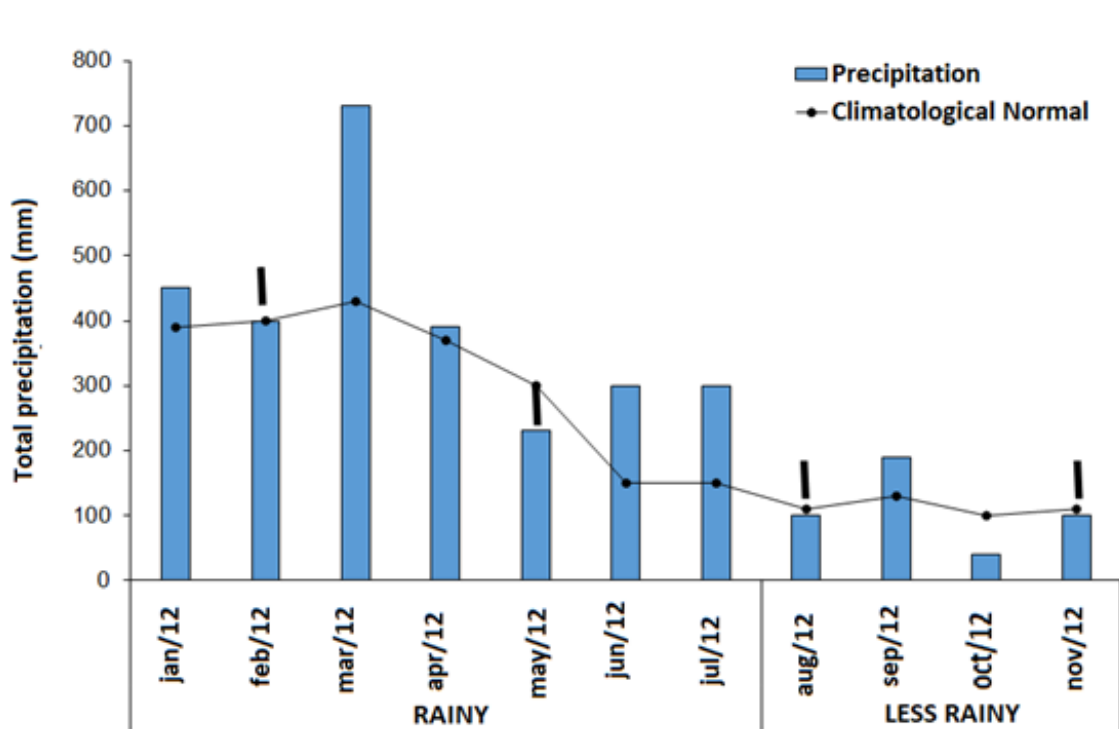


Figure 2 – Total monthly precipitation (bars) for the study period, and normal climatology (lines) for the last 30 years (1989-2019). The arrows indicate collection months.

Source: Based on data from INMET (2012).

Table 1 – Sediment characteristics in collection stations. Rivers: Arrozal (ARZ), Murucupi (MUR), São Francisco (SF), Curuperê-Dende (DEN), Arienga (ARI), and Arapiranga (ARA). Beaches: Caripi (CAR), Itupanema (ITU), Conde (CON), and Beja (BEJ).

Parameter/Period	RIVERS						BEACHES			
	ARA	ARI	ARZ	SF	DEN	MUR	BEJ	CAR	ITU	CON
Rainy period										
Gravel (%)	4.2 ± 0.1	3.9 ± 0.2	3.6 ± 0.1	1.4 ± 0.3	4.1 ± 0.2	0.6 ± 0.2	4.5 ± 0.1	1.4 ± 0.2	2.4 ± 0.1	3.6 ± 0.2
Sand (%)	18.4 ± 0.4	23.1 ± 0.2	20.1 ± 0.1	10.2 ± 0.3	8.8 ± 0.1	24.3 ± 0.1	81.3 ± 0.1	92.3 ± 0.3	63.4 ± 0.4	91.2 ± 0.1
Silt (%)	71.1 ± 0.2	66.2 ± 0.3	71.3 ± 0.1	81.3 ± 0.2	82.1 ± 0.1	68.9 ± 0.1	8.1 ± 0.2	3.6 ± 0.3	26.4 ± 0.2	4.2 ± 0.1
Clay (%)	6.3 ± 0.3	6.8 ± 0.2	5.0 ± 0.3	7.1 ± 0.2	5.0 ± 0.1	6.2 ± 0.2	6.1 ± 0.2	2.7 ± 0.2	7.8 ± 0.1	1.0 ± 0.1
MO (%)	6.3 ± 0.5	9.1 ± 0.3	6.1 ± 0.3	5.6 ± 0.5	8.4 ± 0.3	8.9 ± 0.1	5.1 ± 0.3	3.5 ± 0.2	4.1 ± 0.2	3.4 ± 0.1
Grain size (Ø)	5.4 ± 0.3	5.4 ± 0.2	5.2 ± 0.1	5.6 ± 0.2	6.2 ± 0.1	5.1 ± 0.1	2.4 ± 0.2	2.4 ± 0.1	2.1 ± 0.2	2.1 ± 0.2
Verbal classification	Silt M	Silt M	Silt M	Silt F	Silt F	Silt M	Sand M	Sand M	Sand C	Sand C
Presence of leaflet	+	+	+	+	+	+	-	-	-	-
Less rainy period										
Gravel (%)	1.6 ± 0.1	2.8 ± 0.1	3.1 ± 0.1	3.8 ± 0.1	4.5 ± 0.1	0.5 ± 0.1	2.5 ± 0.2	0.8 ± 0.1	1.6 ± 0.2	2.9 ± 0.1
Sand (%)	10.8 ± 0.2	31.9 ± 0.4	20.2 ± 0.5	17.6 ± 0.2	18.9 ± 0.3	20.5 ± 0.4	84.0 ± 0.3	83.4 ± 0.2	90.8 ± 0.2	95.1 ± 0.3
Silt (%)	84.8 ± 0.2	57.5 ± 0.1	70.5 ± 0.1	75.3 ± 0.1	72.9 ± 0.2	69.0 ± 0.5	8.1 ± 0.2	10 ± 0.2	6.7 ± 0.2	2.4 ± 0.3
Clay (%)	2.8 ± 0.1	7.8 ± 0.1	4.4 ± 0.1	3.3 ± 0.1	3.8 ± 0.1	10.0 ± 0.2	5.4 ± 0.1	5.8 ± 0.2	0.9 ± 0.1	2.5 ± 0.3
MO (%)	5.3 ± 0.3	8.1 ± 0.3	9.1 ± 0.6	5.6 ± 0.5	8.4 ± 0.2	7.9 ± 0.2	3.9 ± 0.1	4.6 ± 0.1	4.00 ± 0.1	4.5 ± 0.2
Grain size (Ø)	5.4 ± 0.3	5.2 ± 0.2	5.3 ± 0.1	5.4 ± 0.2	5.5 ± 0.1	5.2 ± 0.1	1.0 ± 0.2	1.8 ± 0.1	2.0 ± 0.1	2.0 ± 0.1
Verbal classification	Silt M	Silt M	Silt M	Silt M	Silt M	Silt M	Sand M	Sand M	Sand M	Sand M
Presence of leaflet	+	+	+	+	+	+	+	-	-	-

MO= organic matter; Silt F = fine silt; Silt M= medium silt; Sand M= medium sand; Sand C = coarse sand; + = present leaflet; - = without leaflet

Curuperê-Dende and Mucurupi. As to beaches, Itupanema and Conde had a similar structure, whereas all the other comparisons resulted in significant dissimilarity.

PERMANOVA's results did not indicate significant seasonal variation in the structure of assemblies, or the interaction of this factor with spatial factors. Given that, ordination analyzes, SIMPER, and IndVal prioritized spatial patterns (comparisons between seasons/environments and impact sectors).

CLUSTER analysis (Figure 6) showed a separation of the samples of stations of low-impact sectors, considering rivers and beaches. These locations gather samples with greater density and species richness and presented as more common taxa, species of polychaetes (*Namalycastis caetensis*, *Eteone* sp. and *Nephtys fluviatilis*), as indicated by SIMPER (Table 4). Due to its composition and high abundance, the beach Beja exhibited a faunal structure similar to that of rivers. A group of locations (DEN, ITU, CON) of the high-impact sector was also observed. In the SIMPER analysis we noticed that the beaches in this group have *Cirolana* sp. as the most important taxon for sample similarity, and share with the Curuperê-Dende river the high frequency of Nematoda. The rivers Mucurupi and Arrozal form a group with more than 60% similarity, and SIMPER indicates *N.*

caetensis, Chironominae, and Tubificidae as the most common. Caripi was also isolated from the other beaches, and its most frequent taxa were *Eteone* sp., *N. fluviatilis*, and Haplotaxidae.

IndVal values were low: only eight taxa had an indication value greater than 30% and significant (Table 5). Among these, the polychaetes *N. caetensis* and *N. abiurna* were indicatives of the rivers of the medium impact sector. For rivers of the low impact sector, only the larvae of Hydropsychidae were indicators. As to beaches, *Eteone* sp. was an indicator of low-impact beaches, *Cirolana* sp., *Pseudosphaeroma* sp. to the top, and *N. fluviatilis* for medium impact.

Discussion

Composition and spatio-temporal variations

Estuaries are environments of transition between fluvial and marine, characterized by the intense action of physical forcing, with emphasis on the entry of fresh water and tidal fluctuations (ELLIOTT; MCLUSKY, 2002). In tropical estuaries, spatial and temporal variability of salinity, which is influenced by rainfall and fluvial input, is the main macro factor to control biological processes. In Amazon's estuaries, under the influence of large rivers, such as the river Pará, penetration of saline waters from the Atlantic can be largely prevented, giving an

oligohaline character (salinity between 0 and 5) throughout the entire estuary and the year (ROSA FILHO *et al.*, 2018). Accordingly, in the river Pará's stretch and the study period, surface water salinity ranged from 0.01 to 0.05 (ROSÁRIO *et al.*, 2016; COSTA *et al.*, 2016a; 2016b).

Concerning the bottom sediment, organic matter values of rivers and beaches in Pará river estuary, with percentages between 3.4 and 9.1%, are higher than those registered in other Amazonian estuaries, with muddy or sandy sediments (SILVA *et al.*, 2011; AVIZ; CARVALHO; ROSA FILHO, 2012; ROSA FILHO; AVIZ, 2013). This result may indicate enriched substrates in the surroundings of the IPC of Vila do Conde, considering that the release of untreated domestic organic effluents is one of the main contaminants of water in that area (COSTA *et al.*, 2016a; 2016b).

The area's benthic macrofauna was composed of typically freshwater groups (such as insect larvae and oligochaete) (BATZER; BOIX, 2016) and estuarine (such as polychaetes, mollusks, and crustaceans) (ELLIOTT; MCLUSKY, 2002) with numerical dominance of few taxa. This combination is characteristic of estuaries dominated by rivers (SCHUCHARDT; HAESLOOP; SCHIRMER, 1993; ELLIOTT; MCLUSKY, 2002). In the Amazon, macrobenthic communities in estuarine areas are characterized by low taxonomic diversity and dominance of a small number of taxa, attributes that probably are reflections of the stress caused by macrotidal regimes and low salinity, permanent or imposed during some months of the year (ROSA FILHO *et al.*, 2006; ROSA FILHO *et al.*, 2018).

Values of density of organisms (with averages in the collection stations between 484.8 and 3,708 ind. m⁻²) and taxon richness (total

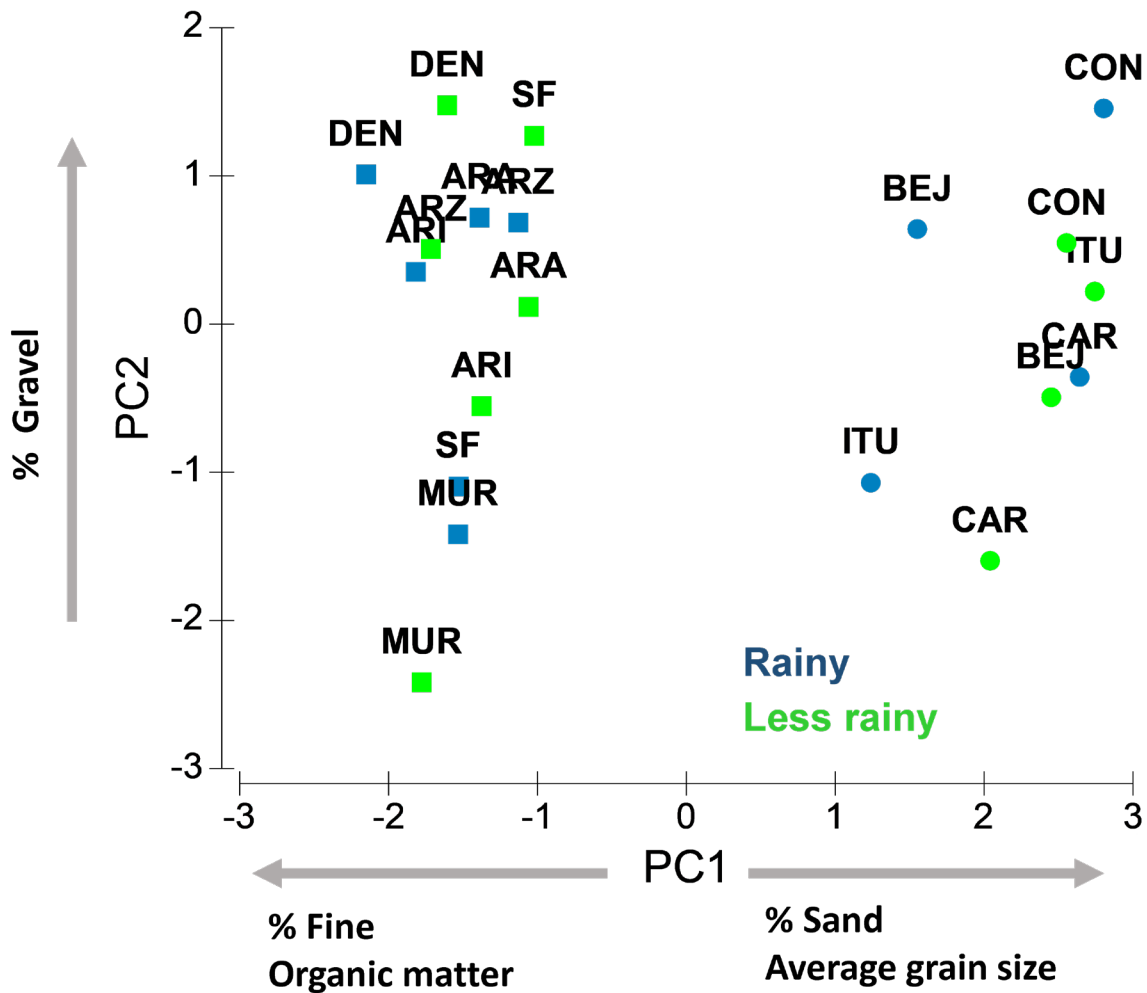


Figure 3 – Result of PCA analysis (PC1 = 62.8%, PC2 = 19.7%) for river sediment data (square) and beaches (circles) of Pará river estuary (Barcarena City, Eastern Amazon). Rivers: Arrozal (ARZ), Murucupi (MUR), São Francisco (SF), Curuperê-Dende (DEN), Arienga (ARI), and Arapiranga (ARA). Beaches: Caripi (CAR), Itupanema (ITU), Conde (CON), and Beja (BEJ). OM: organic matter.

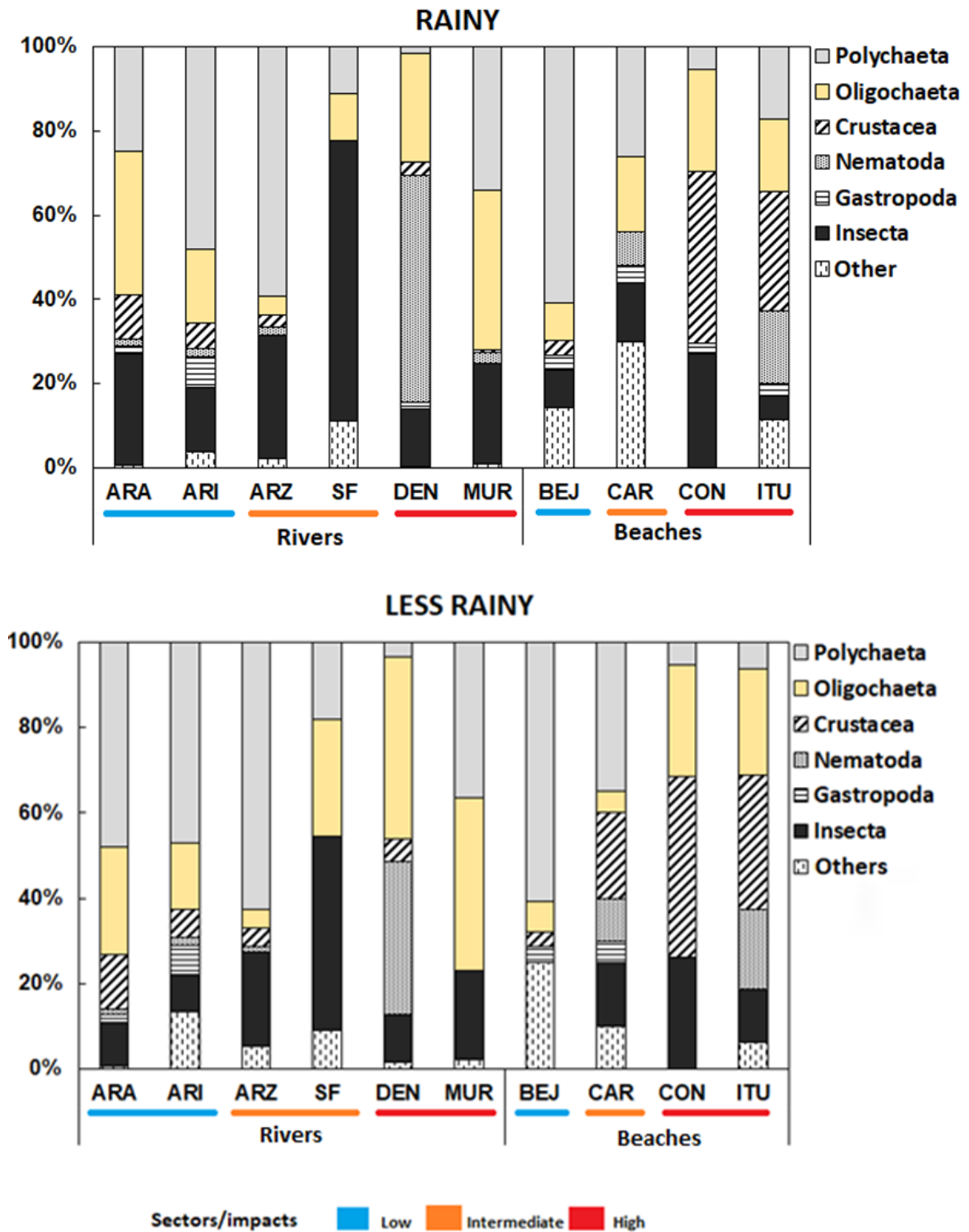


Figure 4 – Relative participation of taxonomic groups in the abundance of organisms in collection stations. Rivers: Arrozal (ARZ), Murucupi (MUR), São Francisco (SF), Curuperê-Dende (DEN), Arienga (ARI), Arapiranga (ARA). Estuarine beaches on the river Pará: Itupanema (ITU); Conde (CON), and Beja (BEJ).

of 30 taxa) from Pará river estuary are consistent with those of other Amazonian estuaries. For example, in Guajará Estuary, with oligohaline waters (< 1), total richness was 22 taxa, and average density varied between 458.3 and 19,290.1 ind. m^{-2} (AVIZ; CARVALHO; ROSA FILHO, 2012). In the same estuary, Rosa Filho and Aviz (2013) recorded a richness of 39 taxa and densities between 403.1 and 5,181.2 ind. m^{-2} . In the estuary of the river Caeté, in a stretch with salinity between 5.1 and 26.5, the total number of taxons was 17, and density varied from 664.6 to 14,399 ind. m^{-2} (ROSA FILHO *et al.*, 2006).

The groups with the highest abundance in the area were Polychaeta, Oligochaeta, Nematoda worms, and Chironomidae larvae. Soft substrates (muddy and sandy-muddy) are especially conducive to wormlike domain, because they facilitate their movement and offer a greater amount of food, especially for deposit-eating species. In turn, Chironomidae larvae can be naturally abundant in continental environments, due to the high diversity of adult larvae in the terrestrial environment, in addition to the plasticity of habitat and tolerance of their larvae, which can use various food resources, including debris (FERRINGTON JR., 2008).

Table 2 – Results of the ANOVA analysis for the descriptors density, richness, and diversity, using the factors environment (river and beaches), collection stations, and seasonal periods.

Variation source	gl.	Density (ind. m^{-2})		Richness (No. taxa)		Diversity (H')	
		F	p-value	F	p-value	F	p-value
Environment (1)	1	35.6	$< 0.01^*$	11.3	$< 0.01^*$	4.6	0.03^*
Station (Environment) (2)	8	88.5	$< 0.01^*$	17.1	$< 0.01^*$	22.2	$< 0.01^*$
Period (3)	1	10.2	$< 0.01^*$	20.3	$< 0.01^*$	0.25	0.61
Interaction (1-3)	1	0.3	0,54	0.04	0.83	0.00	0.99
Interaction (2-3)	8	13.2	0,10	0.82	0.58	0.04	0.99

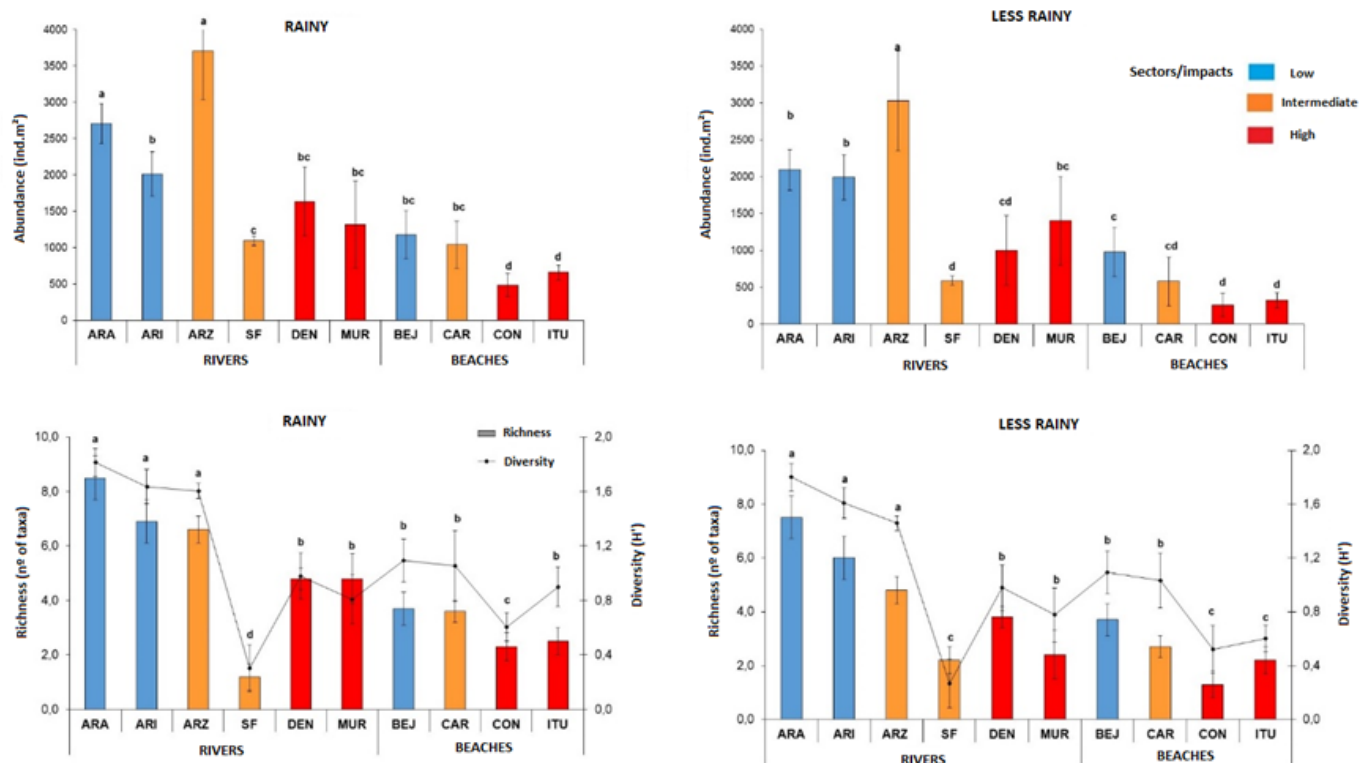


Figure 5 – Mean values (\pm standard error) of density, richness, and diversity of macrobenthic assemblages during different seasonal periods (rainy and less rainy). Rivers: Arrozal (ARZ), Murucupi (MUR), São Francisco (SF), Curuperê-Dende (DEN), Arienga (ARI), and Arapiranga (ARA). Beaches: Caripi (CAR), Itupanema (ITU), Conde (CON), and Beja (BEJ). The letters above the averages represent the Tukey's test results; places that do not share letters differed significantly.

In the river Pará, estuarine beaches were environments with a different composition, lower taxonomic richness, and a lower number of macrobenthic organisms when compared to small tributaries. Those beaches are environments subject to strong tidal currents, with less deposition of fine sediments, and a substrate composed of medium and coarse sands. In turn, small rivers presented more muddy sediment and submerged litter due to low hydrodynamic activity, the influence of soils, and adjacent vegetation. High substrate stability, the greater proportion of fine grains, and the greater supply of food and shelter are factors conducive to the development of benthic organisms (SWARTZ *et al.*, 2019; BOSSLEY; SMILEY, 2019).

In beaches and rivers, the lowest values of richness and density were observed in high-impact sectors. In aquatic environments impacted by deforestation or the release of urban and industrial effluents, macrobenthic fauna tends to reduce density and taxonomic

richness, due to the disappearance or decrease in the numbers of sensitive taxa (MACKINTOSH; DAVIS; THOMPSON, 2015).

In the beaches Conde and Itupanema, high-impact areas, the reduction in biological indexes was associated to more coarse and enriched sediments. In those beaches, the most abundant groups were isopods, tubificids, and chironomids. Tubificidae worms and Chironomidae larvae are known to tolerate habitat modifications, which include contamination by chemical and organic pollutants (PELLETIER *et al.*, 2010; YOSHIDA; ROLLA, 2012; MACKINTOSH; DAVIS; THOMPSON, 2015). In turn, isopods are detritivore, opportunistic organisms, and many species are relevant and useful in environmental monitoring, due to their tolerance to chemical compounds and their great capacity to store metals (GHEMARI *et al.*, 2019).

In addition to the intense urban occupation, the beaches of the river Pará are in an area of a greater influence of port facilities, which

Table 3 – PERMANOVA's results. Rivers: Arrozal (ARZ), Murucupi (MUR), São Francisco (SF), Curuperê-Dende (DEN), Arienga (ARI), and Arapiranga (ARA). Beaches: Caripi (CAR), Itupanema (ITU), Conde (CON), and Beja (BEJ).

Variation source	df	MS	MS	Pseudo-F	P(perm)
Environment	1	19,466	19,466	12.758	0.001*
Stations (Environment)	8	1.479	18,322	12.008	0.001*
Periods	1	2,118.3	2,118,3	13.883	0.206
Environment x Period	1	123.78	123.78	8.11E+02	0.998
Station (Environment) x Period	8	1,304.5	163.06	0.10687	1.000
Res	172	2.62	1,525.8		
Total	191	4.329			
Paired tests					
Groups (Rivers)	t	P(perm)	Groups (Beaches)	t	P(perm)
ARZ, MUR	6.20	0.001*	CAR, ITU	1.86	0.006*
ARZ, SF	4.30	0.001*	CAR, CON	2.67	0.001*
ARZ, DEN	2.98	0.001*	CAR, BEJ	2.84	0.001*
ARZ, ARA	2.44	0.001*	ITU, CON	1.08	0.057
ARZ, ARI	3.47	0.001*	ITU, BEJ	2.50	0.001*
MUR, SF	3.67	0.001*	CON, BEJ	2.82	0.001*
MUR, DEN	1.71	0.100			
MUR, ARA	2.88	0.001*			
MUR, ARI	5.54	0.001*			
SF, DEN	5.75	0.001*			
SF, ARA	4.71	0.001*			
SF, ARI	4.42	0.001*			
DEN, ARA	3.79	0.001*			
DEN, ARI	0.21	0.002*			
ARA, ARI	2.05	0.002*			

can affect the benthic community in different ways (MOSBAHI *et al.*, 2019). Physical facilities, such as support and mooring structures, and pipelines, can alter local hydrodynamic patterns and sediment characteristics, which can include a decrease in local sedimentation (KAWASHIMA *et al.*, 2016). Besides that, contamination of the environment can occur due to oil and fuel leaks from vessels or loss of materials (organic and inorganic) during loading and unloading these vessels (MOREIRA *et al.*, 2019).

The high-magnitude river Arrozal, is in an area of intermediate impact, and presented the highest density of organisms, without a significant drop in the richness of taxa. The site is located downstream the industrial complex and, along its course, it receives discharges of urban effluents (mainly untreated domestic sewage). *N. caetensis* and tubificids, which are deposit consumers, were dominant in this river. Biostimulation can occur in areas with moderate organic enrichment (CULHANE *et al.*, 2019) when organic supply is moderate, or sufficiently diluted; it

does not compromise availability of oxygen for the biota, which contributes to increasing the abundance and the number of benthic species (ROSENBERG; RESH, 1993; AVIZ; CARVALHO; ROSA FILHO, 2012; KRUMHANS� *et al.*, 2015; CULHANE *et al.*, 2019).

Even though the river São Francisco is in an area of intermediate impact, it was the river with the least richness and abundance in the study site, in which Tubificidae, Chironominae, and *N. caetensis* were the dominant taxa. The stations on that river are the most internal to the continent, and the river drains a large continental area, including the urban area of Barcarena City. We believe that the lesser influence of the tides and less dilution of organic loads can contribute to less conducive conditions for the development of the estuarine fauna.

The main seasonal changes observed in macrobenthic assemblages were concerning the density of organisms and the number of species. The increase in the quantity of macrofauna in the rainy season may be associated to the increase in rainfall in the IPC area,

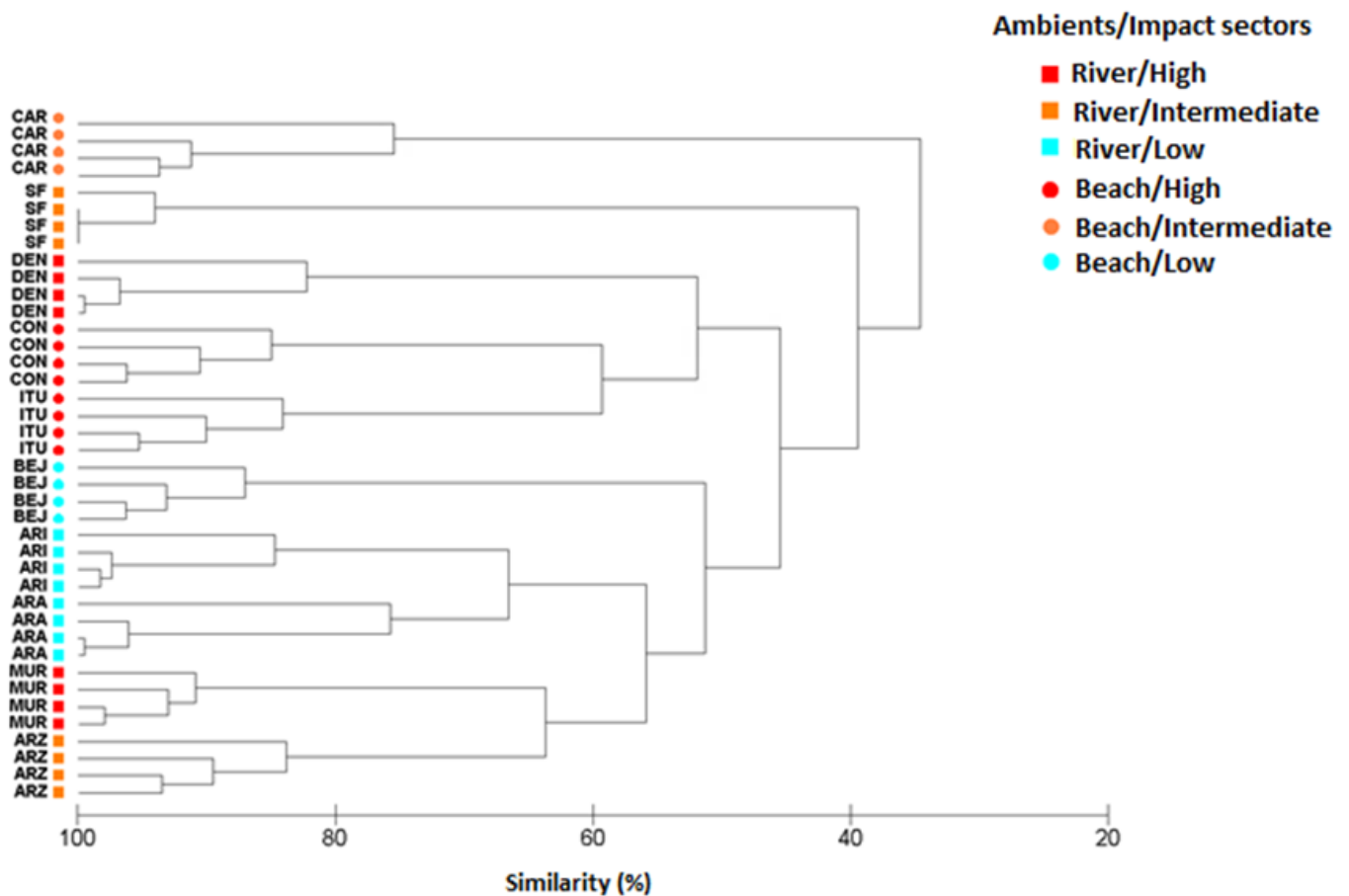


Figure 6 – Result of CLUSTER analysis for samples of benthic macroinvertebrates from Pará River estuary, in different environments and sectors of environmental impact. Rivers: Arrozal (ARZ), Murucupi (MUR), São Francisco (SF), Curuperê-Dendê (DEN), Arienga (ARI), and Arapiranga (ARA). Beaches: Caripi (CAR), Itupanema (ITU), Conde (CON), and Beja (BEJ).

Table 4 – SIMPER results for similarity among the collection sites. Rivers: Arrozal (ARZ), Murucupi (MUR), São Francisco (SF), Curuperê-Dendê (DEN), Arienga (ARI), and Arapiranga (ARA). Beaches: Caripi (CAR), Itupanema (ITU), Conde (CON), and Beja (BEJ).

RIVERS							
Grup ARA				Grup MUR			
Average similarity: 85.1%				Average similarity: 91,4%			
Taxa	Av.S	Cont. %	Cum. %	Taxa	Av.	Cont.%	Cum.%
<i>Namalycastis caetensis</i>	12.5	14.7	14.7	<i>Namalycastis caetensis</i>	32.0	35.1	35.1
Tubificidae	10.3	12.1	26.8	Chironominae	17.6	19.2	54.3
<i>Heleobia</i> sp.	9.9	11.6	38.4	Tubificidae	14.8	16.2	70.5
<i>Eteone</i> sp.	8.2	9.6	48.0	Empididae	9.0	9.9	80.4
Hydropsychidae	7.5	8.8	56.8				
Grup ARI				Grup SF			
Average similarity: 90.1%				Average similarity: 93,9%			
Taxa	Av.S	Cont. %	Cum. %	Taxa	Av.S	Cont.%	Cum. %
<i>Namalycastis caetensis</i>	11.5	12.8	12.8	Chironominae	31.6	33.7	33.7
<i>Eteone</i> sp.	10.8	12.0	24.8	Oligochaeta	19.4	20.7	54.4
<i>Nephtys fluviatilis</i>	10.6	11.8	36.6	<i>Namalycastis caetensis</i>	12.2	13.0	67.4
Tubificidae	10.1	11.2	47.7				
Hydropsychidae	6.8	7.5	55.2				
Grup DEN				Grup ARZ			
Average similarity: 88.3%				Average similarity: 85,9%			
Taxa	Av.S	Cont. %	Cum. %	Taxa	Av.S	Cont.%	Cum. %
Nematoda	30.8	34.9	34.9	<i>Namalycastis caetensis</i>	17.3	20.1	20.1
Chironominae	12.2	13.8	48.7	Tubificidae	17.1	19.9	40.1
<i>Namalycastis caetensis</i>	7.5	8.5	57.2	Chironominae	16.8	19.6	59.6
Tanypodinae	6.1	6.9	64.1	<i>Nephtys fluviatilis</i>	10.9	12.7	72.4
BEACHES							
Grup CAR				Grup ITU			
Average similarity: 86.1%				Average similarity: 84,9%			
Taxa	Av.S	Con. %	Cum. %	Taxa	Av.S	Cont%	Cum. %
<i>Eteone</i> sp.	14.3	16.6	16.6	<i>Cirolana</i> sp.	32.0	37.7	37.7
<i>Nephtys fluviatilis</i>	12.7	14.7	31.3	Nematoda	11.6	13.6	51.3
Haplotaxidae	11.1	12.9	44.2	Tubificidae	11.6	13.6	64.9
Grup CON				Grup BEJ			
Average similarity: 79.5%				Average similarity: 88.4%			
Taxa	Av.S	Cont. %	Cum. %	Taxa	Av.S	Cont.%	Cum. %
<i>Cirolana</i> sp.	28.0	45.8	45.8	<i>Nephtys fluviatilis</i>	31.6	37.3	37.3
<i>Pseudosphaeroma jackobii</i>	10.1	16.5	62.3	<i>Namalycastis caetensis</i>	21.5	25.4	62.7
Nematoda	9.2	11.6	70.2	<i>Cirolana</i> sp.			

which significantly increases organic compounds in the water and can stimulate biota (COSTA *et al.*, 2012a; 2012b). Although rains can naturally contribute to the transport of organic matter with leaching and flooding of forests, the continent's urbanization enhances the entry of nutrients into estuaries (KIMMERER, 2002).

Despite quantitative seasonal changes, the structure of assemblies, *i.e.*, their composition, and the dominant groups were similar in-between the seasonal periods, as indicated by PERMANOVA. The permanent oligohaline condition of the estuary, even with the marked variation in rainfall, seems to keep the faunal structure differently from

what occurs in the estuaries furthest from the mouth of the Amazon River, where salinity throughout the year can vary from 0 to 35 (ROSA FILHO *et al.*, 2018). Additionally, the little seasonal variation in sediment texture can also contribute to greater stability in the benthic communities.

Evaluation of species/bioindicator groups

IndVal obtained a low indication value for most species, due to the low degree of specificity of these species with the impact conditions (high, medium, and low impact sectors). An indicator species must have high fidelity within the assessed ecological status, which is measured by its occurrence percentage (DUFRENE; LEGENDRE, 1997; MCGEOCH; RENSBERG; BOTES, 2002). According to McGeoch, Rensburg and Botes (2002), only species with IndVal values above 70% are characteristic or indicative of ecological status. Species with significant IndVal values, but less than 70%, are considered detectors. Detector species are potential indicators of habitat change because they can change their preferred habitat more quickly than indicator species (VAN RENSBERG *et al.*, 1999). Therefore, in the case of Pará River estuary, all taxa indicated by IndVal are detectors, and studies with longer time series are advised to confirm their use as bioindicators (MCGEOCH; RENSBERG; BOTES, 2002).

One of the most common estuarine organisms in the study site was *Namalycastis caetensis*, a species of polychaete recently described for Pará's estuaries, in sandy-muddy areas of low salinity (ALVES; SANTOS, 2016). In Barcarena, *N. caetensis* was associated by IndVal to intermediate impact rivers, but it was present in practically all collection stations (low, medium, and high impact sectors), with higher densities in rivers with a muddy-sand substrate. The genus *Namalycastis* is generally found in places severely polluted by industrial effluents (rich in heavy metals) and is relatively resistant in toxicity tests that involve hydrocarbons and bioaccumulation of heavy metals (GLASBY, 1999; SARKAR, 2018).

The isopods Cirolanidae, *Cirolana* sp. and *Pseudosphaeroma* sp., were common on the beaches of the river Pará, more frequently in the high-impact sector. Generally, isopods are considered opportunistic taxa and tolerant to environmental impacts, which include metal pollution and organic enrichment (GHEMARI *et al.*, 2019). In Brazil, other species of Cirolanidae, such as *Excirolana brasiliensis*, are considered good monitors of the environmental quality of marine beaches, due to their high resistance to environmental stress, persisting in highly urbanized areas (VELOSO; NEVES; CAPPER, 2011).

Nephtys fluviatilis was a frequent species in rivers and beaches, in a medium- and low-impact sector. IndVal showed a higher frequency of the species for beaches in medium-impact sectors. The polychaete *N. fluviatilis* is an active predator of the macro and meiofauna, and an occasional eater of deposits (SILVEIRA; ORTEGA; DUMONT, 2020), usually registered in Pará's estuaries (ROSA FILHO *et al.*, 2018; ROSA FILHO; AVIZ, 2013). The distribution of the species in the area reflects its preference for sandy-sandy substrates and the minor ability to colonize sandy substrates with thicker grains, such as impacted beaches.

Tubificidae oligochaetes were also a common component in rivers and beaches, with higher densities in rivers. This result is probably related to higher concentrations of fine grains, which commonly favor populations of those worms (RODRIGUEZ *et al.*, 2001). Regarding environmental impacts, oligochaetes had greater participation in the abundance of rivers and beaches in sectors with the greatest impact. Tubificidae is known in literature as a tolerant and opportunistic species, with high densities recorded in places with habitat changes, such as eutrophic water and industrial pollution (ROSENBERG; RESH, 1993). Further efforts are recommended for specific taxonomic identification of the group, which still lacks specialists in the Amazon region.

When comparing polychaetes and oligochaetes, the first group of worms had greater participation in the abundance of rivers and beaches in sectors of greater impact. On the other hand, two other species of

Table 5 – Individual indication value (IndVal) for taxa of benthic macroinvertebrates from Pará River estuary in environments and sectors with different degrees of environmental impact.

Taxa	Indicating valor	p*	Environment (Impact sector)
<i>Namalycastis caetensis</i>	48.3	0.044	River (medium impact)
<i>Cirolana</i> sp.	40.9	0.003	Beach (high impact)
<i>Pseudosphaeroma</i> sp.	29.7	0.008	Beach (high impact)
Hydropsychidae	30.4	0.009	River (low impact)
<i>Nephtys fluviatilis</i>	34.8	0.011	Beach (medium impact)
<i>Namalycastis abiuma</i>	34.5	0.010	River (medium impact)
<i>Eteone</i> sp.	35.1	0.003	Beach (low impact)
Chironominae	30.9	0.021	River (high impact)

*Monte Carlos test.

polychaetes were associated to sectors of medium (*Namalycastis abiuma*) and low impact (*Eteone sp.*). Similarly, Aviz, Carvalho e Rosa Filho (2012), in an Amazon's oligohaline estuary, recorded an increase in the participation of oligochaetes and a decrease in polychaetes in areas impacted by urban effluents.

Insect larvae were highlighted in the fauna structure of rivers and beaches, with different participation according to the order. Chironomidae larvae (Chironominae, Tanypodinae, and Ortocladinae) were present in all collection stations, with emphasis on areas of high environmental impact. In turn, in the rivers Arapiranga and Arienga, the larvae of Trichoptera and other orders (Odonata, Coleoptera, etc.) were more frequent in more preserved hydrographic units.

Traditionally, Chironomidae is considered a tolerant group, and its dominance, when associated with low diversity and the absence, and/or reduction of sensitive taxa, is a characteristic indication of environments with anthropic impacts (ARIMORO *et al.*, 2018). The group's larvae are, in general, resistant to harsh conditions to other organisms, such as waters with high acidity, high temperatures, and low dissolved oxygen levels. (FERRINGTON JR., 2008; MOLINERI *et al.*, 2019). In turn, Trichoptera larvae are considered indicators of good environmental quality, due to their sensitivity to impacts on water quality (HEPP *et al.*, 2010; CHAGAS *et al.*, 2017). Trichoptera larvae are usually more abundant in smaller rivers, with abundant vegetation and well-oxygenated waters (DE CAÍRES SOUZA; FERREIRA; MORAES, 2020), because they depend heavily on submerged leaf litter for protection, cocoon construction, and/or food. The result is that those organisms are extremely sensitive to soil use in watersheds (LECERF; RICHARDSON, 2010; COUCEIRO *et al.*, 2012).

In addition to conditions of better chemical water quality (COSTA *et al.*, 2016a; 2016b), the presence of organisms that are sensitive to impacts (*e.g.*, the Trichoptera), as well as the greater taxonomic richness of the rivers Arapiranga and Arienga, is related to the degree of preservation of marginal vegetation. Forests are not only habitat for the adult forms of insects, but also provide stability to the soils and produce large amounts of leaf litter, which increases habitat heterogeneity (LECERF; RICHARDSON, 2010; SMETI *et al.*, 2019).

Conclusion

Results indicate that the structure of the benthic macroinvertebrate community in the vicinity of the Industrial Port Complex of Vila do Conde responds to the loss of environmental quality, with events of decrease of abundance and taxonomic diversity. The groups found are typically deposit eaters, opportunistic, and adjusted to the oligohaline nature of the estuary. The low richness of the assemblies and the high dominance of few taxa are characteristics that should be considered in the area's environmental monitoring studies, as well as of another Amazon's estuaries, to differentiate the natural attributes of the macrobenthic assemblies from the effects arising from anthropic impacts. The polychaetae *Namalycastis caetensis*, *Nephtys fluviatilis*, *Namalycastis abiuma*, and *Eteone sp.*, the crustaceans *Cirolana sp.* and *Pseudosphaeroma sp.*, and the larvae of Hydropsychidae and Chironominae were identified as potential bioindicators. Nonetheless, in the view of their wide distribution in the area and low fidelity to the impact conditions, further studies with longer time series should be conducted.

Contribution of authors:

Pinto, A.J.A.: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Data curation, Writing — original draft, Writing — review and editing. Tavares, V.B.C.: Supervision, Project administration. Pinheiro, S.C.C.: Supervision, Project administration. Lima, M.O.: Supervision, Project administration. Aviz, D.: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Data curation, Writing — original draft, Writing — review and editing. Lima, A.M.M.: Writing — revision and editing.

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