

Maximum ecological potential of tropical reservoirs and benthic invertebrate communities

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Abstract The Reference Condition Approach (RCA) is now widely adopted as a basis for the evaluation of the ecological quality of water bodies. In accordance with the RCA, the integrity of communities found in a given location should be analyzed according to their deviation

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from the communities that would be expected in the absence of anthropogenic disturbances. The RCA was used here with the aim of defining the Maximum Ecological Potential (MEP) of tropical reservoirs located in the hydrographical basin of the Paraopeba River in the state of Minas Gerais, Brazil. Among the reservoirs, Serra Azul is used as a water supply and is located in a core area of environmental protection where tourism is not allowed and the native vegetation is conserved. The benthic macroinvertebrate communities at 90 sites located in three reservoirs were analyzed and sampled every 3 months over 2 years. The temporal patterns of the communities in the three reservoirs were analyzed (2nd-STAGE MDS and ANOSIM) and were not significantly related to seasonal fluctuations in temperature and precipitation. Twenty-eight sites belonging to the Serra Azul reservoir were selected to define the MEP of these reservoirs because these sites had the lowest human disturbance levels. The macroinvertebrate taxa present in the selected MEP sites are similar to those of natural lakes and different from the communities of disturbed sites. The biological classification of these sites revealed two groups with distinct macroinvertebrate communities. This distinction was related to climatic variables, bottom substrate type, the presence of gravel/boulders, coarse sand, silt, clay or muck, depth, and the shoreline substrate zone. These two subsets of biological communities and respective environmental conditions can serve as a basis for the future implementation of ecological quality monitoring programs for

tropical reservoirs in the study area. This approach can also, however, be implemented in other geographic areas with artificial or heavily modified water bodies.

Keywords Reservoirs · Macroinvertebrates · Reference condition approach · Tropical region

Introduction

An assessment of the ecological quality of an aquatic ecosystem combines information from the traditional monitoring of physical and chemical parameters with monitoring data on the system's biological communities. In accordance with the Reference Condition Approach (Reynoldson et al. 1997; Bailey et al. 2004; Nijboer et al. 2004), the integrity of the communities found in a given location should be analyzed according to their deviations from the communities that would be expected in the absence of anthropogenic disturbances or at minimally disturbed sites. Thus, it is essential to know what the communities of a given ecosystem would be like in the absence of anthropogenic impacts.

In practice, however, the reference conditions for an ecosystem rarely correspond to the concept of "pristine" because reference conditions are most commonly defined based on recently sampled local communities and, for most regions, areas with the total absence of anthropogenic impacts do not exist. Therefore, alternative definitions and approaches have appeared over the last decade (Gibson et al. 2000; Stoddard et al. 2006; Hawkins et al. 2010; Ruse 2010). Moreover, in the case of reservoirs, the concept of pristine cannot be used at all because these are inherently heavily modified water bodies where the environment conditions have shifted from lotic to lentic (Nilsson et al. 2005) with significant consequential changes in the structure of rivers and their hydrological regimes (Tundisi and Matsumura-Tundisi 2003).

In Europe, according to the European Water Framework Directive (WFD; European Commission 2000), the term "Maximum Ecological Potential" (MEP) is used to define the best status that a heavily modified or artificial water body can achieve (European Commission 2003). The MEP status may include permanent hydromorphological changes but only after all mitigation measures have been considered and assuming a suitable water quality (Irmer and Pollard 2006; Lammens et al. 2008). The definition of MEP provided by the WFD is perfectly adaptable to the

tropical reservoirs found in the Paraopeba watershed; therefore, the MEP concept was adopted for this study.

The use of an entire reservoir as a reference or, alternatively, the use of individual sites has been discussed by several authors. Navarro et al. (2009) consider that, due to the difficulty in finding unpolluted reservoirs, the use of a reservoir presenting good ecological quality as a reference for other reservoirs with similar abiotic characteristics is acceptable. We contend, as have other authors (Gibson et al. 2000; Dodds et al. 2006), that the entire reservoir should not be used as a reference without a previous analysis of various sites representing the diversity of the physical, chemical, or even biological characteristics of the overall watershed. Moreover, within a given reservoir, there are heavily impacted regions and others that are not so impacted depending on the level of human activities and proximity to urban centers (Kennedy 2001; Yanling et al. 2009).

There are essentially two ways to group reference sites with similar characteristics for assessment purposes: a priori classification (typology) based on the abiotic characteristics of the sites (e.g., altitude, drainage area, latitude, longitude), which is consistent with the Water Framework Directive (European Commission 2000; Piet et al. 2004; Salas et al. 2006; Teixeira et al. 2007; Puntí et al. 2007), and a posteriori classification, used by the majority of predictive models, in which sites are first grouped based on their biological assemblages (e.g., RIVPACS/AUSRIVAS, BEAST; Reynoldson et al. 1997; Clarke et al. 2003; Bailey et al. 2006; Feio et al. 2007, 2010; Aroviita et al. 2010). Several authors have compared these alternatives, and in a comparative study, Davy-Bowker et al. (2006) concluded that the former approach depends heavily on how well the variables used in the formation of types correlate with the ecological characteristics of the communities. In the scope of this study, and given that reservoirs are less well-studied systems than rivers, the a posteriori classification system based on predictive models was considered more adequate because it provides a prior determination of which environmental variables best explain the communities' distribution.

The aim of the present study is to define the MEP of the tropical reservoirs of Minas Gerais, Brazil from data on abiotic stressors (hydromorphological and water physical–chemical measurements) and the benthic macroinvertebrate communities of least-impacted sites. This information is a useful basis for the development of ecological assessment tools to monitor present and

planned reservoirs in this tropical region. We expect to find most of the least-impaired sites within the reservoir of Serra Azul, which is included in an environmental protected area with dense native vegetation and limited human access where no tourism or fisheries are allowed. Furthermore, we investigated the influence of seasonal variations in the macroinvertebrate communities of the reservoirs to determine the need for defining seasonal MEP values. Benthic macroinvertebrates were used here because they are established as useful bioindicators in the bioassessment of rivers and streams and are also among the biological elements recommended by the WFD (European Commission 2000) for the assessment of lakes and reservoirs.

Methods

Study area

Three reservoirs (Ibitité, Vargem das Flores, and Serra Azul) were studied in the Paraopeba River watershed, Minas Gerais, southeastern Brazil (Fig. 1). The Ibitité reservoir was built in 1968 at an altitude of 773 m above sea level (asl). This reservoir has an area of 2.8 km², a volume of 15,423,000 m³, an average depth of 16 m, and an annual water level fluctuation of 0.70 m (2008–2009). The Ibitité hydrographic basin contains 171,817 inhabitants. The predominant macrophyte in the reservoir is the floating *Eichhornia crassipes*, which is constantly being manually removed (Moreno and Callisto 2006). The landscape is dominated by *Eucalyptus* plantations, a large condominium complex, small farms, and several industrial plants (Pinto-Coelho et al. 2010). The Vargem das Flores reservoir was built in 1971 and is used as a water supply. It is situated at 837 m asl and has a water surface area of 4.9 km², a water volume of 37,000,000 m³, and a maximum depth of 18 m. The maximum level sill spillway is at an elevation of 835 m and has a hydraulic retention time of 365 days; the water level fluctuation in 2008–2009 was 2.54 m. In the littoral region, there are no floating aquatic macrophytes. Approximately 12.3 ha in the proximity of the reservoir were transformed into a state environmental protected area in 2006 (COPASA 2004), but approximately 100,000 people still inhabit the area. Finally, the Serra Azul reservoir is located at an altitude of 760 m asl and has a water surface of 7.5 km², a water volume of 88,000,000 m³, and a maximum depth of 40 m. It has

been operating for approximately 30 years as a source of drinking water. The maximum level sill spillway is at an elevation of 760 m with a hydraulic retention time of 351 days; the fluctuation of the water level in 2008–2009 was 5.71 m. This reservoir is also surrounded by an environmental protected area, established in 1980 with an area of 27,200 ha. Within this area, 3,200 ha belong to COPASA, the water company that manages the reservoir, and no recreational activities or fishing are allowed. The landscape is mostly covered by native vegetation, and an effort has been made to remove exotic plants and replace them with autochthonous vegetation. Only approximately 20 houses, from the period of construction of the reservoir, remain near the reservoir in a constrained area.

Climatic data

The climate of this region is considered tropical sub-humid (Cwb), with summer rains (November to April) and a dry winter (May to October). The average annual temperature is ca. 20 °C (Moreno and Callisto 2006). To analyze the seasonal patterns, the average monthly values of temperature and precipitation were calculated for all sampling periods based on data from the Brazilian National Institute of Meteorology (INMET 2010) for the metropolitan region of Belo Horizonte.

Environmental data

To characterize the natural conditions in the reservoirs and distinguish the various sites with respect to their levels of anthropogenic disturbance, several parameters related to water chemistry and physics, hydro-morphology, and land use were obtained for all sites (Table 1). On each sampling occasion, total dissolved solids (milligrams per liter) were measured in situ using a YSI Model Multiprobe (Table 1). Sub-surface water samples were collected with a Van Dorn-type cylinder for subsequent measurements of total nitrogen, total phosphorus, and orthophosphates in accordance with the “Standard Methods for the Examination of Water and Wastewater” (APHA 1992). The concentration of chlorophyll *a* (Chl_a) 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. (1983), and the Trophic State Index proposed by the Brazilian Society of

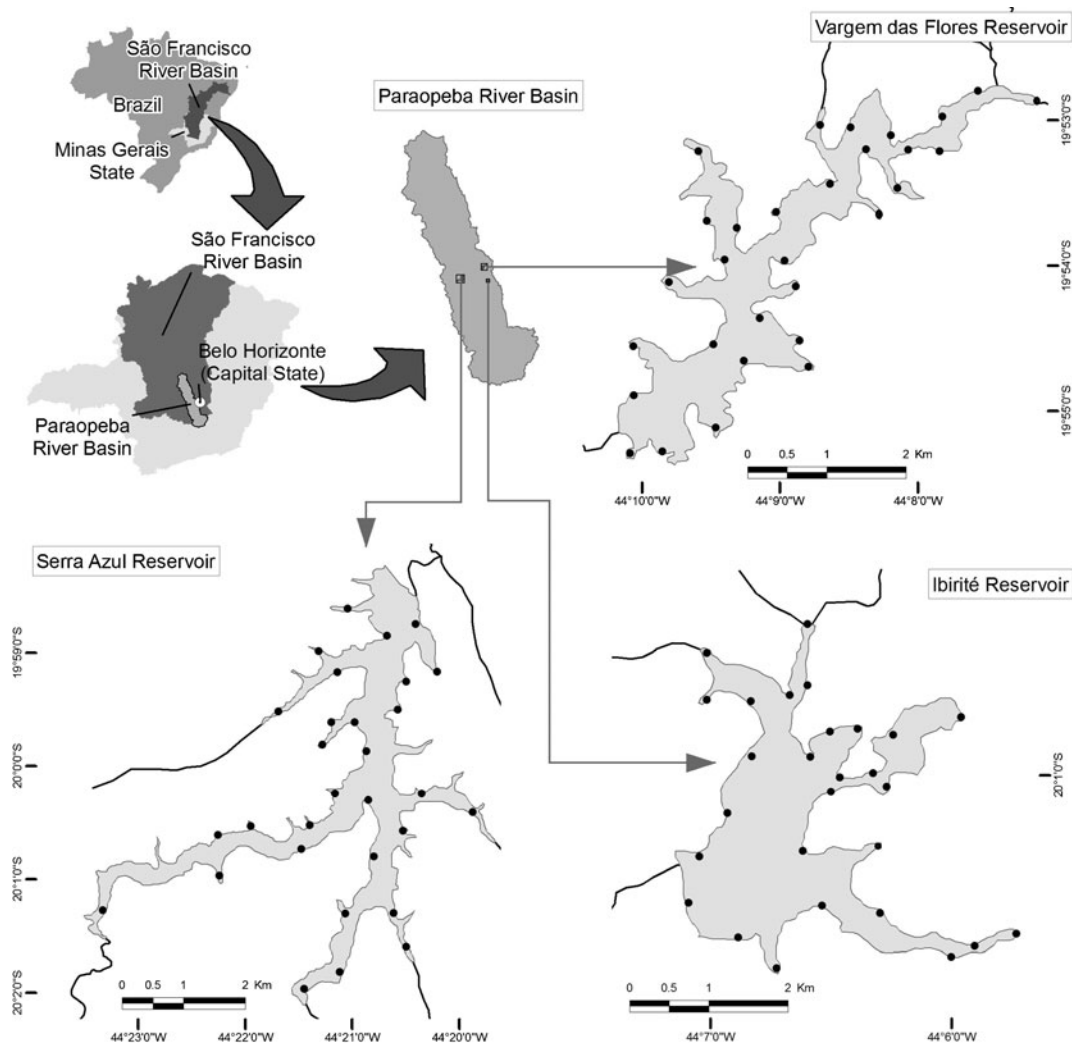


Fig. 1 Locations of the reservoirs of Vargem das Flores, Serra Azul, and Ibitité in the catchment of the Paraopeba River, Minas Gerais, Brazil and distribution of the sampling sites (*black dots*) in the reservoirs

Environmental Agency Technology (CETESB 2000) (TSI2) were calculated for all sites. Each index is composed of sub-indices, which are then weighted to obtain a final value for the trophic status. TSI1 is calculated through the formula

$$TSI1 = TSI(S) + 2 \times [TSI(TP) + TSI(PO_4) + TSI(Chla)]/7,$$

and the sub-indices are obtained as follows:

$$TSI(S) = TSI(S) = 10 \times (6 - (0.64 + 1nS)/1n2))$$

$$TSI(TP) = 10 \times (6 - (1n(80.32/TP)/1n2))$$

$$TSI(PO_4) = 10 \times (6 - (1n(21.67/PO_4)/1n2))$$

$$TSI(Chla) = 10 \times (6 - (2.04 - 0.695 \ln Chla)/1n2))$$

TSI2 is calculated through the formula

$$TSI2 = [TSI(TP) + TSI(Chla)]/2,$$

and the sub-indices are obtained through the following expressions:

$$TSI(TP) = 10 \times (6 - ((1.77 - 0.42) \times 1n(TP)/1n2))$$

$$TSI(Chla) = 10 \times (6 - (0.92 - 0.34) \times 1n Chla)/1n2))$$

TSI1 values ranging from 0 to 44 correspond to oligotrophic, 44–54 to mesotrophic, and >54 to eutrophic

Table 1 Description of the environmental variables measured at all sites

Environmental variables	Description and source	Mean (range)
Stressors		
Total dissolved solids (mgL ⁻¹)	Field measurement (YSI)	106.07 (9.04–324.45)
Chlorophyll a (µgL ⁻¹)	Analysis according to Golterman et al. (1978)	19.35 (0–228.04)
Total nitrogen (µgL ⁻¹)	Analysis according to APHA (1992)	0.19 (0.01–1.37)
Total phosphorus (mgL ⁻¹)	Analysis according to APHA (APHA American Public Health Association 1992)	72.14 (2.35–789.35)
Orthophosphate (µgL ⁻¹)	Analysis according to APHA (APHA American Public Health Association 1992)	2.60 (2.03–284.41)
Color of bottom substrate	Field observation, categories: 1 (brown), 2 (black), 3 (gray), 4 (red), 5 (other), USEPA (2007)	0–1
Odor of bottom substrate	Field observation, categories: 1 (none), 2 (H ₂ S), 3 (anoxic), 4 (oil), 5 (chemical), 6 (other)–USEPA (2007)	0–1
TSI1	Analysis based on Carlson (1977), modified by Toledo et al. (1983)	46.62 (34.90–84.21)
TSI2	Analysis based on CETESB (2000)	62.00 (25.46–91.00)
Buildings (%)	Field observation, categories: 1=absent (0 %), 2=sparse (10 %), 3=moderate (10–40 %), 4=heavy (40–75 %), 5=very heavy (>75 %), USEPA (2007)	1–2
Commercial buildings (%)	Idem	1–3
Docks/boats (%)	Idem	1–4
Dykes (%)	Idem	1–3
Landfills (%)	Idem	1–2
Roads (%)	Idem	1–3
Power lines (%)	Idem	1–3
Row crops (%)	Idem	1–3
Pasture (%)	Idem	1–3
Agriculture (%)	Idem	1–2
Characterization variables		
Gravel/boulders–bottom	Field observation, categories: (>4,000 mm–2 mm) 1=absent (0 %), 2=(<0–20 %), 3=(20–60 %), 4=(<60 %)	1.19 (0.24–6.47)
Coarse sand–bottom	Field observation, categories: (2–0.50 mm) 1=absent (0–15 %), 2=(<15–35 %), 3=(35–45 %), 4=(<45 %)	16.62 (0–51.74)
Fine sand–bottom	Field observation, categories: (0.50–0.062 mm) 1=absent (0–20 %), 2=(<20–50 %), 3=(<50–80 %), 4=(<80 %)	42.52 (0–92)
Silt, clay or muck–bottom	Field observation, categories: (<0.062 mm) 1=absent (0–15 %), 2=(<15–35 %), 3=(<35–45 %), 4=(<45 %)–USEPA (2007)	25.47 (0–85.50)
Bedrock–shoreline	(<15–35 %), 3=(<35–45 %), 4=(<45 %)	1–2
Cobble–shoreline	Field observation, categories: (64–4,000 mm) 1=absent (0–15 %), 2=(<15–35 %), 3=(<35–45 %), 4=(<45 %)	1–2
Gravel–shoreline	Field observation, categories: (2–64 mm) 1=absent (0–15 %), 2=(<15–35 %), 3=(<35–45 %), 4=(<45 %)	1–2
Sand/muck–shoreline	Field observation, categories: (0.062–2 mm) 1=absent (0–15 %), 2=(<15–35 %), 3=(<35–45 %), 4=(<45 %)	1–4
Depth (m)	Field measurement (sonar)	3.92 (0.4–16.20)
Bank steepness	Field observation, categories: 1=flat (<5°), 2=gradual (<5–30°), 3=steep (<30–75°), 4=near vertical (>75°)	1–4

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.

To characterize the sampling sites, we followed the protocol for lentic ecosystems proposed by EMAP-USEPA (Environmental Protection Agency, EUA) (USEPA 2007) modified by Molozzi et al. (2011). Data were recorded in December 2009 at each site in a plot 15 m wide \times 25 m long. This 25 m included 10 m in the littoral and 15 m in the riparian zone. The variables included in the protocol and used in this study are described in Table 1 and are related to land use, type of sediment, and depth. The depth of the water column was estimated using a portable sonar sensor. Sediment collected with an Eckman-Birge dredge was analyzed regarding its granulometric composition and organic matter content according to the methodology as modified by Callisto and Esteves (1996).

Macroinvertebrate sampling

Ninety sites distributed across the reservoirs were sampled quarterly (March, June, September, and December) in 2008 and 2009 with an Eckman-Birge dredge (0.0225 m²), as close as possible to the margin of the reservoir and at a depth varying from 0.4 to 16.2 m (mean depth of 3.92 m). The collected material was fixed with 70 % formalin and transported to the laboratory. Invertebrates were mostly identified to the family level (Peterson 1960; Pérez 1988; Merritt and Cummins 1996; Carvalho and Calil 2000; Fernandez and Domingues 2001; Costa et al. 2006; Mugnai et al. 2010). Chironomidae were treated with a 10 % solution of lactophenol and identified to the genus level under a microscope (\times 400) with the aid of the Trivinho-Strixino (2011) and Epler (2001) taxonomic keys.

Data analyses

Determination of biological seasonal variability

The similarity between communities in different seasons and years was analyzed for each reservoir with a 2nd-STAGE non-metric Multidimensional Scaling Analysis (nMDS) (Clarke and Gorley 2006). This MDS is based on the similarity matrix resulting from a 2nd-STAGE analysis. This procedure calculates a similarity matrix based on the Spearman rank correlation between pairs of Bray–Curtis similarity matrices, each one composed of the biological data collected in a given season and year. Additionally, an analysis of

similarity (ANOSIM) based on rank similarities between samples in the underlying triangular similarity matrix (Clarke and Warwick 2001) was performed to test whether the benthic communities were statistically similar over the sampling period.

Selection of sites with maximum ecological potential

A principal components analysis (PCA) of the stressor data described in Table 1 (normalized data; Clarke and Warwick 2001) was conducted for all sites and samples to determine which sites are least affected by human disturbance and therefore can be used to define the Maximum Ecological Potential of reservoirs in the study area as well as the most relevant stressors in the study area.

Additionally, the distribution of values for each stressor variable was visually inspected with box plots, and the outliers (outlier coefficient 1.5; box range, 25th–75th percentiles) were subsequently removed from the MEP data set. For the final set of MEP sites, the range, mean, and standard deviation of each stressor variable were calculated to define the intervals of acceptable stressor values for these systems.

To determine whether the biological communities of the selected (MEP) sites from the PCA were, in general, distinct from those affected by a higher level of stress, we performed a nMDS ordination with the biological data (square root transformation; Bray–Curtis similarity). Additionally, we performed an ANOSIM to check for significant differences between MEP and disturbed sites (Clarke and Warwick 2001).

Establishment of subsets of communities in MEP sites

An unweighted pair group method with arithmetic mean classification (Bray–Curtis similarity; square root transformation) was carried out to analyze whether there are subsets of reference conditions (groups of sites with similar communities) within the selected MEP sites. The significant differences among the groups were tested with an ANOSIM.

To determine the most representative species of each group and verify whether they differed among the reference groups, we used the SIMPER routine (Primer 6). SIMPER uses a species Bray–Curtis similarity matrix to compute the average dissimilarity between all pairs of intergroup samples and disaggregates this average into separate contributions from each species (Clarke and Warwick 2001). The total number of individuals,

number of species, and Margalef’s richness (Margalef 1969), Shannon–Wiener’s diversity (Shannon and Weaver 1963), and Pielou’s evenness (Pielou 1969) further characterized the different groups found.

Abiotic typology

A stepwise forward discriminant analysis (Alpha-to-Tolerance=0.001 and Alpha-to-Remove=0.10 with Jackknife cross-validation, Hair et al. 1998) was performed to identify the environmental variables that best distinguish the communities in the groups. The potential discriminant variables used in the analysis, such as the type of substrate and the slope of the shoreline (Table 1), describe the morphological characteristics of the system and were selected for being less subject to anthropogenic changes.

All statistical analyses were performed using PRIMER 6.0 software except the discriminant analysis, which was performed in Systat 13.0 (Systat Software).

Results

Seasonal variability

A total of 14,425 organisms belonging to 47 taxa (4 Mollusca, 2 Annelida, and 41 Arthropoda) were

collected from 90 sampling sites over 2 years. Of those, 24 % were Diptera, and *Chironomus* (8 %), *Tanytus* (4 %), and *Coelotanytus* (4 %) were the most representative genera [Electronic supplementary material (ESM) Table 1].

The climatic data for the years 2008 and 2009 confirmed the existence of distinct wet (December and March) and dry seasons (June and September). December 2008 was the month with the highest average rainfall (442 mm), followed by January 2009 (282 mm). The driest periods, with no precipitation, were the months of June 2008 and 2009. The maximum temperatures during the study period were recorded in December 2009 (29.0 °C) and the minimum temperatures in June 2008 (23.9 °C; ESM Fig. 1).

The 2nd -STAGE nMDS was not consistent with the above, showing that there is no pattern of high correlation between the communities sampled in the same month of the year (e.g., December 2008, December 2009) or the same season (dry, wet; Fig. 2), although there is a certain level of segregation by year (2008–2009). The global *R* values of the ANOSIM indicated a wide variability in invertebrate communities within seasons for the three reservoirs (ANOSIM Serra Azul, Global *R*=0.054, *p*=0.001; ANOSIM Ibirité, *R*=0.166, *p*=0.001; ANOSIM Vargem das Flores, *R*=0.113, *p*=0.001), which was confirmed by most pairwise tests (Table 2). Regarding the pairwise tests, for those comparisons with a higher

Fig. 2 Results of 2nd-STAGE MDS for the three reservoirs based on biological data collected in December (*Dec*), March (*Mar*), June (*Jun*), and September (*Sep*) of 2008 (*08*) and 2009 (*09*). **a** Serra Azul, **b** Vargem das Flores, **c** Ibirité

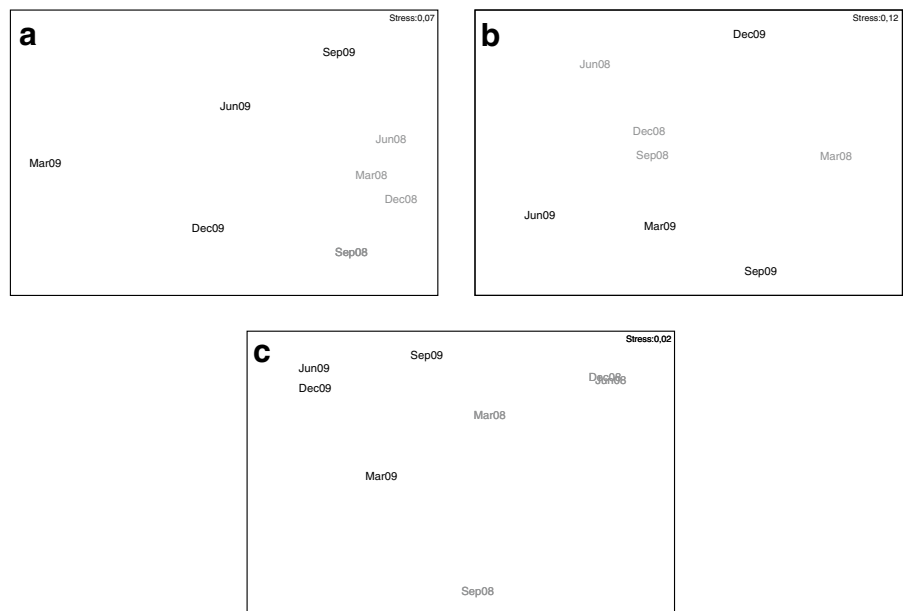


Table 2 Results of the ANOSIM pairwise tests between the samples of the Serra Azul, Vargem das Flores, and Ibitiré reservoirs

Months	Serra Azul (<i>R</i> , <i>p</i>)	Vargem das Flores (<i>R</i> , <i>p</i>)	Ibitiré (<i>R</i> , <i>p</i>)
March 2008–March 2009	0.08, 0.0004	0.18, 0.001	0.21, 0.001
March 2008–June 2008	0.04, ns	−0.01, ns	0.04, 0.04
March 2008–June 2009	0.09, 0.002	0.04, 0.05	0.07, 0.007
March 2008–September 2008	0.02, ns	0.03, ns	0.09, 0.001
March 2008–September 2009	0.09, 0.001	0.06, 0.015	0.39, 0.001
March 2008–December 2008	0.04, 0.025	0.06, 0.015	0.21, 0.001
March 2008–December 2009	0.15, 0.001	0.10, 0.001	0.31, 0.001
March 2009–June 2008	0.05, 0.018	0.20, 0.001	0.20, 0.001
March 2009–June 2009	0.02, 0.094	0.22, 0.001	0.14, 0.001
March 2009–September 2008	0.05, 0.025	0.13, 0.003	0.10, 0.001
March 2009–September 2009	−0.01, 0.665	0.25, 0.001	0.13, 0.005
March 2009–December 2008	0.08, 0.004	0.14, 0.001	0.07, 0.005
March 2009–December 2009	0.02, 0.17	0.34, 0.001	0.20, 0.001
June 2008–June 2009	0.06, 0.015	0.1, 0.001	0.07, 0.007
June 2008–September 2008	0.05, 0.023	0.01, 0.272	0.05, 0.03
June 2008–September 2009	0.04, 0.035	0.15, 0.001	0.22, 0.001
June 2008–December 2008	0.03, 0.038	0.07, 0.015	0.21, 0.001
June 2008–December 2009	0.10, 0.002	0.21, 0.001	0.26, 0.001
June 2009–September 2008	0.04, 0.029	0.09, 0.004	0.11, 0.001
June 2009–September 2009	0.03, 0.054	−0.02, 0.852	0.17, 0.001
June 2009–December 2008	0.08, 0.002	0.12, 0.001	0.25, 0.001
June 2009–December 2009	0.04, 0.036	0.01, 0.222	0.22, 0.001
September 2008–September 2009	0.05, 0.014	0.13, 0.004	0.11, 0.003
September 2008–December 2008	0.01, 0.317	0.02, 0.155	0.09, 0.006
September 2008–December 2009	0.10, 0.001	0.18, 0.001	0.23, 0.001
September 2009–December 2008	0.06, 0.008	0.16, 0.001	0.18, 0.001
September 2009–December 2009	−0.01, 0.643	0.001, 0.375	0.06, 0.007
December 2008–December 2009	0.10, 0.001	0.16, 0.001	0.35, 0.001

ns nonsignificant results ($p>0.05$)

R value ($R>0.2$) and a significant *p* value ($p<0.05$), the differences were also not consistent with the climatic patterns. Therefore, there was no reason to consider different MEP values for different seasons, and in further analyses, the mean taxa abundance was used to characterize sampling sites.

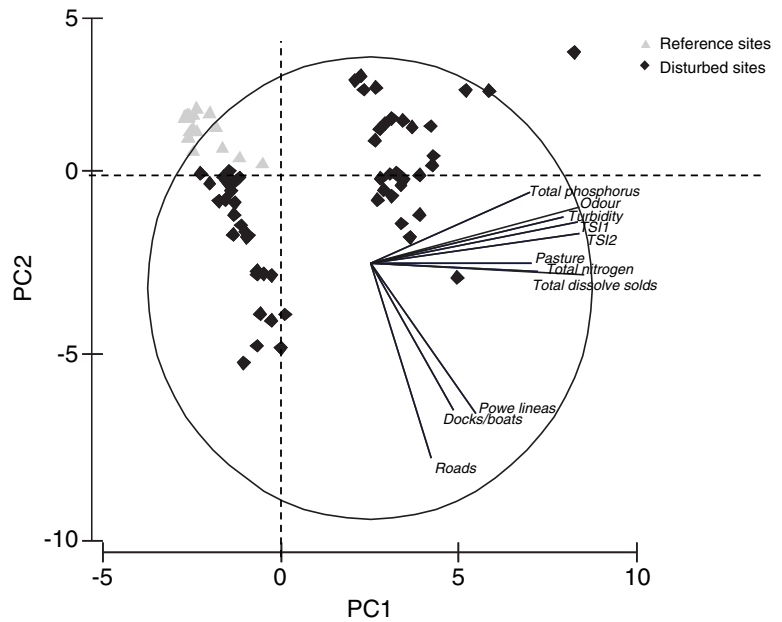
Maximum ecological potential

The first axis of the PCA (Fig. 3) explained 39.4 % of data variability and was correlated primarily with the variables total dissolved solids (0.333), turbidity (0.319), TSI1 (0.326), TSI2 (0.324), and bottom substrate odor (0.329). The second PCA axis explained

17.0 % of data variability and was correlated with the presence of docks/boats (−0.477), roads (−0.360), pasture (−0.325), and power lines (−0.291; Table 3). The sites selected as having less anthropogenic impact and therefore the Maximum Ecological Potential are located on the negative side of PC1 and closer to zero on PC2 (32 sites; Fig. 3).

Three of the sites selected as reference on the PCA (31, 32, and 56) were subsequently eliminated after the examination of box plots because they included outlier values for some stressor variables such as total phosphorus, total nitrogen, and chlorophyll *a*. After that removal, all MEP sites were located in the Serra Azul reservoir. Minimum and maximum acceptable

Fig. 3 Principal component analysis (PCA) based on stressor data from 90 sites sampled in the three reservoirs (see Table 1). Only variables with a Pearson correlation with the axes above 0.7 are shown in the figure



values for all stressor variables for MEP were then calculated (Table 4).

The ordination of the biological data (Fig. 4; stress = 0.20, 2D) confirmed that the communities of the selected MEP sites were, in fact, different from those

of the remaining sites and therefore were conceivably not affected by the examined variables. The ANOSIM indicated significant differences between the MEP communities and disturbed sites (ANOSIM, Global $R=0.463$; $p=0.001$). Taxa such as *Melanoides*

Table 3 Correlations of stressors with PCA axes 1 and 2

Characterization variable	Factorial axes	
	F1	F2
Total dissolved solids (mgL ⁻¹)	0.333	-0.043
Chlorophyll <i>a</i> (µgL ⁻¹)	0.267	0.079
Total nitrogen (mgL ⁻¹)	0.265	-0.027
Total phosphorus (µgL ⁻¹)	0.256	0.162
P-ortho (µgL ⁻¹)	0.161	0.139
Odor of bottom substrate	0.329	0.120
TSI1	0.324	0.078
TSI2	0.326	0.051
Buildings	0.160	-0.370
Commercial buildings	0.095	-0.256
Docks/boats	0.086	-0.477
Dykes	0.044	-0.270
Landfills	0.126	0.109
Roads	0.123	-0.360
Power lines	0.085	-0.291
Row crops	-0.027	-0.271
Pastures	0.026	-0.325
Agriculture	0.248	-0.016

Table 4 Range of acceptable values (minimum–maximum) for stressors based on the sites selected as having Maximum Ecological Potential

Variables	Reference
Total dissolved solids (mgL ⁻¹)	16.34–22.10
Chlorophyll <i>a</i> (µgL ⁻¹)	0.13–3.33
Total phosphorus (µgL ⁻¹)	11.05–29.52
Total nitrogen (mgL ⁻¹)	0.04–0.10
Orthophosphate (µgL ⁻¹)	5.05–12.36
TSI1	29.47–43.91
TSI2	35.02–51.15
Odor	1–2
Buildings	1–2
Walls, dykes, or revetments	1–2
Landfills	1–2
Roads or railroads	1–2
Power lines	1–2
Row crops	1–2
Pastures	1–2
Agriculture	1–2

tuberculatus, Oligochaeta, and *Chironomus* were found in higher proportions in the impacted sites (25.47, 24.83, and 9.27 %, respectively) and in lower proportions in MEP sites (2.62, 3.42, and 1.05 %, respectively). On the other hand, *Fissimentum*, Philopotamidae, *Hydrobiosidae*, and *Procladius* were found in higher proportions in MEP sites (5.45, 0.04, 0.04, and 3.16 %, respectively) and in small amounts or not at all in impacted sites (0.24, 0, 0, 0.09 %, respectively). In the 28 sampling stations classified

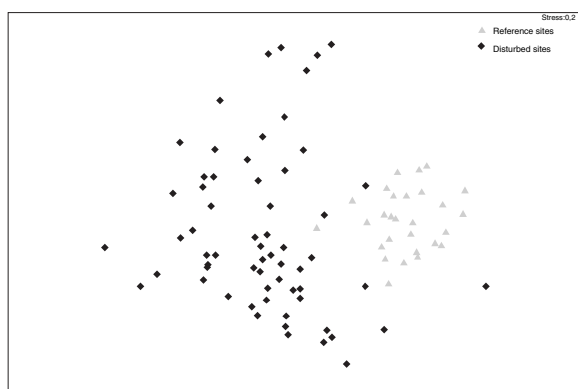


Fig. 4 Multidimensional scaling analysis based on the biological data of Maximum Ecological Potential sites and disturbed sites

as MEP, 2,366 organisms belonging to 39 taxa (1 Mollusca, 2 Annelida, and 36 Arthropoda) were collected.

Subsets of communities in MEP sites

The cluster analysis results indicated the existence of two local groups, G1=5 sites and G2=23 sites, within the MEP sites. The ANOSIM (Global $R=0.686$, $p=0.002$) confirmed the significance of the difference between these two sub-groups (stress=0.21, 2D). Alternative grouping levels were tested, but the groups showed no significant differences (data not shown). Site 60 was excluded because its invertebrate community is highly dissimilar from those of all other sites (ESM Fig. 2).

SIMPER analysis showed that the average Bray–Curtis similarity within each group in terms of benthic macroinvertebrates was similar for both groups (55 % for G1 and 57 % for G2; Table 5). The total abundance and the exclusive presence of the following taxa in group 2 contributed significantly to the dissimilarity between groups (55.52 %): *Tanytus*, *Ablabesmyia*, *Cladopelma*, *Aedokritus*, *Tanytarsus*, *Pseudochironomus*, *Alotanytus*, *Cryptochironomus*, *Stenochironomus*, *Parachironomus*, *Labrundinia*, *Paralauterboniela*, *Manoa*, *Chironomus*, Oligochaeta, Leptoceridae, Gomphidae, Hidracarina, and Hydrobiosidae, whereas group 1 presented no exclusive taxa contributing to the dissimilarity between groups.

Diversity indices also differed between groups, with the sites of group 2 (the largest group) displaying higher values of average taxonomic richness (12.09 taxa) and Margalef’s index (5.30; Table 5) than group 1. Both groups obtained similar Pielou evenness values (G1, 0.78; G2, 0.62).

Abiotic typology

The results of the stepwise discriminant analysis selected three descriptor variables—the bottom substrate (gravel/boulders, coarse sand, and silt/clay/muck), silt/clay/muck of the shoreline, and depth—that best discriminate the two MEP groups ($F=10.66$, $p=0.0001$). A jackknifed cross-validation showed that 100 % of the sites in G1 and 95 % of the sites in G2 are correctly assigned using the selected variables (Table 6). It can be stated that, in general, the sites belonging to group 1 have larger substrate particles (gravel, boulders, and coarse substrate) and are shallower than those in group 2 (Table 6).

Table 5 Average abundance of the taxa that contributed up to 99 % of Bray–Curtis similarity (SIMPER analysis) within sites of the same group

Taxa	Group 1, n=5	Group 2, n=23
Mollusca		
Gastropoda		
Thiaridae		
<i>Melanoides tuberculatus</i> Müller, 1774	23.14	4.82
Annelida		
Hirudinea	0.43	1.34
Insecta		
Odonata		
Gomphidae	0	0.88
Diptera		
Ceratopogonidae	0.32	2.00
Chaoboridae		
<i>Chaoborus</i> Lichtenstein, 1800	37.12	38.26
Chironomidae		
Tanypodinae		
<i>Tanypus</i> Meigen, 1803	0	7.19
<i>Coelotanypus</i> Kieffer, 1913	11.69	12.37
<i>Ablabesmyia</i> Johhansen, 1905	0	5.93
<i>Nimbocera</i> Reiss, 1972	0	1.25
<i>Djalmabatista</i> Fittkau, 1908	8.32	3.86
<i>Procladius</i> Skuse, 1803	7.81	5.83
Chironominae		
<i>Tanytarsus</i> Kieffer, 1921	0	0.20
<i>Chironomus</i> Meigen, 1803	0	1.99
<i>Fissimentum</i> Cranston and Nolte, 1996	3.18	9.81
<i>Pelomus</i> Reiss 1989	0	0.50
<i>Polypedilum</i> Kieffer, 1913	4.99	2.66
Taxonomic Richness	7.33	12.09
Total individual	3.7	12.24
Equitability Pielou's evenness	0.78	0.62
Margalef 's Richness Index	5.30	4.91
Shannon–Wiener Diversity Index	1.54	1.55

Table 6 Variables selected by the stepwise forward discriminant analysis and respective mean values (\pm DS) for the two Maximum Ecological Potential subgroups

Variables	F-to-Renove	Tolerance	Group 1	Group 2
Gravel/boulders–bottom	0.525	0.786	6.26 \pm 14.84	2.81 \pm 11.53
Coarse sand–bottom	23.059	0.428	6.13 \pm 6.23	2.27 \pm 3.64
Silt, clay, or muck–bottom	0.086	0.460	40.25 \pm 23.88	38.93 \pm 17.49
Silt, clay, or muck–shoreline	43.669	0.273	1.33 \pm 0.51	1.90 \pm 0.30
Depth	0.525	0.786	0.61 \pm 0.23	0.63 \pm 0.27

Discussion

In rivers, seasonal climatic variability is usually accompanied by changes in aquatic communities (Sporka et al. 2006; Leunda et al. 2009; Puntí et al. 2009). These changes are known to affect ecological assessments based on reference conditions that represent the systems only for a given season, as has been shown by several authors (e.g., Feio et al. 2006; Aroviita et al. 2010). However, the seasonal variability (precipitation and temperature) observed in the 2 years of sampling was not reflected in changes in the benthic communities of the studied reservoirs. In fact, the seasonal variability of the communities was unpredictable and similar to the inter-annual variability. Other authors in both subtropical (China) and temperate systems (Canada) have observed that rainfall and the flood pulse do not influence the distribution of Chironomidae in reservoirs because they are well adapted to fluctuations in the water level (Zhang et al. 2010; Furey et al. 2006). Therefore, we included in our MEP conditions all of the temporal variability present in our samples.

In this study, we considered sites with MEP to be those that were least impaired within our systems and data set. Those sites also presented values within the acceptable limits for class 1 (waters allocated to the preservation of the natural balance of aquatic communities) according to Brazilian legislation (Brasil 2005, CONAMA/357). As we predicted, all of the selected MEP sites were located in the Serra Azul reservoir, which is located in an area of permanent protection with native vegetation that is characteristic of cerrado forest (COPASA 2004). Moreover, the Water Framework Directive (European Commission 2000) recommends that for reservoirs, the classification of MEP should be assigned when the communities are similar to those of a comparable high quality natural lake. In fact, Ramos (2008) found species richness values of 12 and 23 taxa for the natural lakes Dom Helvécio and Águas Claras, respectively, also in Minas Gerais, Brazil; these values are lower than our results for the MEP sites (39 taxa). Additionally, our list and those lakes had many species in common (e.g., *Coleotanypus*, *Cryptochironomus*, *Fissimentum*, *Goeldichironomus*, *Lauterboniella*, *Polypedilum*, *Procladius*, *Tanytarsus*, *Harnisch*, and *Zavreliella*), and only four taxa present in the natural lakes were absent from our samples. These facts give us additional confidence in our selection of sites and in the

establishment of MEP conditions for tropical reservoirs in the study area and similar regions.

However, the expected taxonomic richness of a reservoir is always lower than the richness of the river before the construction of the reservoir, and simultaneously, the number of exotic species usually increases due to the major changes in the physical and chemical characteristics of the water body (Horsák et al. 2009; Yanling et al. 2009). In our reservoirs, even in sites selected as having MEP, the observed taxonomic richness (51 taxa, 59 % Diptera) was lower than that of the river in the same drainage basin, where 63 taxa were recorded and Ephemeroptera, Plecoptera, and Trichoptera represented 16 % of the total number of individuals (A. Lessa et al., unpublished data).

The colonization of new highly modified habitats such as reservoirs is undertaken by highly resistant species that are adapted to stagnant waters as well as generalist species with small sizes, long life cycles, and frequent reproductive cycles (Prat and Daroca 1983; Rueda et al. 2006; Ruse 2010). In our study, the presence of the exotic species *M. tuberculatus* Müller, 1774 (Thiaridae, Gastropoda) was recorded even at our least disturbed sites, where it should ideally decrease in abundance. Since it was first recorded in Brazil in 1967 (Rocha-Miranda and Martins-Silva 2006), this African-Asian species has extensively invaded Neotropical freshwater ecosystems, settling in various types of substrate (Dudgeon 1989; Clements et al. 2006). However, the densities of *M. tuberculatus* in disturbed habitats are likely to increase and may surpass 10,000 individuals/m² (Santos and Eskinazi-Sant'Anna 2010). In our study, disturbed sites contained approximately 97 % more individuals of this species even though its abundance varied from site to site, which was the main reason for the higher variability in disturbed sites in comparison to MEP sites.

Aside from *Melanoides*, other differences in taxonomic composition between MEP sites and more disturbed sites exist: Oligochaeta, the above mentioned *M. tuberculatus* and *Chironomus* represented 60 % of the total individuals in more disturbed sites, whereas in the reference sites, they accounted for 7 %. Some genera of Chironomidae (*Manoa*, *Pseudochironomus*, *Stenochironomus*, *Zavreliella*, *Lauterboniella*, and *Paralauterboniella*), Philopotamidae, and Hydrobiosidae were found only in reference sites. Several authors have shown that different Chironomidae species have different sensitivities to stress (Davies and Jackson 2006; Arimoro et al. 2007; Roque et

al. 2010). The genus *Fissimentum*, for example, which occurred in high numbers in sites with MEP, is considered an indicator of good water quality (Cranston and Nolte 1996). A high abundance of the genus *Polypedilum* was also recorded in our reference sites. However, species of this genus present high variability in their sensitivities to environmental stress (Roque et al. 2010). Therefore, we think that, contrary to streams where family level is often considered sufficient for monitoring purposes (e.g., Hewlett 2000; Feio et al. 2006; Buss and Vitorino 2010), in reservoirs, it is important to identify individuals to a lower taxonomic level (species).

In our study, MEP sites were divided into two groups based on the classification analysis. Methods of subdividing the reference conditions are necessary to cover the natural variability found in the studied area and, simultaneously, must have biological relevance to make appropriate comparisons (Rawer-Jost et al. 2004). In this study, we found a good correspondence between the biological classification of two groups and some environmental descriptors. Variables related to bottom substrate, the substrate of the shoreline, and depth correctly discriminated 96 % of the 28 reference sites in terms of their respective biological groups. This allowed the construction of an abiotic typology relevant to the invertebrates of the littoral zone of reservoirs. This typology enables future comparisons between the communities of new sites and the reference condition values established based on sites with MEP.

Regarding the selected discriminant variables, Camargo et al. (2005) observed that, similarly to streams and rivers (see Bailey et al. 1998; Rawer-Jost et al. 2004), sediment is a key factor determining the spatial distribution of invertebrates in reservoirs. This fact is corroborated in our study, where substrate was responsible for the differential distribution of Chironomidae genera between the two biological groups in the reference sites. We also found depth to be an important discriminator of reservoir benthic invertebrate communities in our study sites. This is in accordance with other authors who have identified depth as an important factor in structuring the communities of reservoirs (Verneaux et al. 2004; Rossaro et al. 2007; Panis et al. 1996). For example, studies undertaken in both Spanish (Prat et al. 1991) and Brazilian reservoirs (Moretto et al. 2003; Moreno and Callisto 2006) found that Chironomidae have very low abundances in deep areas.

In conclusion, we found within our reservoirs sites that can be considered as having Maximum Ecological Potential, which will be useful for the implementation of future ecological quality monitoring of tropical reservoirs in the study area. This does not render unnecessary the search for better references, although we think that our MEP sites are, in fact, in an advantageous condition regarding land use and water quality compared to other reservoirs in Brazil because they are in a protected area. An investment in the area of taxonomy leading to the identification of Chironomidae to the species level could enable a more sensitive and accurate assessment system. Finally, the MEP approach applied here to tropical reservoirs could be implemented elsewhere for the assessment of other artificial and heavily modified water bodies to provide realistic benchmarks for the assessment and recovery of those types of water bodies.

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