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Benthic macroinvertebrates as bioindicators of water quality in Billings Reservoir fishing sites (SP, Brazil)

Macroinvertebrados bentônicos como bioindicadores da qualidade da água em locais de pesca do reservatório Billings (SP, Brasil)

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Abastract: Aim: The Billings Reservoir is the largest reservoir in the metropolitan region of São Paulo and has multiple uses, including artisanal fishing. Its surroundings present intense occupation, resulting in various environmental impacts. Although the water is degraded, affecting the composition and quality of the fish, this reservoir supports artisanal fishermen who survive through this activity. This study aimed to analyze the water quality in the main sites of artisanal fishing activity, with an emphasis on the benthic community as a bioindicator. **Methods:** Three sampling sites were selected, in which water and zoobenthos samples were collected monthly from April 2012 to March 2013. Water analyses consisted of the determination of pH, temperature, conductivity, turbidity, total dissolved solids, dissolved oxygen, total nitrogen, total phosphorus, and trophic state as well as a principal components analysis. The zoobenthos analysis consisted of determining the relative abundance and total density of taxa, the Shannon-Weaver diversity index, taxa richness, uniformity, Simpson's dominance index, and the benthic community index and conducting a canonical correspondence analysis. The non-parametric Kruskal-Wallis test followed by the Student-Newman-Keuls test were used to investigate the existence of significant differences in the abiotic and biotic variables among the sites. **Results:** The studied sites showed a high degree of eutrophication, with nitrogen and phosphorus totals levels above the limits defined by current Brazilian legislation. Taquacetuba (P1) showed the best environmental conditions for the development of fishing activity, with the presence of sensitive organisms (Polymitarcyidae) and higher levels of dissolved oxygen. Alvarenga (P2) had the worst water quality, with a predominance of tolerant organisms (Oligochaeta) and lower levels of dissolved oxygen. **Conclusion:** In conclusion, the benthic community in association with abiotic metrics proved to be a useful tool as a bioindicator of environmental conditions, indicating that fishing activity at Alvarenga is not recommended due to the degradation of water quality.

Keywords: zoobenthos; environmental impact; eutrophication; artisanal fisheries.

Resumo: Objetivo: O Reservatório Billings é o maior reservatório da Região Metropolitana de São Paulo, com usos múltiplos, incluindo a pesca artesanal. Seu entorno apresenta intensa ocupação, estando sujeito aos mais diversos impactos ambientais. Apesar de a água apresentar-se degradada, comprometendo a composição e a qualidade do pescado, este reservatório conta com a presença de pescadores artesanais que sobrevivem desta atividade. O presente estudo teve como objetivo analisar a qualidade da água nos principais locais de atividade de pesca artesanal, com ênfase na comunidade bentônica como bioindicadora. **Métodos:** Foram selecionados três pontos amostrais, nos quais amostras de água e zoobentos foram coletadas mensalmente de Abril de 2012 a Março de 2013. A Análise da água consistiu de pH, temperatura, condutividade elétrica, turbidez, sólidos totais dissolvidos, oxigênio dissolvido, nitrogênio total e fósforo total e também de Análise de Componentes Principais e estado trófico. A Análise do zoobentos consistiu de abundância relativa e densidade total de táxons, diversidade de Shannon-Weaver, riqueza de táxons, uniformidade, dominância de Simpson e também de Análise de Correspondência Canônica e Índice de Comunidade Bentônica. Os testes não paramétricos de Kruskal-Wallis seguido por Student-Newman-Keuls foram utilizados para investigar a existência de diferenças significativas nas variáveis bióticas e abióticas entre os locais. **Resultados:** Os locais estudados apresentaram alto grau de eutrofização com níveis de nitrogênio e fósforo totais acima dos limites da legislação vigente no Brasil. Taquacetuba (P1) apresentou as melhores condições ambientais para o desenvolvimento da atividade da pesca com presença de organismos sensíveis (Polymitarcyidae) e maiores teores de oxigênio dissolvido. Alvarenga (P2) apresentou a pior qualidade da água, com predomínio de organismos tolerantes (Oligochaeta) e baixos teores de oxigênio dissolvido. **Conclusão:** Concluiu-se que a comunidade bentônica, em conjunto com variáveis abióticas, foi indicadora eficaz das condições ambientais, sinalizando que a atividade pesqueira em Alvarenga não é recomendada em função da acentuada degradação da qualidade da água.

Palavras-chave: zoobentos; impacto ambiental; eutrofização; pesca artesanal.

1. Introduction

The Billings Reservoir is located in the basin of the Tiete River in São Paulo State (Brazil), covering the entirety of six municipalities in the metropolitan region of São Paulo (Diadema, Ribeirão Pires, Rio Grande da Serra, Santo André, São Bernardo and São Paulo). This is the largest water reservoir in the region, with an area of 127×10^6 m^2 and a maximum depth of 19 m. This reservoir shows a history of conflict among its multiple uses in recreation, the professional activity of artisanal fishing, power generation, effluent dilution, flood control and water supply (Cardoso-Silva et al., 2014). Though the water is degraded, affecting the composition and quality of the fish, there are many artisanal fishermen who survive through fishing. According to Alves da Silva et al. (2009) and Castro et al. (2009), between 2005 and 2007 at Billings Reservoir, approximately 100 families lived exclusively on resources from artisanal fisheries, but this number has been decreasing in recent years, which has been attributed principally to the pollution in the reservoir. These authors showed that the most representative species were acará (42.9%) (*Geophagus brasiliensis*, Quoy & Gaimard, 1824), tilapia (25.2%) (especially *Oreochromis niloticus*, Linnaeus, 1758, but also *Tilapia rendalli*, Boulenger, 1897) and lambari (16.3%) (*Astyanax* spp., especially *Astyanax eigenmanniorum*, Cope, 1894 and *Astyanax scabripinnis*, Jenyns, 1842). Although the environment surrounding the reservoir contains areas with good environmental preservation, many regions show urban development and agriculture, which contributes to its degradation, especially by sewage and industrial and agricultural waste. Another environmental contamination factor is the pumping of water from the Pinheiros River into the Billings Reservoir; this transposition occurs for flood control during the rainy season (EMAE, 2014), and the Pinheiros River has a high degree of eutrophication and environmental stress.

The state environmental agency - CETESB (Companhia Ambiental do Estado de São Paulo), through their annual reports, classifies the Billings Reservoir as eutrophic to hypereutrophic, depending on the analyzed compartment (CETESB, 2013). In this context, the use of this reservoir for artisanal fishing is worrisome, and the water quality should be assessed properly.

The methods traditionally used to assess the water quality of aquatic ecosystems based on physical and chemical parameters are considered inefficient (Buss et al., 2008). Bioassessment is widely used in the monitoring and management of environmental quality and the integrity of aquatic ecosystems, complementing traditional physical and chemical methods (Karr, 1999; Linke et al., 2005). Benthic macroinvertebrates are widely employed in impact assessments and are recommended for environmental monitoring (Fonseca-Gessner and Guereschi, 2000), as they possess several features that make them outstanding biological indicators (Hellawell, 1986; Rosenberg & Resh, 1993).

The arguments for the use of benthic macroinvertebrates as a biological tool in biomonitoring programs are numerous. These include the facts that they are cosmopolitan and abundant animals, most have well-known ecological characteristics, they are sessile or have limited mobility and therefore are representative of local conditions, and they have the advantage of characterizing the water quality not only at the moment of sampling but also by reflecting their position over a longer period of time (Moreno & Callisto, 2005).

The analysis of the structure and distribution of the benthic community is an important ecological tool for describing spatial and temporal changes (Callisto et al., 1998; Leal & Esteves, 1999). According to CETESB (2013), by inhabiting the sediment and being sensitive to disturbance in the aquatic environment, the benthic community is considered a good indicator of the ecological quality of water bodies. Some studies (Pamplin et al., 2006; Carew et al., 2007; Jorcin & Nogueira, 2008; Buss & Vitorino, 2010; Cortelezzi et al., 2011; Miserendino et al., 2011) have improved the knowledge of the bio-indicator potential of benthic macroinvertebrates.

The main objective of this study was to use the benthic macroinvertebrate community as a tool in the assessment of the water quality at fishing sites in Billings Reservoir (SP).

2. Materials and Methods

2.1. Study area

The Billings Reservoir has an area of approximately 120 km^2 and has a complex and dendritic format, with a long and narrow central body with eight branches. It has a maximum depth of approximately 18 meters, an average volume of 646,841×10^6 m^3, an average flow of 8.75 m^3·s^{-1} and an approximate water retention time of 720 days (CETESB, 2012, 2013).

The sampling sites (Figure 1) were selected in the areas with the highest intensity of fishing activity. Information about these areas was obtained from the community of traditional fishermen through reports obtained during meetings at Capatazia Z1, São Bernardo do Campo, and data collected by Alves da Silva et al. (2009). According to the Brazilian legislation of São Paulo State (ALESP, 1977 decree #10775) these sites are classified as freshwater of class of use 2, which indicates they are used for fishing or the growing of organisms for intensive consumption purposes.

- Sampling site 1 - P1 (S 23°49'54.0"; W 46°38'26.9") is located in the Taquacetuba branch close to the central body of the reservoir, which is an area with good preservation of the Atlantic Forest in its surroundings along with some agricultural areas because the forming region of the Taquacetuba River occurs in a location with several protected areas. Water collection for transposition into the Guarapiranga Reservoir for public supply purposes occurs in the Taquacetuba branch.
- Sampling site 2 - P2 (S 23°46'01.0"; W 46°37'37.7") is located in the Alvarenga region, an area with the total removal of the Atlantic Forest and great urbanization in its surroundings. This area was chosen because, according to the fishermen, the fish obtained there had unpleasant odor and taste. Thus, it would be possible to infer the effects of the interference of water quality on fish quality. Among the sampling sites, P2 is the closest to the pumping area of the water from the Pinheiros River.
- Sampling site 3 - P3 (S 23°48'53.2"; W 46°32'94.7") is located close to Biguás Island, which is located in the central body of the reservoir close to the fishing colony and the João Basso ferryboat and is an area with Atlantic Forest and low urbanization in its surroundings

2.2. Sampling and data analysis

From April 2012 to March 2013, water and sediment (zoobenthos) samples were collected monthly from three sampling sites located at least 10 meters from the banks. The water was collected 0.5 meters from the bottom using Van Dorn bottles for the analysis of total nitrogen (TN) and total phosphorus (TP) following the methods described by Valderrama (1981), Mackereth et al. (1978) and Strickland & Parsons (1960). In the field, the abiotic variables pH, water temperature (Temp), electrical conductivity (Cond), turbidity (Turb), total dissolved solids (TDS) and dissolved oxygen (DO) were measured with a Horiba U-22 multi-probe at 0.5 meters from the bottom.

A principal component analysis (PCA) was used to verify the ordering of the sampling sites in relation to the intensity of the association among the abiotic variables. To assess the water quality related to nutrient enrichment, the Lamparelli's trophic state index (TSI) for the total phosphorus concentration was used according CETESB (2015).

Macroinvertebrates were sampled by collecting three samples of sediment with an Ekman-Birge

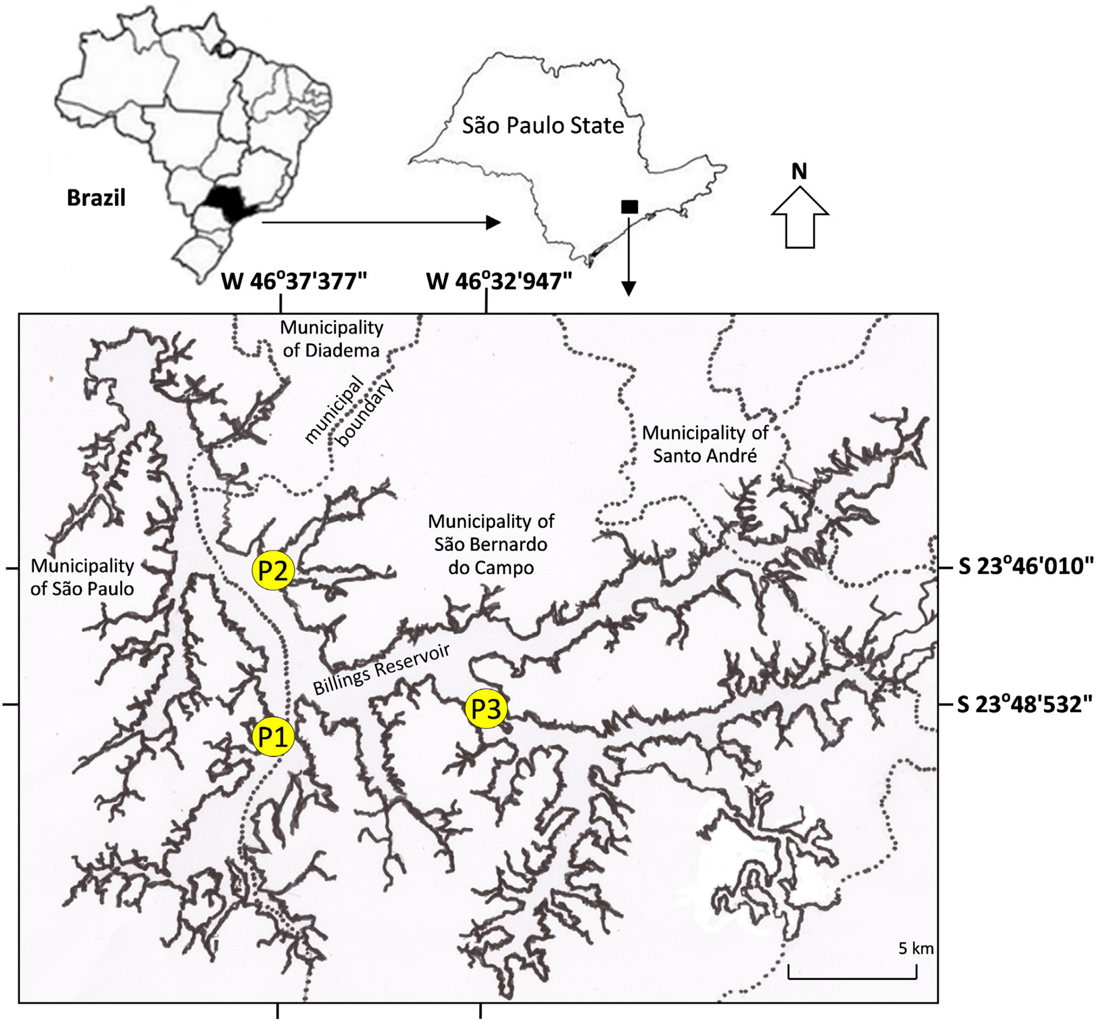

Figure 1. Study area and approximate location of sampling sites at Billings Reservoir (Southeastern region of Brazil, São Paulo State): P1 - Taquacetuba, P2 - Alvarenga and P3 - Biguás Island (dotted lines - municipal boundaries).

grab (225 cm^2) at each sampling site (Pérez, 1996). The samples were washed over a 250 µm mesh and floated following the methods described by Brandimarte & Anaya (1998). The taxonomic identification of organisms was based on Macan (1975), Pennak (1991), Merritt & Cummins (1995), Trivinho-Strixino & Strixino (1995) and Pérez (1996).

The benthic community was analyzed by calculating the relative abundance (%), density of organisms (org·m^{-2}), Shannon-Weaver diversity index (H'), taxa richness (S), uniformity (U') and Simpson's dominance index (C), according to Elliott (1977), Ricklefs (2011) and Begon et al. (2007). The multimetric benthic community index (BCI) was also calculated from the diversity and richness (CETESB, 2013).

The results of the analyses of the abiotic and biotic variables are presented with box-and-whiskers plots, in which the lower and upper limits of the box correspond to the 25th and 75th percentiles, respectively, the whiskers correspond to the minimum and the maximum values, and the center line of the distribution corresponds to the median. The non-parametric Kruskal-Wallis test (H) followed by a Student-Newman-Keuls (SNK) test were used to investigate the existence of significant differences in the abiotic and biotic variables among the sites. A canonical correspondence analysis (CCA) was used to verify the ordering of the sampling sites in relation to the intensity of the association among the abiotic variables. The statistical analyzes followed Hair et al. (2009), Sokal & Rohlf (2009) and Zar (2010). All analyses were carried out using the routines of the PAST 3.x and BioEstat 5.1 programs.

3. Results

3.1. Spatial variation in abiotic variables

The box-and-whiskers plots of the abiotic variables (Figure 2) demonstrate that the pH values were nearly acidic, with variation among the sampling sites of min: 4.9 and max: 6.7. The medians of the pH values were under the CONAMA (2005) #357 limit (between 6.0 and 9.0) at all sites (P1: 5.61, P2: 5.59 and P3: 5.59). The highest values of electrical conductivity (343 $\mu S \cdot cm^{-1}$), turbidity (72.7 NTU), total dissolved solids (223 $mg \cdot L^{-1}$), total nitrogen (15.37 $mg \cdot L^{-1}$) and total phosphorus (0.24 $mg \cdot L^{-1}$) were observed at P2. The total nitrogen and total phosphorus at all sites were above the limits according to the CONAMA (2005) #357 resolution (TN lower than 1.27 $mg \cdot L^{-1}$ and TP lower than 0.03 $mg \cdot L^{-1}$). Regarding the dissolved oxygen, the lowest values were observed at P2 (3.2 $mg \cdot L^{-1}$), which was the only site with values below the limit of the mentioned resolution (DO not lower than 5 $mg \cdot L^{-1}$). The Kruskal-Wallis test (H) and *a posteriori* multiple comparisons using the Student-Newman-Keuls test (Table 1) showed significant differences ($p<0.05$) in electrical conductivity, total phosphorus and dissolved oxygen between P1 and P2 and between P2 and P3, demonstrating that P2 significantly differed from the other sites.

3.2. Principal Component Analysis (PCA)

Regarding the PCA (Figure 3), the first two components explain 61.47% of the total variability in the analyzed data. Table 2 shows the correlation of the variables with the axes. The PCA showed the worst conditions in relation to the water quality at P2. The data cloud for this site was separated from those of the other sites (convex hull), having greater association with electrical conductivity, total nitrogen, total phosphorus and total dissolved solids. On the other hand, sites P1 and P3 showed greater association with DO.

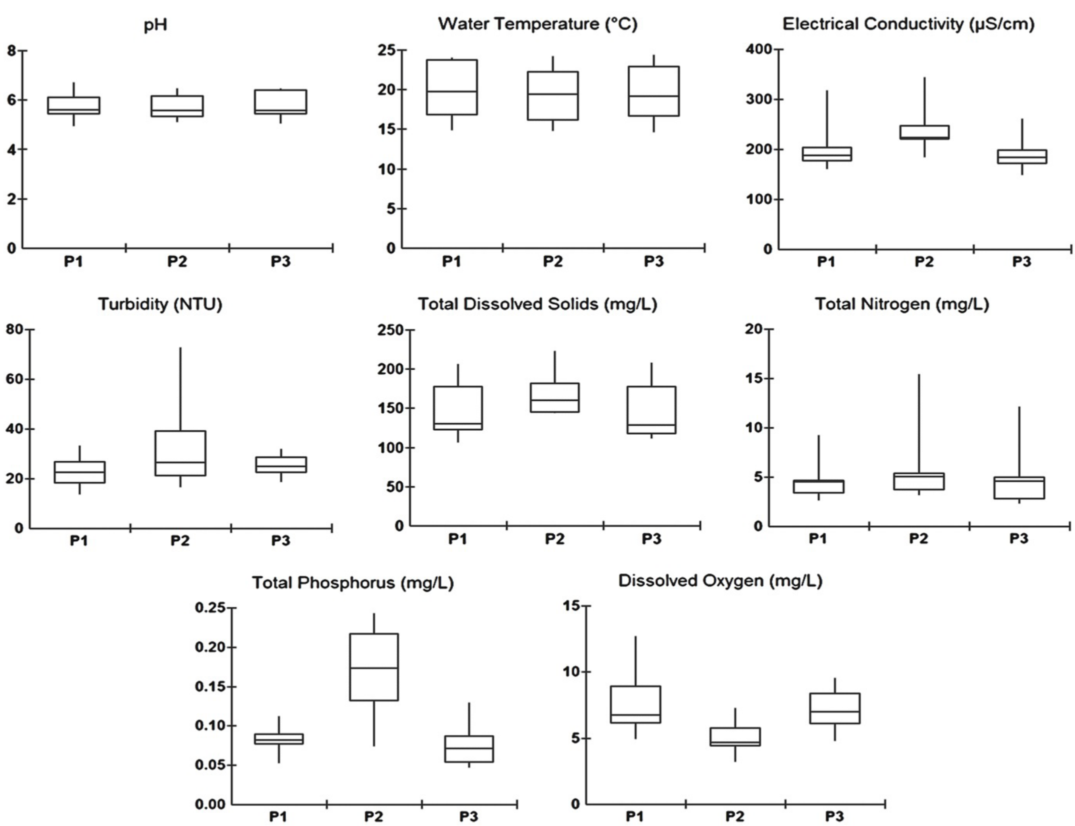

Figure 2. Abiotic variables measured at sampling sites P1 - Taquacetuba, P2 - Alvarenga and P3 - Biguás Island at Billings Reservoir (Brazil, São Paulo State) represented by box-and-whiskers plots where the lower and upper limits of the box correspond to the 25th and 75th percentiles, respectively, the whiskers correspond to the minimum and maximum, and the center line of the distribution corresponds to the median.

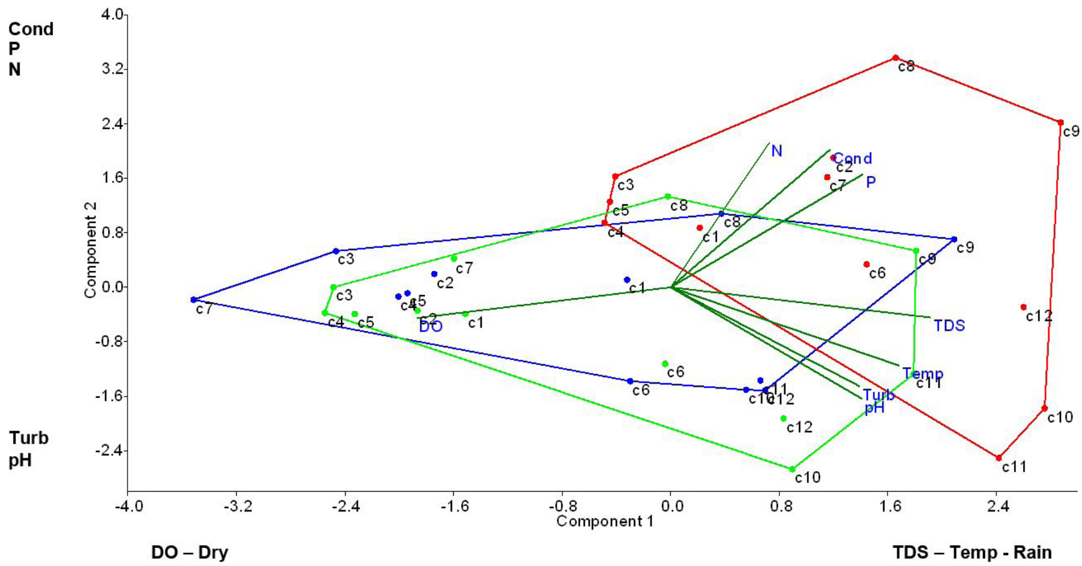

Figure 3. Principal component analysis (PCA) to verify the ordering of the sampling sites in relation to the intensity of the association among the abiotic variables. The numbers represent the collections (samples): C1 (17.04.12), C2 (22.05.12), C3 (26.06.12), C4 (26.06.12), C5 (13.09.12), C6 (24.09.12), C7 (06.11.12), C8 (04.12.12), C9 (18.12.12), C10 (17.01.13), C11 (05.03.13) and C12 (26.03.13). The lines (convex hull) indicate the cloud (data points) represented by the measurements at each site: P1 - Taquacetuba (blue), P2 - Alvarenga (red) and P3 - Biguás Island (green) at Billings Reservoir (Brazil, Sao Paulo State). The vectors (dark green) show the contributions of the variables pH, temperature (Temp), electrical conductivity (Cond), turbidity (Turb), total dissolved solids (TDS), total nitrogen (N), total phosphorus (P) and dissolved oxygen (DO) in the formation of the first two components of the analysis.

Table 1. Results of Kruskal-Wallis test (H) and subsequent Student-Newman-Keuls (SNK) test for abiotic variables among the sampling sites.

	Kruskal-Wallis		Student-Newman-Keuls		
	H	*p*	Sampling sites compared	SNK	*p*
pH	0.299	0.8611			
Water Temperture	0.2662	0.8754			
Electrical Conductivity	**13.0938**	**0.0014***	P1 and P2	**11.8333**	**0.0059***
			P2 and P3	**14.6667**	**0.0006***
Turbidity	2.1495	0.3414			
Total Dissolved Solids	4.9165	0.0856			
Total Nitrogen	1.8396	0.3986			
Total Phosphorus	**18.8996**	**0.0001***	P1 and P2	**14.2917**	**0.0009***
			P2 and P3	**17.5833**	**< 0.0001***
Dissolved Oxygen	**12.8984**	**0.0016***	P1 and P2	**13.4167**	**0.0018***
			P2 and P3	**13.3333**	**0.0019***

P1 - Taquacetuba, P2 - Alvarenga and P3 - Biguás Island at Billings Reservoir (Brazil, São Paulo State), where * indicates significant difference ($p<0.05$).

3.3. Trophic state

The analysis of the trophic state according to CETESB (2015) (Table 3) using the total phosphorus concentrations showed eutrophic conditions at P1 (82 $mg \cdot m^{-3}$) and P3 (71 $mg \cdot m^{-3}$) and supereutrophic conditions at P2 (167 $mg \cdot m^{-3}$). However, the calculation of the trophic state index (TSI) indicated hypereutrophic conditions at all

Table 2. Correlation of abiotic variables with the axes (components) of principal component analysis (PCA) for the variables analyzed for the sampling sites at Billings Reservoir (Brazil, São Paulo State).

	Axis 1	Axis 2	Axis 3	Axis 4
pH	0.5814	-0.5307	-0.147	0.003009
Temp (Water Temperature)	0.6945	-0.3729	-0.4537	0.1335
Cond (Electrical Conductivity)	0.4849	0.6564	0.3081	0.3735
Turbz (Turbidity)	0.5744	-0.4716	0.3413	-0.2601
TDS (Total Dissolved Solids)	0.7909	-0.1442	0.1959	0.4926
N (Total Nitrogen)	0.3002	0.6891	-0.5606	0.01534
P (Total Phosphorus)	0.5842	0.5378	0.265	-0.368
DO (Dissolved Oxygen)	-0.7713	-0.1466	0.1119	0.396

Table 3. Results of Lamparelli's trophic state analysis (CETESB, 2015) based on the total phosphorus concentration and the trophic state index (TSI) (average among samples by site).

Trophic State		Total Phosphorus (mg·m^{-3})	Trophic State Index (TSI)
P1: Supereutrophic		82	69
P2: Hypereutrophic		166	73
P3: Supereutrophic		71	68
Trophic state analysis criterion (CETESB, 2015)			
Trophic state	**Value for weighting (by arithmetic average)**	**Total Phosphorus (mg·m^{-3})**	**Trophic State Index (TSI) criterion for total phosphorus** **TSI (TP) = 10*(6-(1.77-0.42*(lnTP)/ln2))** **ln = natural logarithm = Log$_e$** **and TP = Total Phosphorus**
Ultra-oligotrophic	0.5	TP ≤ 8	TSI ≤ 47
Oligotrophic	1	8 < TP ≤ 19	47 < TSI ≤ 52
Mesotrophic	2	19 < TP ≤ 52	47 < TSI ≤ 52
Eutrophic	3	52 < TP ≤ 120	59 < TSI ≤ 63
Supereutrophic	4	120 < TP ≤ 233	63 < TSI ≤ 67
Hypereutrophic	5	233 < TP	67 < TSI

P1 -Taquacetuba, P2 - Alvarenga and P3 - Biguás Island at Billings Reservoir (Brazil, São Paulo State).

sites: P1 (69), P2 (73) and P3 (68). The assessment using the average of the weighted scores of the results obtained for the total phosphorus (mg·m^{-3}) and the trophic state index shows hypereutrophic conditions at P2 and supereutrophic conditions at the other sites.

3.4. Benthic macroinvertebrates community and biotic indices

The analysis of relative abundance (Figure 4) that was conducted by adding up all samples by site showed a greater presence of Oligochaeta at P2 compared to P1 and P3. *Chaoborus* sp. showed a great abundance at all sites. Chironomini was also observed at all sites and showed higher abundances at P3 and P1. Polymitarcyidae showed a higher abundance at P1.

CETESB (2013) classifies some taxa of the zoobenthos by their tolerance/sensitivity to different environmental impacts. According to this classification, the sensitive group (Polymitarcyidae) was more abundant at P1 (23.27%) compared to the other sites, where it was almost absent (<2.5%); the semi-tolerant group (Chironomini, Tanypodinae, Ceratopogonidae and *Helobdella stagnalis,* Linnaeus, 1758) was more abundant at P3 (88.51%) and P1 (64.43%) compared to P2 (38.44%); and the tolerant group (Oligochaeta) was more abundant at P2 (59.86%) compared to P1 (12.30%) and P3 (9.20%).

The distribution of the biotic index values by site (Figure 5) shows a tendency for higher values of total density, Shannon-Weaver diversity, taxa richness and uniformity at P1 and higher values of Simpson's dominance at P2 and P3. In general,

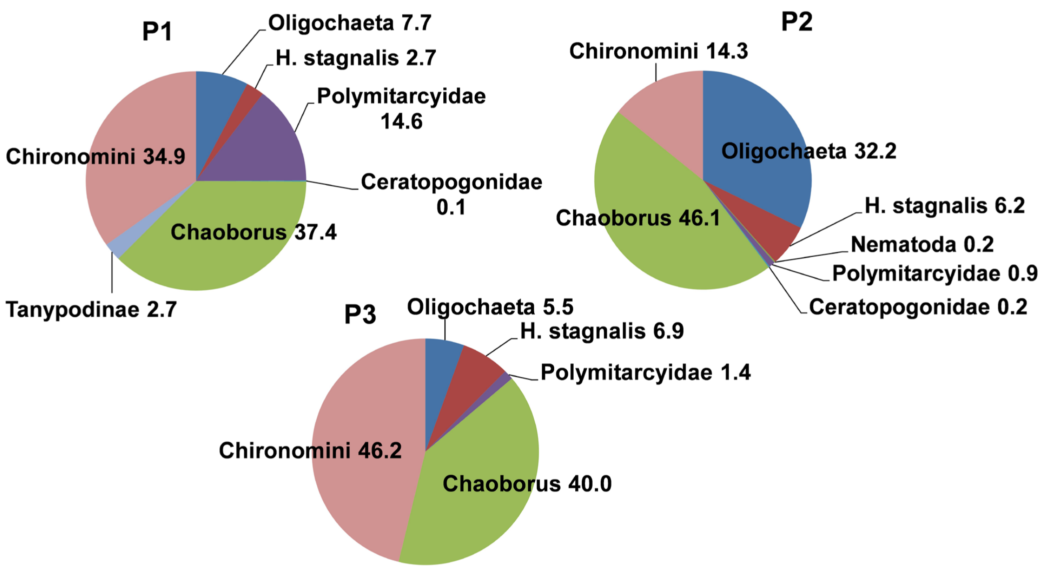

Figure 4. Relative abundances (%) of benthic macroinvertebrates by sampling site (P1 - Taquacetuba, P2 - Alvarenga and P3 - Biguás Island) at Billings Reservoir (Brazil, São Paulo State).

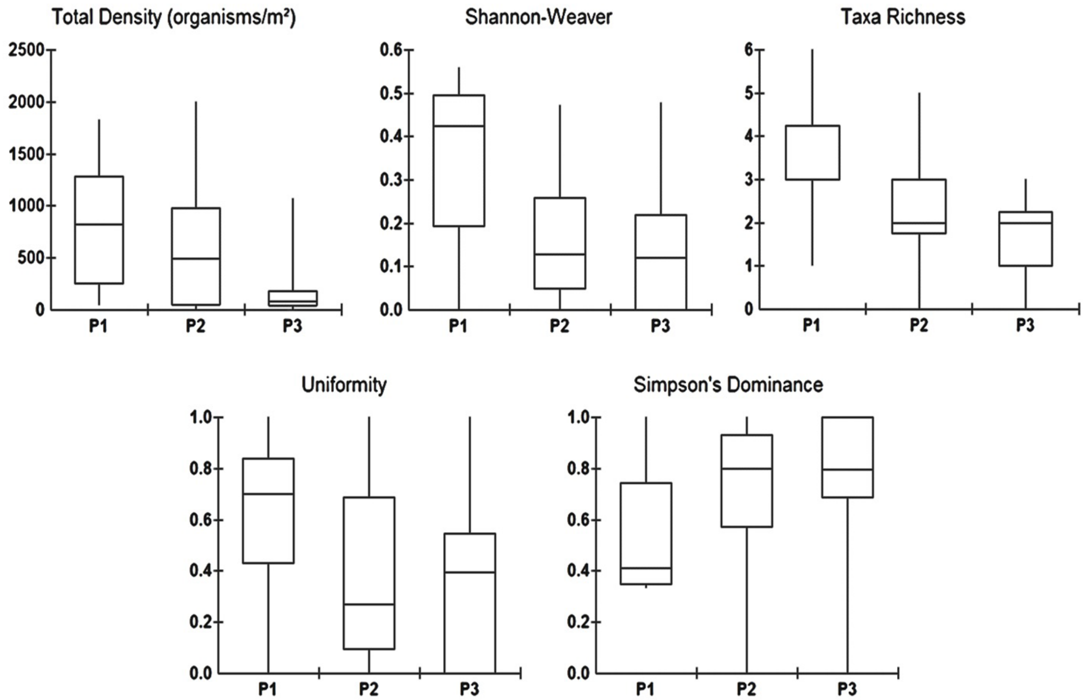

Figure 5. Biotic indices by sampling site (P1 - Taquacetuba, P2 - Alvarenga and P3 - Biguás Island) in Billings Reservoir (Brazil, São Paulo State), represented by box-and-whiskers plots, where the lower and upper limits of the box correspond to the 25th and 75th percentiles, respectively, the whiskers correspond to the minimum and maximum, and the center line of distribution corresponds to the median.

all the samples showed low values of total density (lower than 2000 org·m^{-2}), Shannon-Weaver diversity (lower than 0.56), and taxa richness (lower than 6). The medians of the uniformity values were lower than 0.5 at P2 and P3, and the medians of the dominance values were higher than 0.5 at P2 and P3.

The Kruskal-Wallis test (H) and *a posteriori* multiple comparisons using the Student-Newman-Keuls test (Table 4) show significant differences (p<0.05) for the total densities between P1 and P3, the Shannon-Weaver diversity between P1 and P2 and between P1 and P3, and the taxa richness between P1 and P3. This analysis therefore demonstrates that P1 significantly differs from the other sites.

The multimetric benthic community index, BCI (CETESB, 2013), which is calculated using the Shannon-Weaver diversity and taxa richness values, classified P1 as regular and the other sites as bad (Table 5).

3.5. Canonical Correspondence Analysis (CCA)

The results of the CCA are shown in Figure 6. The first two axes explain 88.82% of the total variability in the analyzed data. The substantial overlap of the dispersion of the data clouds (convex hull) of the three sites indicates that they have similar conditions in relation to water quality and the composition of the zoobenthos. However, P1 showed better water quality, indicated by its greater association with dissolved oxygen and Polymitarcyidae.

4. Discussion

All of the sites sampled in this study are in a high degree of eutrophication and an accelerated process of degradation. The benthic community showed relative abundance values of organisms that are tolerant or semi-tolerant to environmental impacts of higher than 76% at P1 and higher than 97% at the other sites, indicating environmental stress and a high degree of eutrophication. The high values of TN and TP, which were in non-compliance with CONAMA (2005) Resolution #357, also indicated a high degree of eutrophication.

The sensitive taxa of the order Ephemeroptera and family Polymitarcyidae were fifteen times more abundant at P1 than P2 and ten times more abundant at P1 than P3, indicating that P1 has better environmental conditions, which was corroborated by higher DO values. Most species of Ephemeroptera show a preference for apparently clean water with high concentrations of oxygen (Wetzel, 1993).

Better environmental conditions at P1 were also indicated by the benthic community indices,

Table 4. Results of Kruskal-Wallis test (H) and subsequent Student-Newman-Keuls test (SNK) for biotic indices among the sampling sites.

	Kruskal-Wallis		Student-Newman-Keuls		
	H	*p*	Sampling sites compared	SNK	*p*
Total Density	**8.7737**	**0.0124***	P1 and P3	**12.625**	**0.0033***
Shannon-Weaver	**8.5848**	**0.0137***	P1 and P2	**9.9583**	**0.0206***
			P1 and P3	**11.6667**	**0.0067***
Taxa Richness	**9.5706**	**0.0137***	P1 and P3	**12.75**	**0.003***
Uniformity	4.5128	0.1047			
Simpson's Dominance	3.8303	0.1473			

P1 - Taquacetuba, P2 - Alvarenga and P3 - Biguás Island at Billings Reservoir (Brazil, São Paulo State), where * indicates significant difference ($p<0.05$).

Table 5. Results of the benthic community index (BCI) based on the diversity and richness (average among samples by site).

Benthic Community Index	Shannon-Weaver	Taxa Richness
P1: REGULAR	1.16	3.58
P2: BAD	0.53	2.33
P3: BAD	0.44	1.75

Benthic Community Index criterion (CETESB, 2013)

Classification	Value for weighting (by arithmetic average)	Shannon-Weaver (H')	Taxa Richness (S)
GREAT	1	H' > 2.50	S ≥ 21
GOOD	2	2.50 ≥ H' > 1.50	20 > S > 14
REGULAR	3	1.50 ≥ H' > 1.00	6 > S > 13
BAD	4	1.0 ≥ H'	5 ≥ S

P1 - Taquacetuba, P2 - Alvarenga and P3 - Biguás Island at Billings Reservoir (Brazil, São Paulo State).

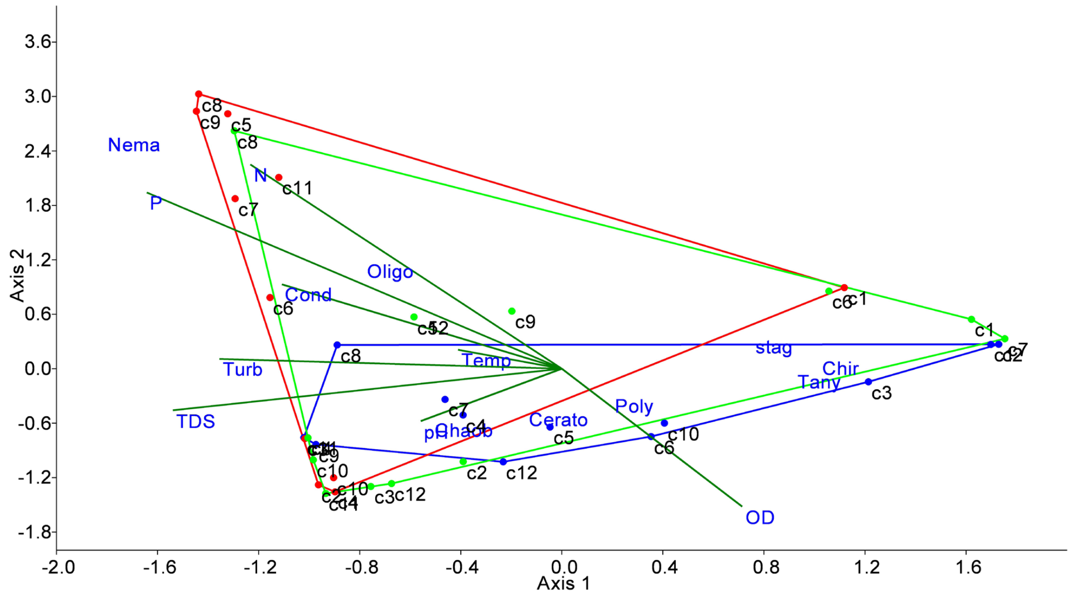

Figure 6. Results of canonical correspondence analysis (CCA) to verify the ordering of the sampling sites in relation to the intensity of the association among the abiotic and biotic variables. The numbers represent the collections (samples): C1 (17.04.12), C2 (22.05.12), C3 (26.06.12), C4 (26.06.12), C5 (13.09.12), C6 (24.09.12), C7 (06.11.12), C8 (04.12.12), C9 (18.12.12), C10 (17.01.13), C11 (05.03.13) and C12 (26.03.13). The lines (convex hull) indicate the cloud (data points) represented by the measurements at each site: P1 - Taquacetuba (blue), P2 - Alvarenga (red) and P3 - Biguás Island (green) in Billings Reservoir (Brazil, São Paulo State). The vectors (dark green) show the contributions of the variables pH, temperature (Temp), electrical conductivity (Cond), turbidity (Turbz), total dissolved solids (TDS), total nitrogen (N), total phosphorus (P) and dissolved oxygen (DO), and the Chironomini (Chir), Tanypodinae (Tany), Chaoborus (Chao), Ceratopogonidae (Cerato), Polymitarcyidae (Poly), Nematoda (Nema), *Helobdella stagnalis* (stag) and Oligochaeta (Oligo) in the formation of the axes.

which showed higher values of Shannon-Weaver diversity and taxa richness at this site. This analysis is corroborated by the results of the multimetric benthic community index (BCI), which classified P1 as regular and the other sites as bad.

The relative abundance of Oligochaeta at P2 was four times higher than at the other sites; these organisms are very resistant and are able to survive under oxygen depletion (Heller & Ehrlich, 1995), being associated with eutrophic water (Lang, 1990), again indicating the poor environmental conditions at this site. In a study carried out by Pamplin et al. (2006) at Americana Reservoir, which shows hypereutrophic characteristics, 73% of the invertebrates collected were oligochaetes.

The abiotic variables confirm the worst water quality at P2, the site with the most accelerated eutrophication. This site had significantly higher values of electrical conductivity, total phosphorus and lower dissolved oxygen concentrations than the other sites and was the only site with dissolved oxygen values in non-compliance with current legislation in Brazil (CONAMA, 2005, #357). Cardoso-Silva et al. (2014) found values of electrical conductivity close to those found in this study at Taquacetuba and higher values at Pedreira, a location near Alvarenga and under the influence of the pumping of the Pinheiros River. The same author found values of DO in non-compliance with current legislation in Brazil at Pedreira, showing the worst conditions at sites under the influence of the pumping of the Pinheiros River.

The PCA showed that P2 had a greater association with abiotic variables indicative of environmental stress, and the trophic state index indicated a high degree of eutrophication at all sites with worse conditions at P2 (hypereutrophic) compared to the other sites (supereutrophic). Various authors associate the worst conditions at Alvarenga to deficiencies in sewage collection (Moschini-Carlos et al., 2009; Cardoso-Silva et al., 2014).

The study of Hortellani et al. (2012), conducted at Billings Reservoir fishing sites, corroborates this study, demonstrating that the Billings Reservoir has a high degree of degradation with accelerated eutrophication conditions in addition to being contaminated by metals in sediment. Alvarenga proved to be the site with the highest degree of eutrophication and higher metal contamination in sediment, which directly reflects the quality of the water and fish. Hortellani et al. (2012) mention that Alvarenga is a critical area for the accumulation of metals in sediment and also that a slight increase in mercury at Taquacetuba and Biguás Island occurs during the rainy period.

The abiotic assessment differentiated Alvarenga (P2) from the other sites, showing that this site had the highest degradation of water quality compared to the other sampled sites. On the other hand, the macroinvertebrate analyses improved the assessment of the water quality, setting apart the sites with higher values of dissolved oxygen, especially Taquacetuba (P1), where sensitive taxa occurred. Therefore, in conclusion, the benthic community in association with abiotic metrics proved to be a useful tool as an indicator of environmental conditions. The results indicated that the fishing activity at Billings Reservoir must be done with caution at the three sampling sites, but fishing is especially not recommended at Alvarenga due to the worst water quality occurring at this site, as shown by the values of the abiotic variables, the TSI, and the higher abundance of tolerant organisms.

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