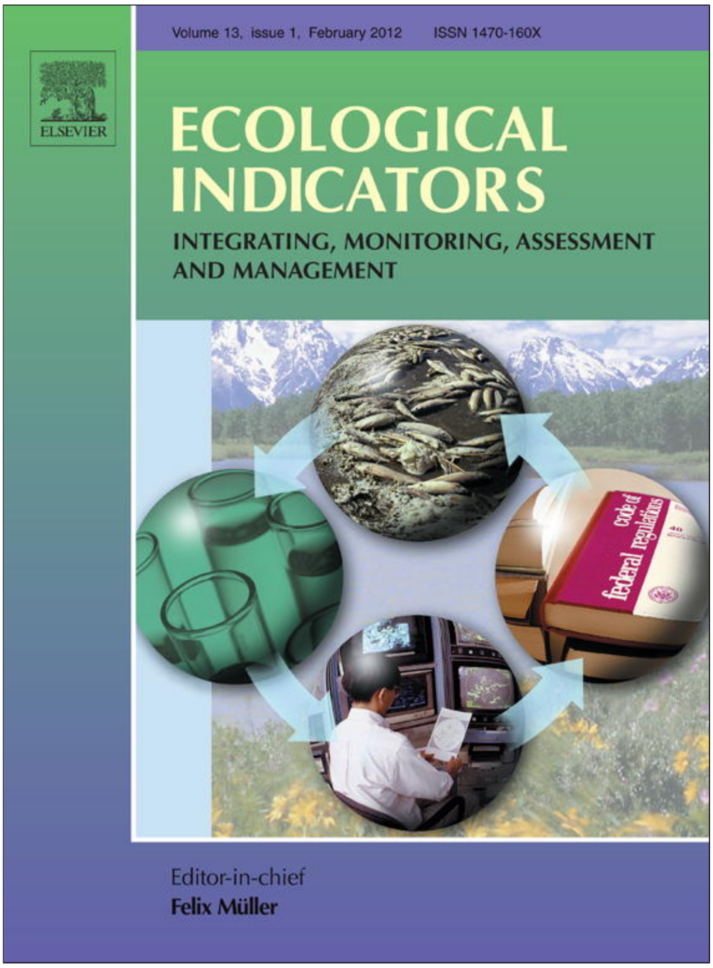

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Original article

Development and test of a statistical model for the ecological assessment of tropical reservoirs based on benthic macroinvertebrates

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ABSTRACT

Reservoirs are heavily modified lentic ecosystems. In spite of their differences from natural lakes, it is important to maintain and improve their chemical and ecological status. In the present study, we tested the value of an assessment tool based on the structure of benthic macroinvertebrate communities, to evaluate the Ecological Potential (EP) of tropical reservoirs. We designed a conceptual assessment scheme based on the Reference Condition Approach, and developed a statistical model based on 28 sites classified as having Maximum Ecological Potential, localized in the reservoir of Serra Azul, Minas Gerais, Brazil. Sixty-two disturbed sites from three reservoirs were used to test the model. A classification system based on three EP classes was found to be the best option, and tracked different levels of total dissolved solids, turbidity, total nitrogen and trophic status. This study confirmed the utility of benthic macroinvertebrates as an indicator group of biological quality in reservoirs, and the statistical model applied was effective in providing a measure of the ecosystem health of the reservoirs. As a further improvement, the level of taxonomic resolution for certain groups such as chironomids could be increased, because knowledge of the species composition may provide a better discrimination of intermediate degradation levels.

1. Introduction

Nowadays, most biomonitoring methods of aquatic systems are based on the Reference Condition Approach (Reynoldson et al., 1997; Directive, 2000/60/EC, 2000; Bailey et al., 2004; Stoddard et al., 2006). In accordance with the Reference Condition Approach (Reynoldson et al., 1997, 2001; Bailey et al., 2004; Stoddard et al., 2006), which is currently used worldwide, the integrity of communities found in one location should be analyzed according to the diversion they represent to expected communities in the absence of anthropogenic disturbances (Ruse, 2010; Hawkins et al., 2010). Following this principle, the first predictive models were developed for rivers based on macroinvertebrate communities (Wright et al., 1996; Reynoldson et al., 1995, 1997; Wright, 2000). These predictive methods, such as the RIVPACS (River Invertebrate Prediction Classification System; Wright, 2000; Clarke, 2000; Reynoldson and Wright, 2000), BEAST (Benthic Assessment of Sediment; Reynoldson et al., 1995, 2000) and ANNA (Assessment by Nearest Neighbour Analysis; Linke et al., 2005) allow for the direct classification of water quality and are used to monitor the quality of a site over time. More recently, other biological elements have been used in the development of models (e.g., Joy and Death, 2002; Kennard et al., 2006; Feio et al., 2007a), and the methodology has also been adapted to other systems, such as lakes and swamps (e.g., Johnson and Sandin, 2001; Rennie et al., 2005; Tall et al., 2008).

Traditionally, the environmental status of reservoirs in lakes has been assessed mostly through physical and chemical parameters and chlorophyll *a* (e.g., Canfield and Bachmann, 2001; Carrillo et al., 2003) since the main concern was to avoid algae blooms and maintain a reasonable water quality for domestic and agricultural purposes. In Brazil, some indices based on chemical parameters are presently used, such as the Water Quality Index (WQI) (CETESB, 2005) and the Trophic State Index (TSI) (Carlson, 1977 modified by Toledo et al., 1993), but biological elements are not considered. Recently in Europe, and especially following the development of the European Water Framework Directive (WFD; Directive, 2000/60/EC, 2000), phytoplankton community-based approaches began to be developed (e.g., Elliott et al., 2005; Cabecinha et al., 2009), and other studies have focused on the potential of using fish communities as bioindicators for reservoirs (Adams et al., 1999; Terra and Araújo, 2011).

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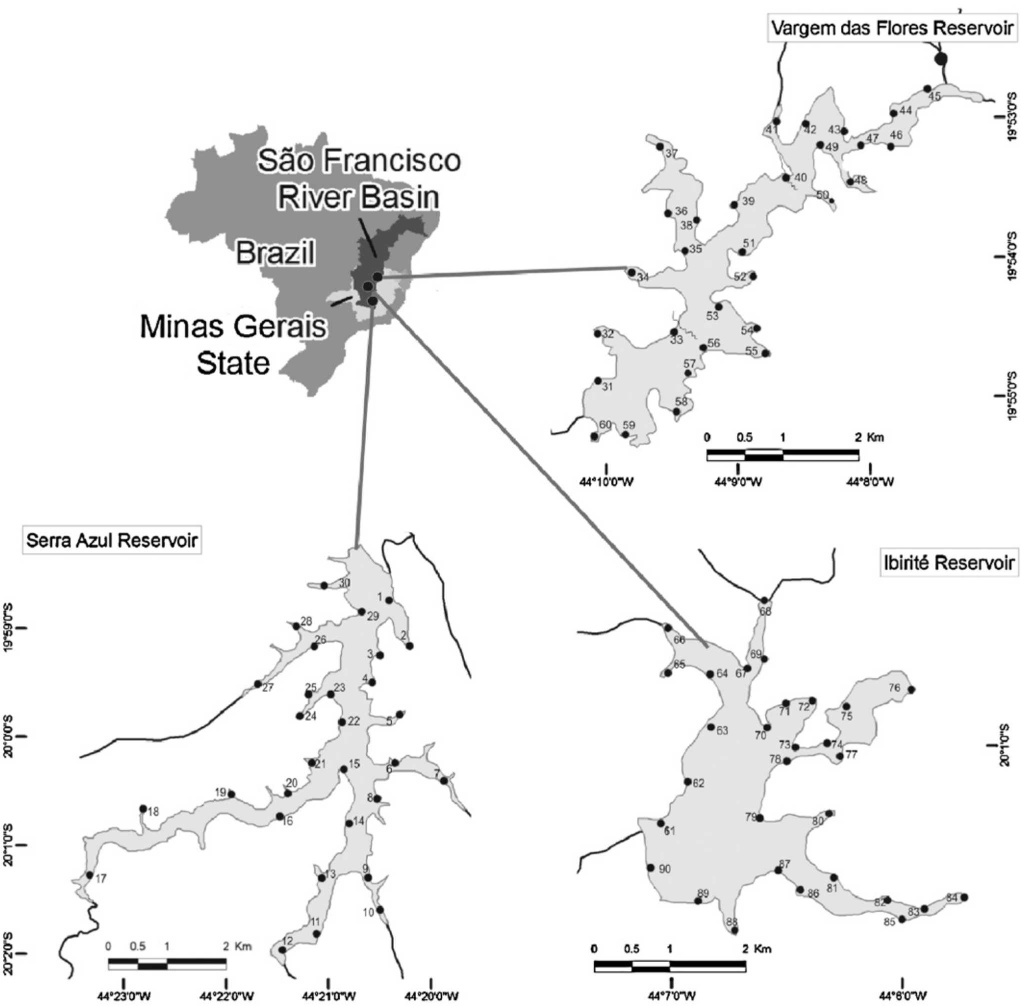

Fig. 1. Location of the reservoirs Vargem das Flores, Ibirité and Serra Azul in the catchments of the Paraopeba River, Minas Gerais, Brazil and distribution of the sampling sites in the reservoirs, and respective codes.

Freshwater macroinvertebrates are widely used as bioindicators in running waters, since these organisms have limited mobility, are more sensitive to local disturbances than pelagic organisms, are capable of detecting structural changes and habitat loss, and different species have different degrees of stress tolerance (Hellawell, 1977; De Pauw and Vanhooren, 1983; Karr, 1991; Barbour et al., 1996). Recently some efforts have been also made, in temperate zones, to develop biological evaluation tools for lakes and reservoirs based on benthic invertebrates (Johnson and Sandin, 2001; Blocksom et al., 2002; Martin and Rippey, 2008; O'Toole et al., 2008; Peeters et al., 2009).

The aims of the present study were: (1) to demonstrate that benthic macroinvertebrate communities in littoral areas (in shallow waters) are a useful biological indicator of the degradation of tropical reservoirs; and (2) to propose and test a conceptual scheme and a statistical model for this evaluation, based on the Reference Condition Approach.

The approach proposed here follows closely the Canadian BEAST models (Reynoldson et al., 1995, 1997), since it considers that a site is disturbed when its community is dissimilar from the communities found in reference sites or, in this case, with Maximum Ecological Potential (MEP), even though the statistical methods are different. Our model was based on 28 sites that were previously classified as having Maximum Ecological Potential (Molozzi, 2011), from three reservoirs in the metropolitan region of Belo Horizonte, Minas Gerais State, Brazil. Additionally, 62 disturbed sites from these reservoirs were used to test if the model explains the existing gradient of environmental degradation caused by human activities.

2. Materials and methods

2.1. Study area

This study was based on data collected at 90 sites distributed through three reservoirs (Serra Azul, Vargem das Flores and Ibirité) located in the Paraopeba River watershed, an affluent of the São Francisco River in Minas Gerais State, southeastern Brazil. In this region the climate is tropical sub-humid (Cwb), with summer rains (October to March) and a dry winter (April to September). The mean annual temperature is ca. 20 °C (Moreno and Callisto, 2006) (Fig. 1).

Serra Azul reservoir (19°59′24.92″S; 44°20′46.74″W; sampling sites 1–30), located at an altitude of 760 m, has a water surface of 7.5 km^2, water volume of 88,000,000 m^3 and a maximum depth of 40 m. It has been operating for approximately 30 years as a source of drinking water for the metropolitan region of the state capital, Belo Horizonte (ca. 4.8 million people). The reservoir has a hydraulic retention time of 351 days. Surrounding this reservoir is an environmental protection area established in 1980, with an area of 27,000 ha. Inside this area, 3.2 ha belong to COPASA (2004) (Companhia de Saneamento de Minas Gerais), the water company that manages the reservoir (Decree 20.792 on 07/08/80), and no recreational activities or fishing are allowed.

Vargem das Flores reservoir (19°54′25.06″S; 44°09′17.78″W; sampling sites 31–60) has a surface area of 4.9 km^2, contains 37,000,000 m^3 of water and has a maximum depth of 18 m. The maximum height of the sill spillway is 837 m and the reservoir has a hydraulic retention time of 365 days. An area of about 12.3 ha of the area around the reservoir was transformed into a state environmental protection area in 2006 (Decree 20.793 on 07/08/80).

The Ibirité reservoir (20°01′13.39″S; 44°06′44.88″W; sampling sites 61–90) was constructed in 1968 at an altitude of 773 m. This reservoir has an area of 2.8 km^2, a volume of 15,423,000 m^3 and a mean depth of 16 m. The hydrographic basin of the Ibirité Reservoir extends over two municipalities, Ibirité (148,535 inhabitants) and Sarzedo (23,282 inhabitants).

2.2. Macroinvertebrate sampling

The macroinvertebrate samples were collected in 90 sites located in the littoral zone of the three reservoirs. Samples were collected quarterly over a period of two years, 2008 and 2009 (March, June, September, December) with an Ekman–Birge dredge (0.0225 m^2), in the littoral area. The material collected was fixed in 70% formaldehyde and subsequently identified to family, or genus level in the case of Chironomidae (Peterson, 1960; Merritt and Cummins, 1996; Mugnai et al., 2010; Trivinho-Strixino, 2011).

2.3. Abiotic data

On each sampling occasion, and for each sampling site, the following water physical and chemical parameters were measured, using a YSI model Multiprobe: electrical conductivity, turbidity, total nitrogen (TN), and pH. In addition, groundwater samples were collected with a Van Dorn-type cylinder for subsequent measurement of total phosphorus (TP) and orthophosphates (PO_4), in accordance with “Standard Methods for the Examination of Water and Wastewater” (APHA, 1992). The concentration of chlorophyll *a* (Chla) was obtained according to Golterman et al. (1978). Transparency was estimated using a Secchi disc (S).

The Carlson (1977) trophic state index (TSI1), modified by Toledo et al. (1993), and the Trophic State Index proposed by CETESB (2000) (TSI2) were calculated for all sites. Each index is composed by sub-indices, which are then weighted to obtain a final value of the trophic status. The TSI1 is calculated through the formula:

$$\text{(a)}\quad \mathrm{TSI1} = \mathrm{TSI(S)} + 2 * \left[\frac{\mathrm{TSI(TP)} + \mathrm{TSI(PO_4)} + \mathrm{TSI(Chla)}}{7}\right],$$

and the sub-indices are obtained as follows:

$$\mathrm{TSI(S)} = 10 * \left(\frac{6 - (0.64 + \ln S)}{\ln 2}\right)$$

$$\mathrm{TSI(TP)} = 10 * \left(\frac{6 - \ln(80.32/\mathrm{TP})}{\ln 2}\right)$$

$$\mathrm{TSI(PO4)} = 10 * \left(\frac{6 - (\ln(21.67/\mathrm{PO4})}{\ln 2}\right)$$

$$\mathrm{TSI(Chla)} = 10 * \left(\frac{6 - (2.04 - 0.695 \ln \mathrm{Chla})}{\ln 2}\right)$$

The TSI2 is calculated through the formula:

$$\text{(b)}\quad \mathrm{TSI2} = \frac{\mathrm{TSI(TP)} + \mathrm{TSI(Chla)}}{2},$$

and the sub-indices are obtained through the expressions:

$$\mathrm{TSI(TP)} = 10 * \left(\frac{6 - ((1.77 - 0.42) * \ln(TP)}{\ln 2}\right)$$

$$\mathrm{TSI(Chla)} = 10 * \left(\frac{6 - (0.92 - 0.34) * \ln \mathrm{Chla}}{\ln 2}\right)$$

TSI1 values ranging from 0 to 44 correspond to oligotrophic, 44–54 to mesotrophic, and >54 to eutrophic waters. TSI2 values ranging from 0 to 23 correspond to ultraoligotrophic, 24–44 to oligotrophic, 44–54 to mesotrophic, 54–74 to eutrophic, and >74 to hypereutrophic conditions.

The relative abundance of gravel/boulders, coarse sand, and silt/clay/muck substrate types, according to Suguio (1973), in the bottom of the reservoir and silt/clay/muck near the shore was also assessed at each site, according to diversity of habitats protocol proposed by Baker et al. (1997) and USEPA (2007), as the granulometric data is needed to determine the typology of test sites (see below; variables described in Table 1).

2.4. Maximum Ecological Potential sites

Twenty-eight sites were previously classified as Maximum Ecological Potential (MEP; Molozzi, 2011). All of the sites with MEP come from Serra Azul Reservoir, which is located in a protected area with an area of 27.200 ha, mostly covered with native vegetation, and where no recreational or fishing activities are allowed. The potential MEP sites were initially checked against human degradation indicators (pressure variables) and those selected presented the lowest levels of human disturbance, based on the evaluation of land use (e.g., % of land occupied by roads, crops, pastures, buildings), water chemistry and physics (e.g. Total N, Total P, Chorophyll a, Trophic indices) in the littoral, transition zone and riparian zone. The communities of the selected sites were statistically different from those of non-MEP sites (checked through multivariate methods – Multidimensional Scaling Analysis and ANOSIM; Molozzi, 2011), which assured us that their biological communities could be used as a benchmark in the evaluation of other sites.

Subsequently these MEP sites were split into two types of sites, based on their biological communities which corresponded to different natural abiotic characteristics: in G1 the community is dominated by the Diptera *Chaoborus*, *Djalmabatista*, *Procladius* and *Polypedilum* and the sites have a higher percentage of coarser substrates (gravel, boulders and coarse sand) that those of G2 (Table 1). In G2, the Diptera Ceratopogonidae, Tanypus, Coelotanypus, Ablabesmyia and Fissimentum dominate the community.

Table 1 shows the mean values for typological variables, those that best discriminate the two groups of sites with MEP (96% correct discrimination after cross-validation), according to a previous study (Molozzi, 2011).

2.5. Data analysis

2.5.1. Model construction and assessment of test sites

The conceptual approach followed is shown schematically in the flow diagram (Fig. 2). The first step consists of building a classification system based on the within-groups Bray–Curtis dissimilarity of the MEP-site communities, using the SIMPER routine (Clarke and Warwick, 2001; Primer 6). Biological data were previously averaged by site and transformed by square root. The remaining similarity gradient was divided by two or three equal intervals, in order to obtain a 3- or 4-class quality-assessment system, to test for the best classification system. The class intervals are presented in dissimilarity percentages to MEP groups (100-similarity). The 4-class quality system, also used by Reynoldson et al. (1997) and Feio

Table 1
Environmental variables used in the present study. The pressure variables were previously used to select the Maximum Ecological Potential sites (MEP; Molozzi, 2011) and here to evaluate the sensitivity of the models to anthropogenic disturbance. The discriminant variables were used to determine test sites type. The mean values (±SD) for pressure and discriminant variables of the two MEP types (G1, G2) are also indicated.

Environmental variables	Description and source	G1 – Mean (SD)	G2 – Mean (SD)
Pressure variables			
Total dissolved solids (mg L^{-1})	Field measurement (YSI)	134.07 ± 75.48	75.05 ± 62.92
Chlorophyll a (μg L^{-1})	Analysis according to Golterman et al. (1978)	28.39 ± 32.76	10.71 ± 19.51
Total nitrogen (μg L^{-1})	Analysis according to APHA (1992)	0.25 ± 0.17	0.15 ± 0.09
Total phosphorus (mg L^{-1})	Analysis according to APHA (1992)	116.75 ± 170.85	36.64 ± 32.85
P-ortho (μg L^{-1})	Analysis according to APHA (1992)	19.12 ± 43.88	7.64 ± 1.46
Colour of bottom substrate	Field observation, categories: 1(brown), 2(black), 3(gray), 4(red), 5(other), USEPA (2007)	1	1
Odour of bottom substrate	Field observation, categories: 1(none), 2(H2S), 3(anoxic), 4(oil), 5 (chemical), 6(other) - USEPA (2007)	1	1
TSI1	Analysis based on Carlson (1977), modified by Toledo et al. (1983)	59.18 ± 15.28	48.06 ± 12.20
TSI2	Analysis based on CETESB (2000)	48.33 ± 11.93	37.85 ± 6.00
Buildings (%)	Field observation, categories: 1 = absent (0%), 2 = sparse (10%), 3 = moderate (10–40%), 4 = heavy (40–75%), 5 = very heavy (>75%), USEPA (2007)	0	0
Commercial buildings (%)	Idem	0	0
Docks/boats (%)	Idem	0	0
Dykes (%)	Idem	0	0
Landfills (%)	Idem	0	0
Roads (%)	Idem	0	0
Power lines (%)	Idem	0	0
Row crops (%)	Idem	0	0
Pasture (%)	Idem	0	0
Agriculture (%)	Idem	0	0
Characterization variables			
Gravel/boulders (2–4000 mm) – bottom (%)	Fraction % of a sediment sample collected with Eckman–Birge dredge (0.0225 m^2). Granulometry measured according to Suguio (1973)	6.16 ± 14.84	2.81 ± 11.53
Coarse sand (0.50–2 mm) – bottom (%)	Fraction % of a sediment sample collected with Eckman–Birge dredge (0.0225 m^2). Granulometry measured according to Suguio (1973).	6.13 ± 6.23	2.27 ± 3.64
Silt, clay or muck (<0.062 mm) – bottom (%)	Fraction % of a sediment sample collected with Eckman–Birge dredge (0.0225 m^2). Granulometry measured according to Suguio (1973).	40.25 ± 23.88	38.93 ± 17.49
Silt, clay or muck (0.062–2 mm) – shoreline (%)	Fraction % of a sediment sample collected with Eckman–Birge dredge (0.0225 m^2). Granulometry measured according to Suguio (1973).	1.33 ± 0.51	1.90 ± 0.30
Depth (m)	Field measurement (Sonar).	0.61 ± 0.23	0.63 ± 0.27

et al. (2007a,b), is composed by: class 1 (Equivalent to Maximum Ecological Potential), class 2 (Moderately Different from Maximum Ecological Potential), class 3 (Different from the Maximum Ecological Potential), and class 4 (Very Different from the Maximum Ecological Potential). Alternatively, we created a 3-class system where: class 1 is Equivalent to Maximum Ecological Potential, class 2 means that the site is Different from the Maximum Ecological Potential, and class 3 means that the site is Very Different. Each class corresponds, therefore, to an interval of similarity to the MEP group. Although for streams and rivers, 5- or 4-class systems are more common, we assumed that for naturally poor systems (for invertebrate communities) such as the reservoirs, this number of classes might be too large to show the results of disturbance on the communities.

The second step consists of determining the adequate type (subgroups of MEP) for each test site, in order to make the most appropriate comparison. In this case we have only two MEP groups defined for these tropical reservoirs, so, in practice we have two models, one for each type. This step is accomplished by running a complete discriminant analysis (Systat 13.0, Hair et al., 1998) based on abiotic data, with MEP and test sites, with the types as grouping variables and using as discriminating variables the typological variables, i.e., the abiotic variables that characterize and distinguish the reference groups (Table 1) according to previous work (Molozzi, 2011). The discriminant analyses returns the probabilities of membership for each test site. The type to which a higher probability of membership is attributed is the most adequate type for comparison (next step). Sixty-two disturbed sites (thirty from Vargem das Flores, thirty from Ibirite, and two Serra Azul reservoirs) were used to test the models.

In the third step, the biotic data from each test site are compared to the appropriate MEP subgroup (determined in step 2) by calculating their average Bray–Curtis dissimilarity through a SIMPER analysis (Clarke and Warwick, 2001; PRIMER 6 Version 6.0, Ltd. 2004). The dissimilarity between each test site and MEP subgroup was also visually inspected with a non-metric Multi-Dimensional

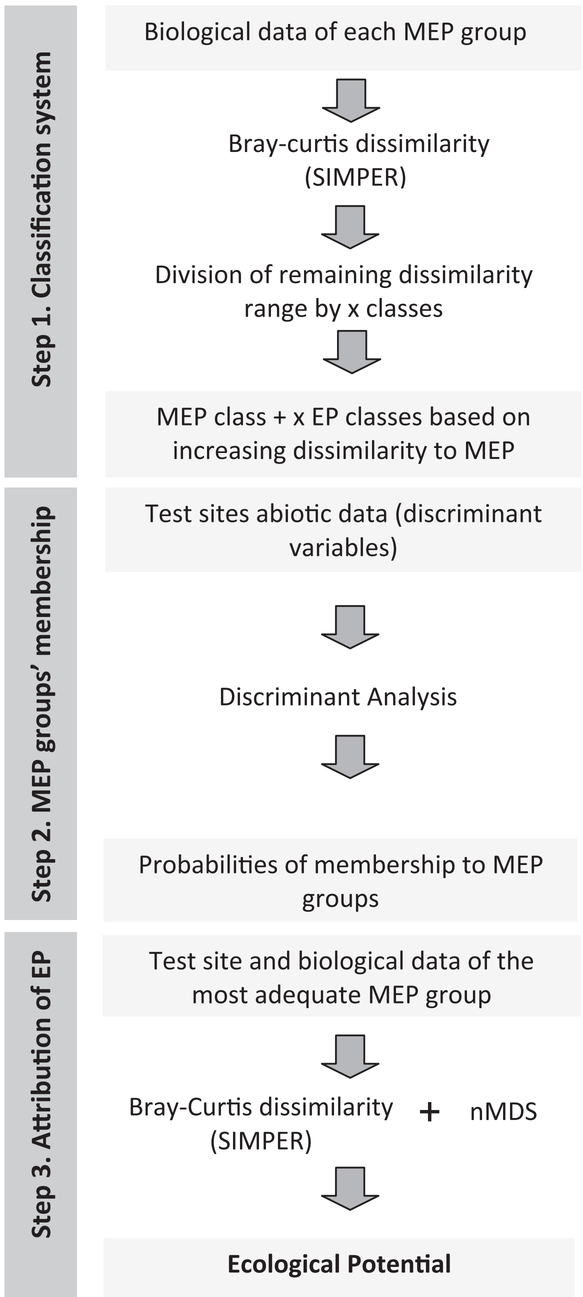

Fig. 2. Methodology used to develop the model for the ecological assessment of the reservoirs of Vargem das Flores, Ibirité and Serra Azul (Paraopeba River catchment, Minas Gerais, Brazil), using benthic invertebrates of region littoral. MEP stands for Maximum Ecological Potential and EP for Ecological Potential.

Scaling analysis (nMDS, PRIMER-6 Version 6.0, Ltd., 2004). Finally, a quality class is attributed to test sites according to their dissimilarity and according to the classification system constructed in step 1. For each test site we obtained, therefore, two quality classifications according to the system being tested (3 or 4 class quality system).

2.5.2. Evaluation of model response to anthropogenic disturbance

In order to determine which is the most useful quality system (3 or 4 classes), we repeated all the following tests for both systems. First, to check that the level of abiotic degradation is different between classes, we used a PERMANOVA test with 9999 permutations (Permutational Multivariate Analysis of Variance; Anderson, 2001a,b; Anderson and Braak, 2003; Anderson et al., 2008; software package PERMANOVA + for PRIMER, 2006, with normalized pressure data). This routine is a multivariate permutational non-parametric test, analogous to the univariate ANOVA. We also used the PERMANOVA to check that each quality class corresponded to similar levels of abiotic degradation classes when using the two different reference groups, i.e., if class 1 attributed by the model based on reference group A was similar in terms of abiotic degradation, to class 1 attributed by the model based on reference group B, and so on.

Using Box–Whisker plots, we evaluated graphically if there was a progressive increase of anthropogenic degradation of test sites with the increase of class, for each pressure variable measured, i.e., if sites with class 2 in fact showed a higher level of degradation than sites in class 1, and so on (Statistic 7.0).

Then, in order to see if the distribution of sites by quality classes corresponds to differences in overall disturbance, we performed a Canonical Analysis of Principal Components (CAP) on normalized pressure data (Clarke and Warwick, 2001) (PERMANOVA + for PRIMER, 2006). The CAP analysis provides a constrained ordination that maximizes the differences among *a priori* groups (Anderson and Braak, 2003), which in our case are the quality classes. It also shows the strength of the association between the multivariate data cloud (based on site pressures) and the hypothesis of differences between quality classes. Additionally it calculates the probability associated with differences between multivariate groups, in the form of a misclassification error using the “leave one out allocation of the observation groups” approach. We therefore used it to compare the percentage of correct classifications to the class attributed by the model, in the 3 and 4 class systems. Finally, to find which pressures best characterize the differences between classes, we superimposed vectors corresponding to Spearman correlations of individual pressures with the CAP axes.

3. Results

At the 90 sampling sites (reference and disturbed), 47 taxa (4 Mollusca, 2 Annelida and 41 Arthropoda), were collected over two years, and from those, 36 taxa were recorded (4 Mollusca, 2 Annelida and 30 Arthropoda) at the 62 test sites (Table 2). The complete discriminant analysis, based on the abiotic predictors (typological variables), attributed 34 test sites to G1, and 28 sites to G2. Five sites had a similar probability of belonging to the two groups and therefore, they were first ordinated (nMDS, not shown) with the MEP sites from both groups, and finally compared to the closest group (Table 4).

The average Bray–Curtis similarity (SIMPER analysis) of benthic macroinvertebrate communities was similar in both MEP subgroups (55.28–57% in G1–G2). These values were converted in average dissimilarity (100-average similarity) and the remaining dissimilarity gradient (from the reference sites dissimilarity to 100% of dissimilarity) was divided in 2 or 3 equal intervals, to form 3 or 4 quality classes (Table 3).

The calculation of Bray–Curtis dissimilarity between each test site and the respective MEP subgroup and subsequent allocation to the previously established classes resulted, for the 3-class quality system, in one site (1.6%) was classified as Equivalent to Maximum Ecological Potential (Class 1), 26 sites (58.2%) as Moderately Different from MEP (Class 2) and 25 sites (40.3%%) as Very Different from MEP (Class 3) (Table 4).

Using the 4-class quality system, one site (1.6%) was also classified as Equivalent to Maximum Ecological Potential (Class 1), 17 sites (27.4%) were classified as Moderately Different from MEP quality status (Class 2), 37 sites (59.7%) as Different from MEP (Class 3), and 7 sites (11.3%) were classified as Very Different from MEP (Class 4) (Table 4).

Table 2
List of taxa and respective abundance (number of individuals) and frequency (%) collected in the three reservoirs, at the 28 MEP sites and 62 test sites, during the two years of sampling.

Taxon	Maximum Ecological Potential ($n = 28$)		Test sites ($n = 62$)	
	Abundance (n)	Frequency (%)	Abundance (n)	Frequency (%)
Gatropoda				
Thiaridae – *Melanoides tuberculatus* Müller, 1774	62	2.62	3078	25.47
Planorbiidae – *Biomphalaria straminea* Dunker, 1848	0	0	18	0.14
Ampullariidae – *Pomacea haustrum* Reeve, 1856	0	0	10	0.08
Bivalvia				
Corbiculidae – *Corbicula fluminea* Müller, 1774	0	0	34	0.28
Annelida				
Hirudinea	17	0.71	167	1.38
Oligochaeta	81	3.42	3000	24.83
Ephemeroptera				
Polymirtacyidae	9	0.38	12	0.09
Baetidae	2	0.08	2	0.01
Leptoceridae	5	0.21	15	0.12
Odonata				
Gomphidae	12	0.50	6	0.04
Trichoptera				
Odontoceridae	0	0	1	0.008
Hydrophilidae	1	0.04	1	0.008
Philopotamidae	1	0.04	0	0
Hydrobiosidae	1	0.04	0	0
Coleoptera				
Elmidae	1	0.04	14	0.11
Acari				
Hydracarina	1	0.04	0	0
Diptera				
Chaoboridae – *Chaoborus* Lichetenstein, 1800	1345	56.84	2914	24.12
Simuliidae	0	0	8	0.06
Ceratopogonidae	31	1.31	36	0.29
Chironomidae				
Labrundinia Roback, 1987	1	0.04	0	0
Coelotanypus Kieffer, 1913	165	6.97	367	3.03
Ablabesmyia Johhansen, 1905	48	2.02	21	0.17
Djalmabatista Fittkau, 1968	73	3.08	27	0.22
Procladius Skuse, 1803	75	3.16	12	0.09
Tanypus Meigen, 1803	145	6.12	443	3.66
Chironominae				
Dicrotendipes Kieffer, 1913	0	0	2	0.01
Beardius Reiss & Sublette, 1985	0	0	1	0.008
Aedokritus Roback, 1958	6	0.25	480	3.97
Chironomus Meigen, 1803	25	1.05	1120	9.27
Cladopelma Kieffer, 1921	6	0.25	2	0.01
Cryptochironomus Kieffer, 1918	2	0.08	1	0.008
Fissimentum Cranston & Nolte, 1996	129	5.45	29	0.24
Goeldchironomus Fittkau, 1965	1	0.04	36	0.29
Harnischia Kieffer, 1921	0	0,00	0	0
Lauterboniella Lenz, 1941	7	0.29	0	0
Paralauterboniella Lenz, 1941	1	0.04	0	0
Pelomus Reiss, 1989	10	0.42	78	0.14
Polypedilum Kieffer, 1913	66	2.78	5	0.04
Stenochironomus Kieffer, 1919	1	0.04	0	0
Zavreliella Kieffer, 1920	14	0.59	0	0
Nilothauma Kieffer, 1921	1	0.04	1	0.008
Alotanypus Roback, 1971	2	0.08	0	0
Parachironomus Lenz,1921	1	0.04	2	0.01
Manoa Fttkau, 1963	1	0.04	0	0
Pseudochironomus Mallock, 1915	2	0.08	0	0
Tanytarsus van der Wulp, 1984	8	0.34	97	0.80
Cladotanytarsus Kieffer, 1924	0	0	1	0.008
Nimbocera Reiss, 1972	7	0.29	18	0.15

The PERMANOVAs demonstrated that: (1) for both 3- and 4-class quality systems, there are no differences between G1 and G2 for the same class (Pseudo-$F_{2.719} = 3.45$, $p = 0.592$, Pseudo-$F_{2.719} = 2.63$, $p = 0.548$, for 4- and 3-class quality systems, respectively); and (2) for both 3- and 4-class systems, there were significant differences ($p < 0.001$ and $p < 0.05$) between classes concerning the abiotic degradation of sites (Table 5).

The Box–Whisker plots showed increasing values of abiotic degradation for both 3- and 4-class classification systems. The clearest patterns were observed for similar pressure variables (total dissolved solids, turbidity, total nitrogen, TSI1 and TSI2) (Figs. 3 and 4).

The Canonical Analysis of Principal Components (CAP) confirmed that the distribution of sites by quality classes corresponds

Table 3
Quality class intervals, for 3- and 4-class classification systems, based on Bray–Curtis dissimilarity.

3-classes quality system			4-classes quality system		
	G1	G2		G1	G2
1. Equivalent to MEP	≤44.72	≤43.00	1. Equivalent to MEP	≤44.72	≤43.00
2. Different from MEP	44.73–72.36	43.01–71.50	2. Moderately Different from MEP	44.73–63.15	43.01–62.00
3. Very Different from MEP	>72.37	>71.51	3. Different from MEP	63.16–81.58	<62.01–81.00
			4. Very Different from MEP	>81.59	>81.01

Table 4
Attribution of test sites to their Maximum Ecological Potential (MEP) group of sites (type) and respective quality class, based on their dissimilarity % to the MEP group, according to 3- and 4-class quality assessment systems for all test sites.

Test sites	Group membership and % of similarity with MEP group (group,%)	Quality class (3-classes system)	Quality class (4-classes system)
17	G1, 52.07	2	2
29	G1, 41.29	1	1
31	G2, 56.33	2	2
32	G2, 58.90	2	2
33	G1, 56.93	2	2
34	G2, 68.90	2	3
35	G2, 49.97	2	2
36	G1, 63.25	2	3
37	G2, 65.22	2	3
38	G2, 64.61	2	3
39	G2, 56.45	2	2
40	G2, 63.79	2	3
41	G1, 69.49	2	3
42	G2, 69.96	2	3
43	G1, 74.70	3	3
44	G1, 67.32	2	3
45	G1, 77.42	3	3
46	G1, 70.30	2	3
47	G2, 61.55	2	2
48	G1, 68.61	2	3
49	G1, 68.84	2	3
50	G1, 75.70	3	3
51	G2, 57.09	2	2
52	G2, 52.42	2	2
53	G2, 57.36	2	2
54	G2, 60.49	2	2
55	G1, 74.08	3	3
56	G2, 79.70	3	3
57	G2, 56.14	2	2
58	G2, 56.15	2	2
59	G2, 71.17	2	3
60	G2, 58.98	2	2
61	G1, 72.66	3	3
62	G1, 68.97	2	3
63	G1, 66.03	2	3
64	G1, 77.55	3	3
65	G1, 74.85	3	3
66	G1, 82.35	3	4
67	G1, 68.74	2	3
68	G1, 80.72	3	3
69	G1, 90.80	3	4
70	G2, 60.16	2	2
71	G2, 59.40	2	2
72	G1, 72.58	3	3
73	G1,79.39	3	3
74	G1, 90.35	3	4
75	G1, 92.36	3	4
76	G1, 89.47	3	4
77	G1, 98.39	3	4
78	G2, 80.65	3	4
79	G2, 63.37	2	3
80	G1, 74.21	3	3
81	G2, 53.42	2	2
82	G1, 81.56	3	3
83	G1, 74.54	3	3
84	G2, 72.34	2	3
85	G2, 63.17	2	3
86	G1, 72.41	3	3
87	G1, 72.09	2	3
88	G1, 73.18	3	3
89	G2, 72.55	3	3
90	G2, 73.67	3	3

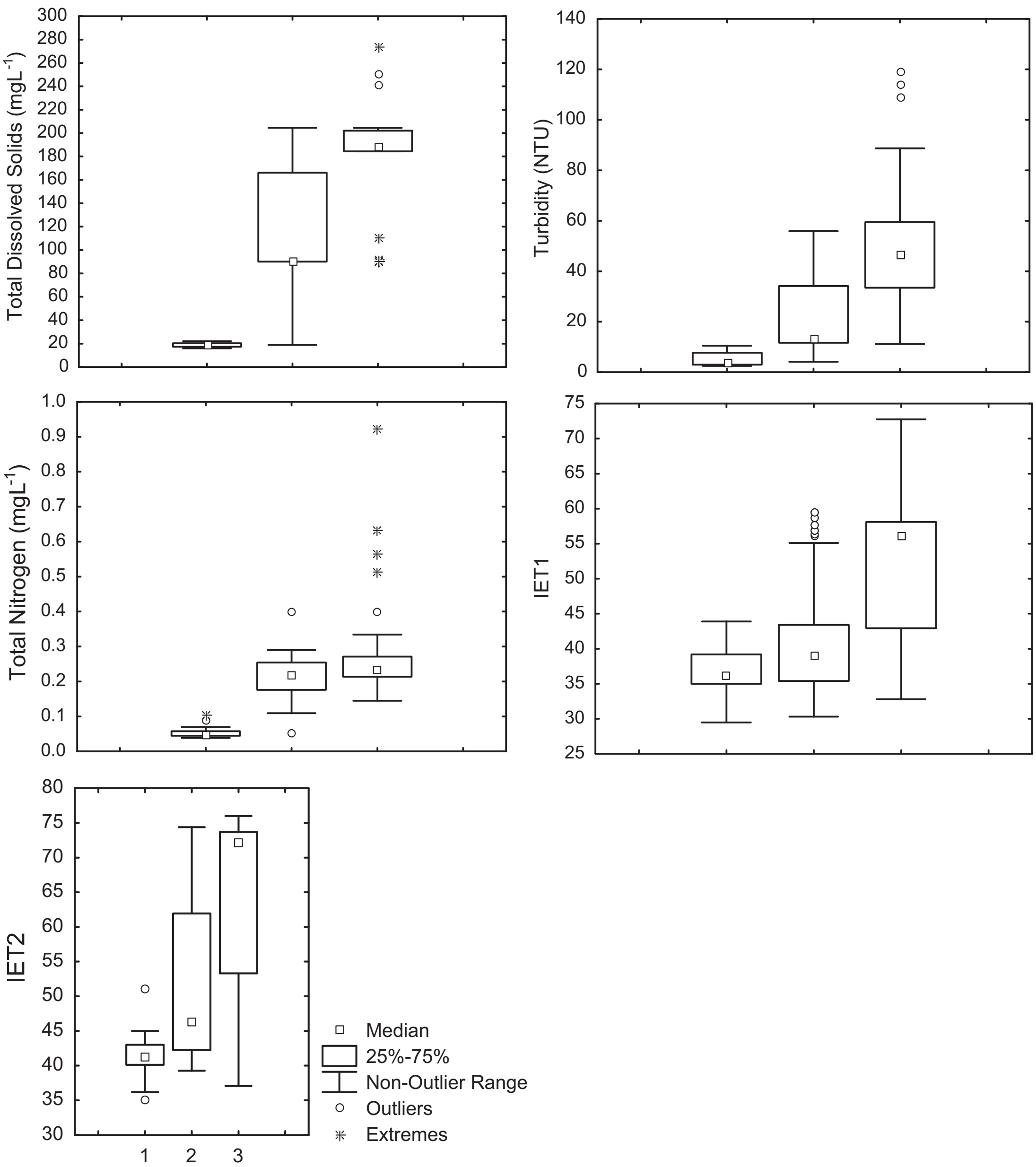

Fig. 3. Box–Whisker plots of the best responsive pressure variables (Total Dissolved Solids, Turbidity, Trophic State Indices 1 – TSI1 and 2 – TSI2, P-orthophosphates, Total Nitrogen), to the classes attributed by the models using the 3-classes quality system. Outliers are 1.5 times outside the 25th and 75th percentile.

to differences in overall disturbance. The first two canonical correlation axes showed good strength for the association between the multivariate patterns based on all pressure variables and the quality classes attributed by the 3-class system ($\delta 1 = 94\%$, $\delta 2 = 65\%$). The pressure variables total phosphorus, TDS, buildings (0.69, 0.28, 0.25, respectively) were better correlated with CAP axis 1, and the variables chlorophyll a, TSI2, P-ortho (−0.53, −0.48, −0.346 respectively) with axis 2 (Fig. 5). Regarding the 4-class system, the first two canonical correlation axes also showed very good strength for the association between the multivariate data in the Canonical Analysis of Principal Components (CAP) and the quality classes ($\delta 1 = 86\%$, $\delta 2 = 63\%$). The pressure variables chlorophyll a, buildings, roads (−0.56, 0.31, 0.37, respectively) were better correlated with CAP axis 1, and the variables total nitrogen, commercial buildings and TDS1 (−0.63, 0.46, 0.34, respectively) with axis 2 (Fig. 6).

Table 5
Results of PERMANOVA test (t-statistics for the pairwise comparison and p-significance level) for differences in pressure level between quality classes attributed to test sites by the two classification systems.

	3-Classes (t, P(perm))	4-Classes (t, P(perm))
1–2	4.94; 0.001	4.49; 0.001
1–3	6.48; 0.001	5.83; 0.001
1–4		7.63; 0.001
2–3	2.80; 0.001	1.47; 0.047
2–4		2.83; 0.001
3–4		2.56; 0.001

4. Discussion

In the present work, it was shown that predictive models based on macroinvertebrate communities can also be used for the bioassessment of reservoirs, as they respond well to the human degradation and that response can be translated into quality classes. Nevertheless, some adjustments from models used in

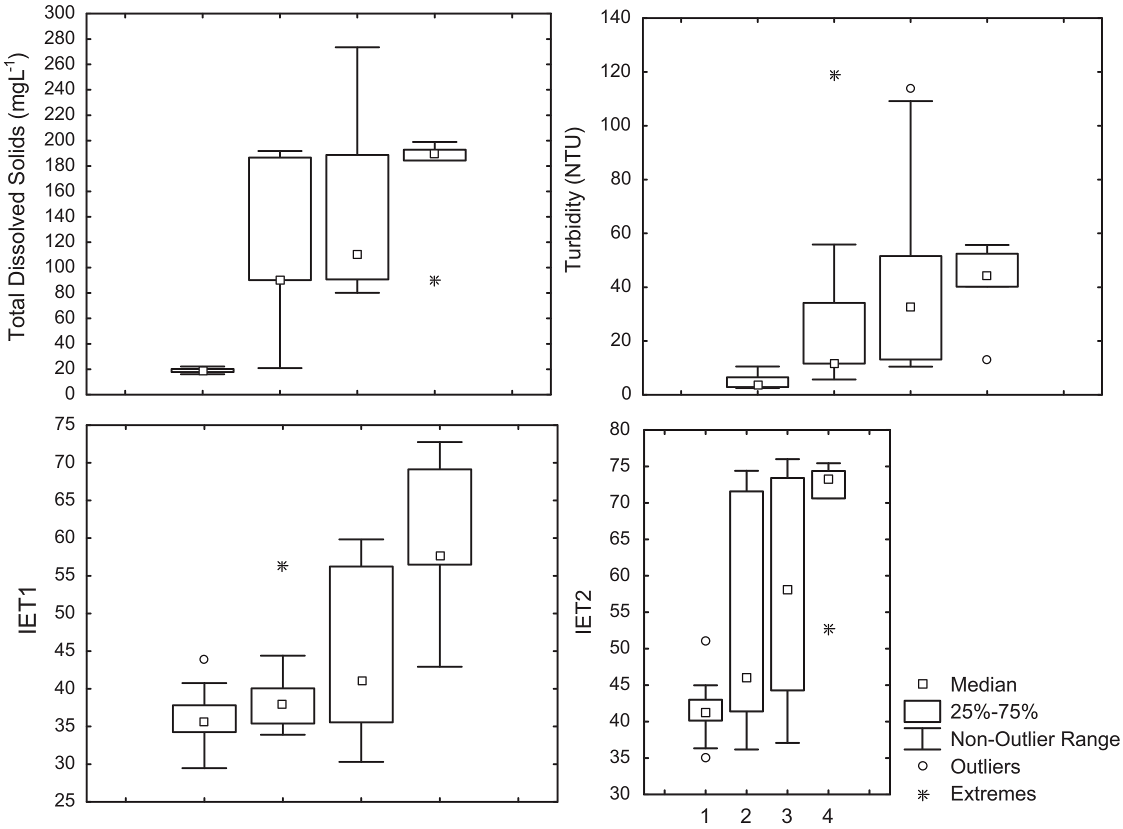

Fig. 4. Box–Whisker plots of the best responsive pressure variables (Total Dissolved Solids, Turbidity, Trophic State Indices 1 – TSI1 and 2 – TSI2, P-orthophosphates, Total Nitrogen), to the classes attributed by the models using the 4-classes quality system. Outliers are 1.5 times outside the 25th and 75th percentile.

streams and rivers bioassessment (e.g., Norris and Norris, 1995; Reynoldson et al., 2001; Feio et al., 2007b; Hawkins et al., 2010) are needed due to constrains imposed by the characteristics of these systems.

The first initial constrain in the models development, which is based on multivariate patterns of the fauna, could be the potentially low taxa diversity of the macroinvertebrate communities in the reservoirs. However, in the study sites it was recorded a relative high diversity of taxa (47). Still, the taxa could have a low sensitivity to human degradation that would not allow the detection of impairment. In fact, from the EPT (Ephemerotera, Plecoptera and Trichoptera), the traditional bioindicator groups, only three Ephemerotera and four Trichoptera families were detected (Table 2). Moreover, the last are rare taxa (only one individual) and have therefore little influence in the similarity calculations. From the remaining, only the Leptoceridae is considered sensitive taxa and may have contributed to the detection of human disturbance in our models. So, the discrimination ability of our models was mainly due to the 29 genus of the Chironomidae family. In opposition to rivers bioassessment, in reservoirs their use seems to be particularly important for bioassessment. The model sensitivity

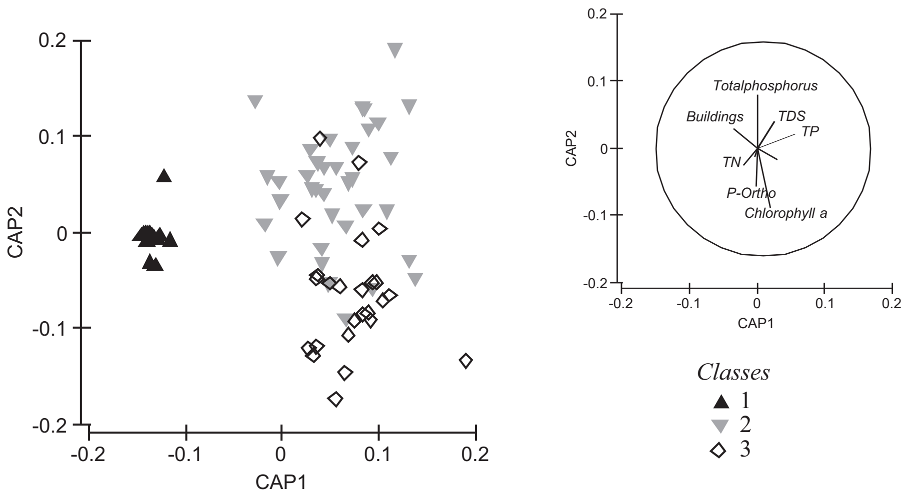

Fig. 5. Canonical analysis of principal coordinates analysis (CAP) showing the distribution of sites according to their general degradation and respective classification, using the 3 classes-system. The top right image shows the vector overlay of Spearman rank correlations of individual pressures variable vectors with the CAP axes, which indicate which are the pressures responsible to the distribution of sites.

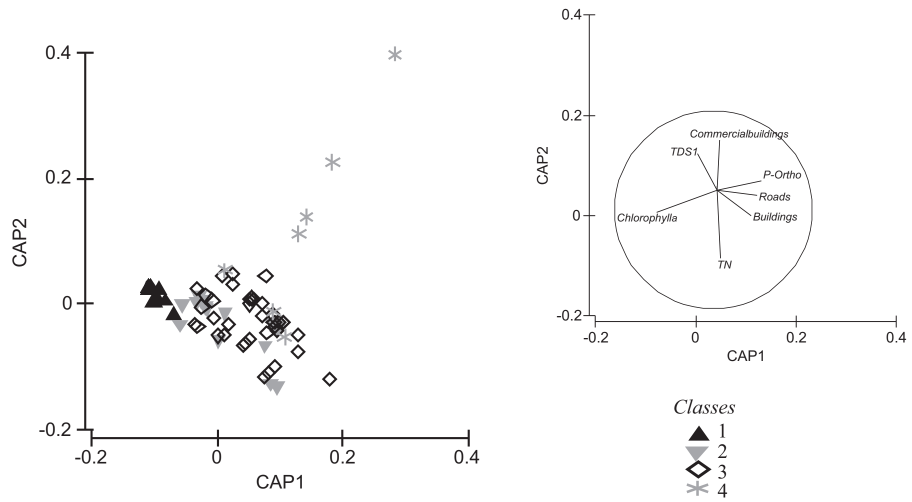

Fig. 6. Canonical analysis of principal coordinates (CAP) showing the distribution of sites according to their general degradation and respective classification, using the 4 classes-system. The top right image shows the vector overlay of Spearman rank correlations of individual pressures variable vectors with the CAP axes, which indicate which are the pressures responsible to the distribution of sites.

could even be improved if the chironomids were identified to species level, as other studies indicate that species of the genus *Procladius*, *Chironomus*, *Tanytarsus*, found with higher abundances in the study sites, have different sensitivities to chemical, organic, and metal contaminants (Mousavi, 2002; Mousavi et al., 2003; Puntí et al., 2009). A higher resolution could however have some disadvantages, such as requiring a higher level of expertise from technicians and being more time consuming (Feio et al., 2006; Stribling et al., 2008; Buss and Vitorino, 2010).

Regarding the choice of classification system, the test sites evaluation based on 3- and 4-classes system resulted in identical classifications for one site of Class 1 (equivalent to MEP), and to 47% of the sites attributed to class 2 (Table 4) while the remaining sites shifted from class 2 to class 3 in the 4-classes system. For class 3, the classifications were identical in 72% and the remaining sites shifted to class 4. So, in general the 4-classes system leads to lower quality classifications. However, with the present data set, the classification system with only 3 quality classes (Equivalent to Maximum Ecological Potential, Different, and Very Different from Maximum Ecological Potential) seems more accurate, as it is more effective in showing the distinction between classes regarding individual pressures level (Figs. 3 and 4) and general anthropogenic degradation (Figs. 5 and 6). This is not unexpected, since the remaining dissimilarity gradient from a Maximum Ecological Potential community is already small and its division into 3 more classes results in intervals of dissimilarity smaller than 20% (Table 3).

In conclusion, the predictive modeling approach tested, based on macroinvertebrate communities, showed to be an effective tool for the bioassessment of the studied reservoirs. Moreover, this approach, could also be applied to other heavily modified water bodies elsewhere, provided that a Maximum Ecological Potential can established and that there is a good taxa diversity, which, in the absence of numerous families from EPT taxa, can also be obtained by a using a higher taxonomic resolution of taxonomic diverse groups, often neglected in bioassessment, such as the Chironomidae.

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