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Defining a fish bio-assessment tool to monitoring the biological condition of a cascade reservoirs system in tropical area

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ABSTRACT

We studied five tropical reservoirs in the cascade system of the Tietê River (State of São Paulo, Brazil) in order to evaluate the suitability of a fish multimetric index (Reservoir Fish Assemblage Index—RFAI) as a bio-assessment tool. For this purpose, we investigated the spatial and temporal variability of the RFAI with the objectives of testing the effects of the unit measure (catch in numbers or individual weight) of abundance and trophic metrics categories on the index response and, to identify the best sampling season for a bio-assessment program. We located seventy-two sampling sites in three different reservoir zones (fluvial, transition, and lacustrine) and collected samples by habitats – lateral (L), tributary mouth (M) and central (C) – inside each reservoir zone. We recorded the fish assemblages and several physicochemical and habitat variables in the dry and rainy seasons. We tested candidate metrics for range, responsiveness, and redundancy and selected nine for inclusion in the final indices ($RFAI_N$, the index calculated when we expressed fish in number of individuals, per species, and $RFAI_W$, calculated when we expressed fishes in weight, per species). We used a discrete scoring criterion (1,3,5), and the final indices varied from 9 to 45. $RFAI_N$ and $RFAI_W$ were highly correlated and able to detect a clear spatial variability among the five reservoirs. $RFAI_N$ showed a higher temporal variability especially in the smaller reservoirs of the system (Bariri, Ibitinga and Nova Avanhandava), allowing us to consider the dry season as the preferable sampling period for the development of bio-assessment programs. $RFAI_W$ appeared to be more responsive than $RFAI_N$, and was in agreement with the environmental gradients observed when analyzing the physicochemical and habitat variables collected in the present study. These findings indicate that the fish multimetric approach is a suitable tool for the assessment of the biological conditions of these reservoirs.

1. Introduction

Many hydrological systems of the world are impounded or heavily modified to satisfy human demands, such as electricity production, irrigation, water regulation, drinking water provisioning and navigation (Welcomme and Marmulla, 2008). Despite their importance in providing a useful service to humans, the assessment of their biotic condition after their formation has received little attention (Irz et al., 2006). In the near future, in order to allow water multiple and sustainable use, their maintenance and restoration will be a dominant society objective. For this reason, the development of tools for their assessment and monitoring is of scientific interest. In this context, multimetric indices are considered very useful tools (Zhai et al., 2010), and one of the most popular is the Index of Biotic Integrity (IBI) (Karr, 1981). The IBI arose in the 1980s in response to the requirements of the USA Clean Water Act (CWA) which specifically declared the goal of "restoring and maintaining the chemical, physical and biological integrity of the Nation's waters" (Novotny et al., 2005). This Act promoted the development and application of bio-assessment as a routine tool for the monitoring and assessment of aquatic habitats in many US states. IBI adaptations are available for different typologies of

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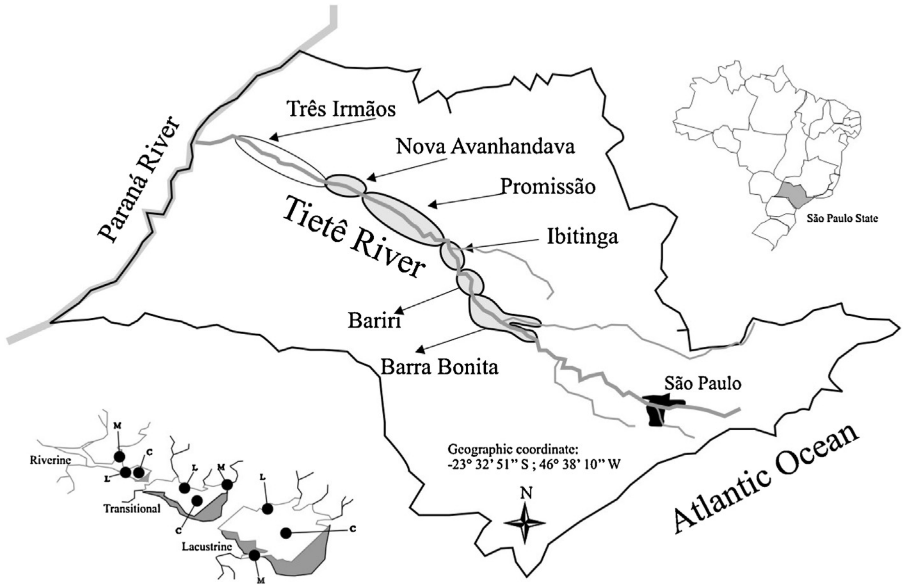

Fig. 1. Study area: Tietê cascade reservoir system, São Paulo (Brazil).

aquatic habitats such as streams, large rivers, lakes, estuarine habitats and reservoirs, and for different taxonomic groups such as periphyton, macro-invertebrates, fish, and aquatic plants (Hughes and Oberdorff, 1999; Simon, 1999; Yoder and Kulik, 2003; Roset et al., 2007). Regarding reservoirs, Jennings et al. (1995) emphasized that the term "biotic integrity" is not formally appropriate for environments that are a direct result of human interference. Consequently, "Reservoir Fish Assemblage Index" (RFAI) was considered a more suitable term, and was adopted in the present study as in Petesse et al. (2007).

The more recent European Water Framework Directive (WFD) (European Community, 2000), similar to the Clean Water Act (USA), mandates the restoration and maintenance of aquatic habitats using bio-assessment tools and, in relation to artificial and heavily modified water bodies supplying specific human necessities, facilities or services, declares the objective of obtaining a "Good Ecological Potential" (GEP). In particular, GEP refers to the achievement of a reasonable condition for habitats that cannot objectively be brought back to their pristine status. For this purpose, the WFD specifies that a water body shows a GEP when there are slight changes in the biological elements from those found at the Maximum Ecological Potential (MEP). The MEP biological conditions are equivalent to reference conditions for heavily modified water bodies and should reflect, as much as possible, the biological conditions of the closest comparable natural water body type (Borja and Elliott, 2007). Man-made water bodies are considered as lake-type water bodies, but the great heterogeneity among reservoir characteristics and, their intermediate nature between lakes and rivers, makes it difficult to identify the "closest natural water bodies" whose MEP should be used as a reference. In this context and in an attempt, to respond to the European WFD indications, Irz et al. (2006) compared fish assemblages similarities among reservoirs, river and lakes to identify the closest natural water body from which to derive reference conditions for reservoirs. The authors recognized the reservoir ichthyofauna as dissimilar to that of the rivers, but affirmed that the *a priori* choice of natural lakes as a reference for reservoirs may be questionable, highlighting the complexity of these systems and the difficulty in classifying them with the criteria derived from natural habitats. Nevertheless, the impossibility of finding adequate reference conditions for reservoirs is common for other natural habitats subjected to strong anthropogenic pressures (Stoddard et al., 2006). This is even more difficult in cascade reservoir systems where the downstream reservoirs are influenced by the upstream ones and where the water management is rigorously controlled to respond solely to human exigencies. Thus, cascade reservoirs might be considered as "unique" in their typology, and looking for natural reference condition or to a "Maximum Ecological Potential" (in agreement with the European WFD) may be conceptually a "nonsense" (Moss, 2008).

In Brazil, many fluvial systems have been dammed for hydroelectric purposes. In the Paraná River Basin (MMA, 2006), a large portion of the lateral tributaries (Tietê, Paranapanema, Grande) have been transformed into a sequence of reservoirs. This profoundly altered the original riverine environment and compromised the possibility of recovering the original fluvial condition due to the societal need of water usage. The impact of the impoundment on the biotic communities and fish assemblages, in particular, has been studied over the last 30 years (Agostinho et al., 1999, 2007; Tundisi and Straškraba, 1999), but the biological status of these "cascade systems" has not been sufficiently analyzed especially for monitoring purposes.

This study evaluates the suitability of a fish multimetric index as a bio-assessment tool for the cascade reservoir system of the Tietê River located in the State of São Paulo, Brazil. To allow this, we used a "response-oriented" approach in which the environmental conditions are assessed on the basis of the status of the responding organism (Abbasi and Abbasi, 2012). As specific issues, we analyzed the spatial and temporal variability (within and between reservoirs) of the index with the objective of testing the effect of the unit measure of abundance and trophic metrics (fish catch expressed in numbers or weight) on the index response, and to identify the better "index period", which is defined as the more proper sampling season for bio-assessment program development.

2. Materials and methods

2.1. Study area

The Tietê River basin (71,988 km^2) is completely contained in the State of São Paulo. From its middle stretch to its confluence with the Paraná River, it was transformed into a cascade of six reservoirs: Barra Bonita (BB), Bariri (B), Ibitinga (I), Promissão (P),

Table 1
Morphological variables of the Tietê cascade reservoir system Source: AES-Tietê, www.aestiete.com.br, accessed 2010 May 12.

	Barra Bonita	Bariri	Ibitinga	Promissão	Nova Avanhandava
Reservoir area (km^2)	310	63	114	530	210
Reservoir water volume ($\times 10^6$ m^3)	3622	607	1100	8111	2830
Maximum regulation level (m asl)	451.5	427.5	404	384	358
Watershed area (km^2)	32330	36430	43500	57590	62300
Average depth $\bar{z}$ (m)	11.68	9.63	9.64	15.30	13.47
Perimeter (km)	525	203	375	1423	462
Discharge (m^3/s)	402	443	525	640	688
Sinuosity Index (SI)*	8.41	7.21	9.91	17.44	8.99
Mean water residence time (days)	69	16	24.4	135	48.7
Mean water level fluctuation (m/y)**	5.04	0.40	0.33	4.15	0.47
Year of filling	1962	1965	1969	1975	1982
Reservoir age at the sampling year of the present study	42	43	39	34	26
Generating power capacity (MW)	140.7	136.8	131.4	264	347.4

(*): Sinuosity index calculated as: $SI = p/(\sqrt{4\pi * S})$, where p is the reservoir perimeter (m) and S is the reservoir surface area (m^2).
(**): Calculated from the difference between the maximum and minimum water levels during the year. The mean value was obtained analyzed the reservoirs daily water level data from the historical series of: 1969–2002 (BB = Barra Bonita); 1988–2007 (B = Bariri); 1988–2007 (I = Ibitinga); 1984–2008 (P = Promissão); 1988–2007 (NA = Nova Avanhandava) (Petesse et al., unpublished results).

Nova Avanhandava (NA) and Três Irmãos (TI) (Fig. 1). The upper five reservoirs are currently managed by the AES-Tietê Electric Company and are the subject of this study. Water use in the area, in order of priority, is as follows: electricity production, navigation, public and industrial water abstraction, domestic and industrial sewage dumping, crop irrigation and fishing.

Some morphological and limnological characteristics of these reservoirs are summarized in Table 1. They were built between the 1960s and 1980s and exhibit a similar composition in their native fish species as a result of the homogenization process (Rahel, 2002) promoted by the aging of the reservoir and fish species introductions (Agostinho et al., 1999, 2007; Petesse and Petrere, 2012). None of the dams allow for the upstream passage of fish, and in the 1980s a fish-stoking program with non-native species of commercial interest (*Pterygoplichthys anisitsi*, *Tilapia rendalli, Oreochromis n. niloticus*, *Plagioscion squamossissimus*, *Cyprinus carpio* and *Cichla* spp.) was carried out (Torloni et al., 1993). A total of 14 non-native species, voluntarily or involuntarily introduced, are now resident in the system. Based on the hydraulic management, these water bodies can be grouped into reservoirs with high water level fluctuation (Barra Bonita and Promissão–mean water level fluctuation: 4–5 m/y), and reservoirs with low water level fluctuation (Bariri, Ibitinga and Nova Avanhandava—mean water level fluctuation: <0.50 m/y) (Table 1).

The Tietê River catchment basin is strongly affected by anthropogenic sources of pollution, as twenty-six million people live in the catchment section upstream of the Barra Bonita reservoir (90.5% of the total Tietê River Basin population), which is equivalent to 70% of the population of the State of São Paulo (IBGE, 2010). This basin section is also characterized by a large industrialy concentration and by intensive soil cultivation, especially sugar cane. Conversely, in the catchment section surrounding the downstream reservoirs (Promissão and Nova Avanhandava), the main input of pollution is from non-point sources such as citric cultivation (orange and lemon) and cattle breeding (CETESB, 2008). In relation to the reservoirs trophic state the CETESB (2008) classifies the Barra Bonita reservoir as eutrophic, Bariri, Ibitinga and Promissão as mesotrophic and Nova Avanhandava as oligotrophic, demonstrating that the reservoir chain acts as a "nutrient trap" that contribute to the reduction of the organic charge principally originating in the basin section upper the Barra Bonita reservoir.

2.2. Fish sampling

Fish sampling was carried out in the dry (August, winter) and rainy (February, summer) periods, which represent the dominant seasons in this tropical region (dry season—April to September, and rainy season—October to March). We sampled 72 sites for a total of 144 samples. In each sampling the fish were caught with a set formed by 10 gillnets with mesh sizes ranging from 3 to 12 cm between opposite knots (stretched mesh). The total area of the fishing gears was 358 m^2. The nets were set in the afternoon (17:00–19:00 h) and removed in the following morning (06:00–08:00 h). The sampling effort was stratified by reservoir zones: fluvial, transition and lacustrine, and, inside each zone, by habitat type: central-benthic (C, corresponding to the deepest point, far from the reservoir shoreline), lateral (L, corresponding to the reservoir littoral margin) and tributary mouth (M, corresponding to the tributary margin proximal to its entrance into the reservoir) (see box in Fig. 1). The number of sampling sites in each reservoir was proportional to its surface area (large—Promissão, medium—Barra Bonita and Nova Avanhandava, and small—Bariri and Ibitinga) and shape (sinuosity index and presence of large tributary) in order to maximize the variability in the fish assemblage compositions (Gerritsen et al., 2003). The independence of the sampling sites, inside each reservoir zones, was preserved ensuring a distance of a minimum 3–6 km among habitat typologies. The numbers of sampling sites considered in each reservoir was as follows: 24 in Barra Bonita; 6 in Bariri; 6 in Ibitinga; 24 in Promissão; and 12 in Nova Avanhandava. At the Barra Bonita reservoir, we considered two fluvial zones corresponding to its forming rivers (Tietê and Piracicaba). The Barra Bonita reservoir was sampled in 2003 (winter-dry) and 2004 (summer-rainy); the Promissão reservoir was sampled in 2009, and the remaining reservoirs were sampled in 2007 (winter-dry) and 2008 (summer-rainy). The use of data from different sampling years is not considered a limitation in this study because the vertebrate community is less subject to interannual variations compared with other aquatic organisms, such as macro-invertebrates (Fausch et al., 1990; Loreau et al., 2002). Thus, dramatic changes in the fish composition and organization between years were not expected.

2.3. Environmental aspects

At each sampling site, the following variables were registered prior to the removal of the set of gillnets: water depth (m) and temperature (°C), dissolved oxygen (mg/L), transparency (m), pH and conductivity (µS/cm). The water depth and transparency were recorded with a graduated rope and a Secchi disk, respectively. The chemical variables and water temperature were measured at 20 cm below the water surface with an electronic multi-parameter probe (Horiba U-10). The dissolved oxygen content was determined in the field using Winkler' method. Habitat traits, such as riparian forest, and emerged, floating and submerged macrophytes, were assessed by visual inspection recording the percentages for each sampling site. Habitat variables were recorded to identify and quantify environmental traits that may be important sources of food, nursery habitat and shelter for fish. With the purpose of detecting the presence of an environmental gradient among the reservoir sequence, we subjected the physicochemical and habitat variables collected in the field to a Principal Component Analysis (PCA). Due to the different units of measurement involved, the analysis was accomplished based on the correlation matrix (Hammer et al., 2001).

2.4. Index construction

Previous to the index construction, each sample was analyzed in relation to abundance and species richness. Following Lyons (1992), samples with fewer than 15 individuals or when only a single species was caught were excluded. This avoids that sites with very low catches could bias the scoring of the metrics (Lyons, 1992). Consequently, 48 samples, from the 24 central habitats sampling sites, were not considered in the analyses.

Following Roth et al. (2000), Lyons et al. (2001) and Magalhães et al. (2008), we randomly divided the remaining 48 sampling sites into two parts: a development set (corresponding to 2/3 of the sampling sites), and a validation set (corresponding to 1/3 of the sampling sites). The development set ($n = 64$) was used to select candidate metrics, to define metrics scoring and index calculation, while the validation set ($n = 32$) was used for an independent evaluation of the final index.

The reservoir fish assemblage index (RFAI) was developed from 24 candidate metrics grouped into four groups: (i) species composition, (ii) numerical and weight abundance, (iii) trophic guild, and (iv) well being/health/behavioral categories. We excluded richness metrics because they are influenced by the river catchment or lake surface; in our case all the reservoirs (with the exception of Barra Bonita) are isolated and the richness metrics should be biased by the introduction of alien species. Each candidate metrics represents a measurable characteristic of the biological subject that changes in a predictable way, according to environmental stressors (Karr et al., 1987; Karr, 2006).

The description of each candidate metrics is as follows.

Number of Characiformes species (Var1)—This metrics was used by Araújo et al. (2003) for the evaluation of the water column habitat. It is justified because these are species that rely on vision to find food, are capable of large and small displacements, and are widely distributed in the habitat. In general they are *r*-strategists and opportunistic.

Number of Siluriformes species (Var 2)—The choice of this group is justified because Siluriformes are adapted to benthic habitats and are highly specialized. This metrics was originally used by Hugueny et al. (1996) for African rivers, and by Araújo et al. (2003), Bozzetti and Schulz (2004), Petesse et al. (2007), Terra and Araújo (2011) for Brazilian rivers and reservoirs.

Number of Cichlidae species (Var3)—This metrics is indicative of the degradation of the littoral habitat, as these species use it for spawning and nest construction (Hugueny et al., 1996). In our study it was considered to be negative (increasing with the degradation) because this group is entirely composed of non-native species.

Number of species comprising 80% of the abundance (Var4)—This metrics intends to measure the relative importance of the species in the assemblage. Assemblages with a large numbers of species comprising 80% of the individuals show a high diversity and fewer differences between species that are rare and abundant. This is typical of a mature community with high environmental diversity (Rossaro, 1993).

Number of tolerant species (Var5)—This metrics represents species whose abundance and distribution increases with degradation. The species considered for this metrics, were selected by observing their abundance distributions in the cascade system and by a literature survey (Araújo et al., 2003; Barrella and Petrere, 2003; Smith et al., 2003). The following species were considered tolerant: *Hoplosternum littorale, Pterygoplichthys anisitsi, Hypostomus ancistroides, Pimelodus maculatus, Oreochromis niloticus, Satanoperca pappaterra* and *Geophagus brasiliensis*.

Percent of dominance (Var6)—This metrics is based on the evidence that only a few and tolerant species increase their relative abundance in degraded environments (Hughes and Oberdorff, 1999). This was considered to be a negative metrics, with high percentages being indicative of degraded conditions.

Total number (N) and weight (W) of individuals (Var7 and Var8)—Abundance and weight metrics are a gross measure of fish production (Ganasan and Hughes, 1998) and are historically based on the assumption that, the total number or weight of individuals is expected to be lower in a disturbed environment compared to an undisturbed environment (Hugueny et al., 1996; Roth et al., 2000; Griffith et al., 2005). The range of values of these metrics was preliminarily analyzed in relation to a disturbance gradient to verify their variability in relation to cultural eutrophication. Because there is a great amount of the non-native species in the study area, we also decided to assess separately the contribution of native and non-native metrics.

Total number (N) and weight (W) of native individuals (Var9 and Var10)—We justify the choice of these metrics because the native fish composition among the reservoirs had increased in similarity from the 2000s (Petesse and Petrere, 2012), and because it is now important to monitor their abundance/biomass evolution in comparison to that of non-native species.

Total number (N) and weight (W) of non-native individuals (Var11 and Var12)—The introduction of these metrics is justified by the observation that tolerant, opportunistic or non-native species can be found in great abundance under impaired conditions. In the current adaptation, we assessed these metrics because non-native species tend to be more abundant in reservoirs and could easily spread throughout the river basin, impairing assemblages far from the impoundment site (Havel et al., 2005). For this reason, Kennard et al. (2005) caution that non-native species may represent a symptom and a cause of decline in the integrity of the fish assemblage, thereby being responsible for the loss of native species, pathogens diffusion, and habitat structure modifications (Clavero and García-Berthou, 2005; Ricciardi, 2007; Thomaz et al., 2012). We defined the abundance and weight of the non-native species metrics as a negative trait (high values indicating a strong negative alteration from the original assemblage). This is based on the evidence that in a disturbed or artificial environment, non-native species are favored by human actions (voluntary or accidental introductions) and by their own biological advantages (feeding opportunism, higher tolerance and effective reproductive strategies). These metrics were also assessed as *Percent of non-native individuals in number and weight (Var13 and Var14)*.

Percent of individuals larger than 30 cm (Var15)—This metrics was assessed with the objective of evaluating the relative abundance of medium to large individuals. It is intended to assess the impoundment's impact on larger migratory fish and the aging process in reservoirs that exhibits a reduction in large and long-lived species (k-selection) in favor of small and short-lived fish (*r*-selection) (Agostinho et al., 1999). A reduction in fish mean length is also considered an index of over-fishing (Hilborn and Walters, 1992).

Percent in number and in weight of omnivores (Var16 and Var17)—Omnivore metrics are used for IBI adaptation worldwide. Originally proposed by Karr (1981), such metrics assess food chain alterations. A high proportion of these species, normally opportunists and *r*-strategist species, indicates a simplification of the food chain. This metrics includes species without a specialized diet, for which the alimentary supply varies according to availability.

Percent in number (N) and in weight (W) of top carnivores (Var18 and Var19)—Top carnivores are highly specialized species that are long-lived, and sensitive to physicochemical stress (e.g., bio accumulation of toxic substances) or human impact (e.g., over-fishing). In face of its apex position atop the trophic chain, the percent abundance of this group is widely used in multimetric indices because these species respond to every change occurring at the lower trophic levels. Therefore, high values were associated with high environmental quality.

Percent in number (N) and in weight (W) of iliophagous (Var20 and Var21)—Iliophagous fishes are considered herein because in the tropics, they are specialized species exploring a very thin and muddy substratum (Lowe-McConnell, 1987). In the present study, their abundance varied greatly among the reservoirs when compared to omnivores and, the highest abundances were associated with organic enrichment originating from cultural eutrophication. Consequently, a high abundance of iliophagous species was considered indicative of poor environmental conditions. The Curimatidae and Prochilodontidae families are representatives of this trophic group (Fugi et al., 2001).

Percent (by the number) of individuals with anomalies, lesions or eroded fins (Var22)—This metrics is based on the evidence that healthy individuals are found in a healthy habitat. In severely degraded conditions the chance of developing tumors or body malformations in fish increases. Eroded fins can be attributed to specific predatory behaviors of species or structural habitat degradation. Under these conditions, the chance of a fish contracting mycosis or infection with weakening effects increases. This metrics is extensively used for IBI adaptations worldwide (Hughes and Oberdorff, 1999).

Numbers of migratory species (Var23)—Migratory species are the most affected by dams (Agostinho et al., 2007) as their routes are cut off. Particularly affected are the large migratory species that have slowly disappeared in the dammed river cascade. However, some small and medium-sized species (such as *Pimelodus maculatus*, *Prochilodus lineatus*, and *Rhinelepis aspera*) and some species of genera *Leporinus* and *Astyanax* adapted to the reservoir habitat are found performing short-distances migrations, looking for suitable reproduction habitats or better living conditions. In the reservoir cascade system of the Tietê River, there is no upstream passage for fish, thus, when present, the migrations occur in lateral tributaries.

Number of species with parental care (Var24)—This metrics was assessed because in reservoirs, these species are favored and their populations tend to increase over time (Agostinho et al., 1999). We considered this metrics as negative (high values indicate poor conditions). Species displaying parental care were selected based on a literature survey (Vazzoler and Menezes, 1992; Nakatani et al., 2001; Suzuki et al., 2004).

The RFAI index was calculated in fish numbers ($RFAI_N$) and weight ($RFAI_W$) to verify the effect of the unit measures of abundance and trophic guild categories on the response of the index. In general, the counting metrics (number of individuals) is preferred in streams and rivers because it is considered a more direct expression of the system's productivity (Griffith et al., 2005; Hugueny et al., 1996), but some authors suggest the use of the weight measure for lakes in particular (Drake and Pereira, 2002; Drake and Valley, 2005; Gassner et al., 2003; Minns et al., 1994).

Candidate metrics were selected based on three criteria: (i) *range test*: we retained those metrics with sufficient variability among sampling sites. In agreement with Whittier et al. (2007), we discarded metrics with range values less than or equal to 4 and percentage metrics with a range less than 10%. If at least 75% of a given metrics had equal values, it was considered inadequate to detect differences among the samples, and it was subsequently discarded; (ii) *responsiveness*: we verified the metrics' responsiveness to physicochemical and habitat variables recorded in the field by Spearman's correlation. Those metrics that displayed two or more significant Spearman correlations with the water quality and habitat variables were considered responsive and passed the test (McCormick et al., 2001); (iii) *redundancy*: metrics were considered redundant if the Spearman correlation coefficient between pairs of metrics was $r_s > 0.70$ (Whittier et al., 2007). According to McCormick et al. (2001), for redundant pairs, those metrics with a smaller number of significant correlations obtained from the responsive test, were excluded.

2.5. Metrics scoring

As cascade reservoirs can be considered "unique" in their typology, and due to the impossibility of finding reference conditions from a similar natural environment, the metrics scoring was based on the best observed condition criteria in agreement with Jennings et al. (1995), McDonough and Hickman (1999) and Stoddard et al. (2006). To this end, first we examined the box-whiskers plot distribution of the value of each metrics in the calibration data sets to individualize the 5th and 95th percentiles. After this, scores intervals were defined by trisecting the box-whiskers plot after omitting the 5th and 95th percentiles to exclude the effects of outlier or extreme values (McDonough and Hickman, 1999). A discrete scoring criterion was chosen (Gerritsen et al., 2003; Joy and Death, 2004), and to each metrics was assigned a score of 1, 3, or 5 depending on whether the value strongly departed from (1), moderately departed from (3) or minimally departed from (5), the best observed condition range. In this way, the upper third, for metrics in which high values indicate high quality, received a score of 5, values below or within the first third received a score of 1, and values within the middle third received a score of 3. For those metrics in which low values indicated high quality, an inverse scoring criterion was used.

2.6. Scoring correction

Before the assignment of the score, each selected metrics was assessed for score correction or calibration. This procedure is normally applied to richness metrics because as stated by Fausch et al. (1990) and Klemm et al. (2003), these are influenced by morphologic characteristics such as catchment area, river order, lake surface or elevation. In our study we extend this procedure to all the selected metrics, because, reservoirs show heterogeneous environments and biological organizations on their own longitudinal gradient (Agostinho et al., 2007; Tundisi and Straškraba, 1999) due to the intermediate nature between rivers and lakes (Thornton, 1990). The calibration was applied observing the box-plot distribution of the metrics values by zones (fluvial, transitional and lacustrine). When a clear longitudinal increasing or decreasing tendency in the metrics distribution was observed, we trisected the metrics values separately for each zone (McDonough and Hickman, 1999). This provision had the effect of increasing the precision of the index.

2.7. Final RFAI

The final RFAI index was calculated by summing the partial scores assigned to each selected metrics; four categories of biological status were successively defined: very poor, poor; acceptable and good.

Table 2
Characteristics of the data set (development and validation) used to develop and validate the RFAI for the Tietê reservoirs system. Values are the mean ± SD and, inside parenthesis, minimum and maximum values.

	Development set ($n = 64$)	Validation set ($n = 32$)
Depth (m)	3.6 ± 1.6 (1.2; 9)	3.8 ± 1.5 (1.5; 8)
Water Temperature (ºC)	24.0 ± 3.5 (18.8; 29.6)	24.0 ± 3.5 (18.8; 29.0)
pH	7.9 ± 0.7 (6.8; 9.7)	7.9 ± 0.7 (6.3; 9.3)
Transparency (m)	1.1 ± 0.6 (0.1; 3.0)	1.2 ± 0.4 (0.4; 2.2)
Conductivity (μS/cm)	173.9 ± 67.6 (53.0; 447.0)	157.9 ± 47.5 (29.0; 275.0)
O_2 (mg/L)	6.1 ± 3.3 (0.7; 14.6)	5.8 ± 2.8 (1.4; 12.2)
Riparian forest (%)	49.8 ± 31.9 (0.0; 100)	58.4 ± 32.86 (10.0; 100)
Emergent macrophytes (%)	33.4 ± 18.3 (0.0; 70.0)	26.9 ± 13.3 (0.0; 60.0)
Floating macrophytes (%)	32.6 ± 23.4 (0.0; 60.0)	38.86 ± 22.7 (0.0; 80.0)
Submerged macrophytes (%)	15.0 ± 25.8 (0.0; 90.0)	15.6 ± 19.76 (0.0; 60.0)

The index variability among the reservoirs and sampling season was assessed through the coefficient of variation ($CV = (s/\bar{x}) \times 100$) (Gerritsen et al., 2003). Ideally, the CV should be small, perhaps an arbitrary CV < 15% would be a good choice.

2.8. RFAI validation

We performed the index validation as follows: (i) we first verified the ability of the index to produce repeatable scores from an independent data set. According to Astin (2007), this is the preferred method to validate a bio-assessment index. For this purpose, we used the validation data set initially separated from the full data set and scored the metrics using the intervals previously defined by the development set. Finally, the Mann-Whitney *U*-test was employed to detect significant differences between the development and validation data sets (Magalhães et al., 2008). (ii) We tested the responsiveness of the final indices ($RFAI_N$ and $RFAI_W$) to the selected metrics and physicochemical and habitat variables by Spearman correlations and, finally, (iii) we verified if whether the first two PCA ordination axes, representative of the observed environmental gradient previously obtained analyzed the physicochemical and habitat variables collected in the field, it were correlated with the RFAI scores.

The package Statistica 7.0 was used for the analyses.

3. Results

3.1. Data set description

The analyzed data set was composed of a total of 15,158 individuals of 51 species. The development set was composed of 51 species and 10,589 individuals, whereas the validation set included 43 species and 4569 individuals. Only slight differences were detected in the environmental characteristics between the development and validations data set (Table 2).

3.2. Index development

Table 3, for each candidate metrics, shows the tendency to increase or decrease with human interference and the steps of the selection process.

All the metrics passed the range test; however, eight were excluded for low responsiveness to the physicochemical and

Table 3
Candidate metrics, metrics codes, expected response to environmental degradation, and selection stages process.

	Metrics code	Expected response	1-Range test	2-Responsiveness	3-Redundancy
I. Species composition metrics					
Number of Characiformes species	Var1	Increase	Passed the test	–	
Number of Siluriformes species	Var2	Decrease	Passed the test	Passed the test.	–
Number of Cichlidae species	Var3	Increase	Passed the test	–	
Number of species comprising 80% of the abundance	Var4	Decrease	Passed the test	–	
Number of tolerant species	Var5	Increase	Passed the test	Passed the test	Passed the test
II. Abundance/weight metrics					
Percent of dominance	Var6	Increase	Passed the test	Passed the test	Passed the test
Total number of individuals	Var7	Decrease	Passed the test	–	
Total weight of individuals	Var8	Decrease	Passed the test	Passed the test	–
Numbers of native individuals	Var9	Decrease	Passed the test	Passed the test	Passed the test
Weight of native individuals	Var10	Decrease	Passed the test	Passed the test	Passed the test
Number of non-native individuals	Var11	Increase	Passed the test	Passed the test	Passed the test.
Weight of non-native individuals	Var12	Increase	Passed the test	Passed the test	Passed the test.
Percent of non-native individuals by number	Var13	Increase	Passed the test	–	
Percent of non-native individuals by weight	Var14	Increase	Passed the test	–	
15. Percent by number of individuals with total length >30 cm	Var15	Decrease	Passed the test	–	
III. Trophic guild metrics					
Percent of omnivores individuals by number	Var16	Increase	Passed the test	Passed the test	Passed the test
Percent of omnivores individuals by weight	Var17	Increase	Passed the test	Passed the test	Passed the test
Percent of top carnivores individuals by number	Var18	Decrease	Passed the test	Passed the test	Passed the test
Percent of top carnivores individuals by weight	Var19	Decrease	Passed the test	Passed the test	Passed the test
Percent of iliophagous individuals by number	Var20	Increase	Passed the test	Passed the test	Passed the test
Percent of iliophagous individuals by weight	Var21	Increase	Passed the test	Passed the test	Passed the test
IV. Health/Migratory/Reproduction metrics					
Percent of individuals (by number) with lesion or anomalies	Var22	Increase	Passed the test	Passed the test	Passed the test
Number of migratory species	Var23	Decrease	Passed the test	Passed the test	Passed the test
Number of species with parental care	Var24	Increase	Passed the test	–	

Table 4
Selected metrics and score intervals for the final $RFAI_N$ and $RFAI_W$ of Tietê cascade reservoir system.

Metrics code	Metrics	Calibration by Reservoir zone	Index scores			Best	Worst
			1	3	5		
Metrics commons to $RFAI_N$ and $RFAI_W$							
Var5	Number of tolerant species	–	>3.3	1.6–3.3	<1.6	0	7
Var6	Percent of dominance	–	>53.8	38.4–53.8	<38.4	19	75.7
Var22	Percent of individuals (by number) with lesion or anomalies	–	>23.1	12.0–23.1	<12.0	0	40.9
Var23	Number of migratory species	–	<2.3	2.3–3.5	>3.5	6	1
Metrics of $RFAI_N$							
Var11	Number of non-native individuals	Fluvial	>90	46–90	<46	2	164
		Transition	>86.2	47.6–86.2	<47.6	5	228
		Lacustrine	>96.5	55.6–96.5	<55.6	8	145
Var9	Number of native individuals	–	<123	123–212	>212	365	3
Var16	Percent of omnivores individuals by number	–	>48.8	27.4–48.8	<27.4	0	78.7
Var18	Percent of top carnivores individuals by number	Fluvial	<13.7	13.7–27.5	>27.5	51.8	0
		Transition	<25.5	25.5–46.9	>46.9	84.2	2.7
		Lacustrine	<29.3	29.3–52.7	>52.7	83.3	3.5
Var20	Percent of iliophagous individuals by number	Fluvial	>46.2	24.7–46.2	<24.7	0	81.2
		Transition	>40.18	20.1–40.2	<20.1	0	66.8
		Lacustrine	>15.9	7.9–15.9	<7.9	0	58.5
Metrics of $RFAI_W$							
Var12	Weight of non-native individuals	Fluvial	>10.3	5.3–10.3	<5.3	0.1	17.7
		Transition	>12.1	6.8–12.1	<6.8	0.36	33.6
		Lacustrine	>12.8	7.3–12.8	<7.3	0.9	23.7
Var17	Percent of omnivores individuals by weight	–	>40.2	23.2–40.2	<23.2	0	79
Var21	Percent of iliophagous individuals by weight	Fluvial	>32.7	17.7–32.7	<17.7	0	51.9
		Transition	>26.7	13.4–26.7	<13.4	0	47.3
		Lacustrine	>18.9	9.5–18.9	<9.5	0	38.5
Var19	Percent of top carnivores individuals by weight	Fluvial	<22.3	22.3–44.7	>44.7	71.4	0
		Transition	<30.8	30.8–54.1	>54.1	80	7
		Lacustrine	<33.4	33.4–55.6	>55.6	79	8.9
Var10	Weight of native individuals	–	<6.2	6.2–11.2	>11.2	21.7	0.2

habitat variables. Among them, the metrics “number of native individuals—Var9” was retained instead of “number of total individuals—Var7” because it was the only metrics assessing the importance of native species in the fish assemblages of the reservoirs system. The remaining metrics were subjected to a redundancy evaluation, and two additional ones were excluded (the “number of Siluriformes species”—Var2, and the “total weight of individuals”—Var8). The final RFAIs ($RFAI_N$ and $RFAI_W$) resulted from nine metrics: four were common between them and the others were specific to each one of the indices (Table 4). Three metrics for $RFAI_N$ and the corresponding ones for $RFAI_W$ (“Percent of top carnivores individuals” by number and weight—Var18 and Var19, “Percent of iliophagous individuals” by number and weight—Var20 and Var21 and “Number/Weight of non-native individuals”—Var11

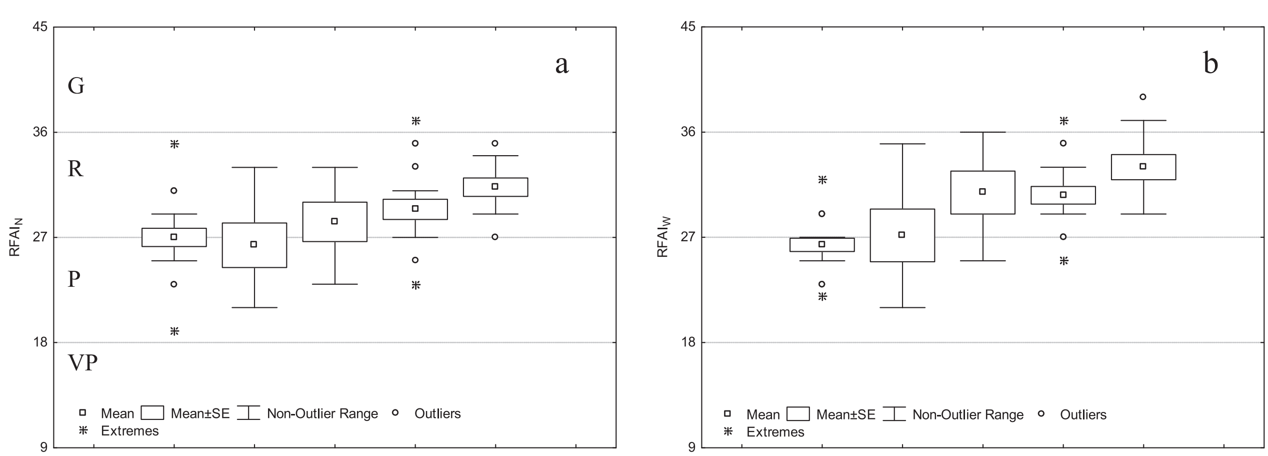

Fig. 2. Spatial variability of the $RFAI_N$ (a) and $RFAI_W$ (b) indices in the cascade reservoir system of the Tietê River. Index categories of biological condition: G = Good; R = Reasonable; P = Poor; VP= Very Poor. Reservoir codes: BB = Barra Bonita; B = Bariri; I = Ibitinga; P = Promissão; NA = Nova Avanhandava.

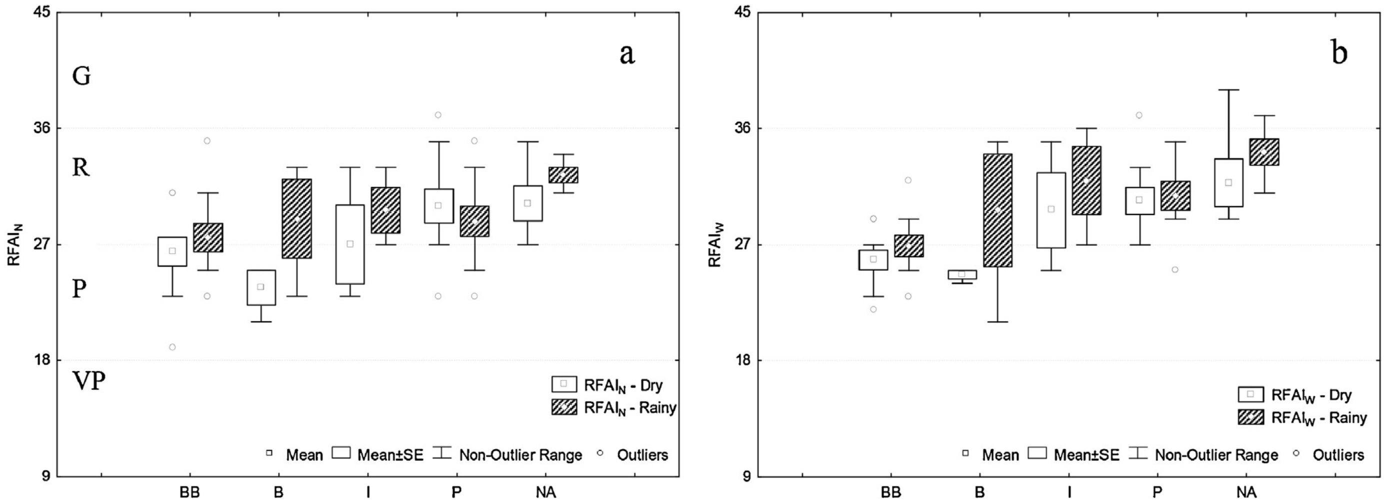

Fig. 3. Box-plot of the $RFAI_N$ (a) and $RFAI_W$ (b) scores from the dry and rainy seasons. Index categories of biological condition: G = Good; R = Reasonable; P = Poor; VP = Very Poor. Reservoir codes: BB = Barra Bonita; B = Bariri; I = Ibitinga; P = Promissão; NA = Nova Avanhandava.

and Var12), require calibration of the score intervals by zone (Table 4). The final indices varied inside a possible range of 9–45. This interval was divided into four categories representing the biological conditions of the fish assemblages in the cascade reservoirs system as follows: very poor (9–18), poor (19–27), reasonable (28–36) and good (37–45).

3.3. Final RFAI

The mean values of the final index scores showed very little difference between $RFAI_N$ (28.7) and $RFAI_W$ (29.5). The minimum values were 19 and 21 for $RFAI_N$ and $RFAI_W$, respectively. The maximum value, for both indices, was 39. The samples classified as "poor" were 48.4% for $RFAI_N$ and 40.6% for $RFAI_W$, and none was classified as "very poor". Only one sample for $RFAI_N$ and three samples for $RFAI_W$ were classified as "good". The remaining samples (50.0% for $RFAI_N$ and 54.7% for $RFAI_W$) were classified as "reasonable".

$RFAI_N$ and $RFAI_W$ were highly correlated ($r_s = 0.86$; $n = 64$; $p < 0.01$), and both showed a slight tendency for score improvments from the Barra Bonita to Nova Avanhandava reservoirs (Fig. 2 a, b). In fact, the main portion of the sites in the biologically poor category for both RFAIs belongs to the Barra Bonita and Bariri reservoirs.

In relation to the temporal variability, the two indices showed better scores in the rainy season (Fig. 3 a, b), but the differences were not significant (Man-Whitney U test: $U_{RFAI\text{-}N} = 429.0$, $p = 0.26$, $n = 32$; $U_{RFAI\text{-}W} = 391.0$, $p = 0.10$, $n = 32$). For $RFAI_N$, Bariri Ibitinga and Nova Avanhandava reservoirs showed high variability of CV between the seasonal index values in relation to the other reservoirs (Fig. 4a). In the case of $RFAI_W$, higher variability was observed only for the Bariri and Nova Avanhandava reservoirs (Fig. 4b).

3.4. Validation

There was no difference in the RFAI scores between the development and validation data sets (Fig. 5), as confirmed by the Man-Whitney U test which showed no statistical significance for the two indices ($U_{RFAI\,N} = 954.0$, $p = 0.58$; $U_{RFAI\,W} = 888.5$, $p = 0.29$).

The final $RFAI_N$ scores had little correlation with the physicochemical and habitat variables (Table 5), showing significant Spearman correlation with water temperature (positive) and conductivity (negative) only. In contrast, $RFAI_W$ was found to be significantly and positively correlated to water temperature, pH, dissolved oxygen and percentage of submerged macrophytes, and negatively to conductivity. In relation to the selected metrics, each RFAIs was responsive to four out of nine metrics. $RFAI_N$ was

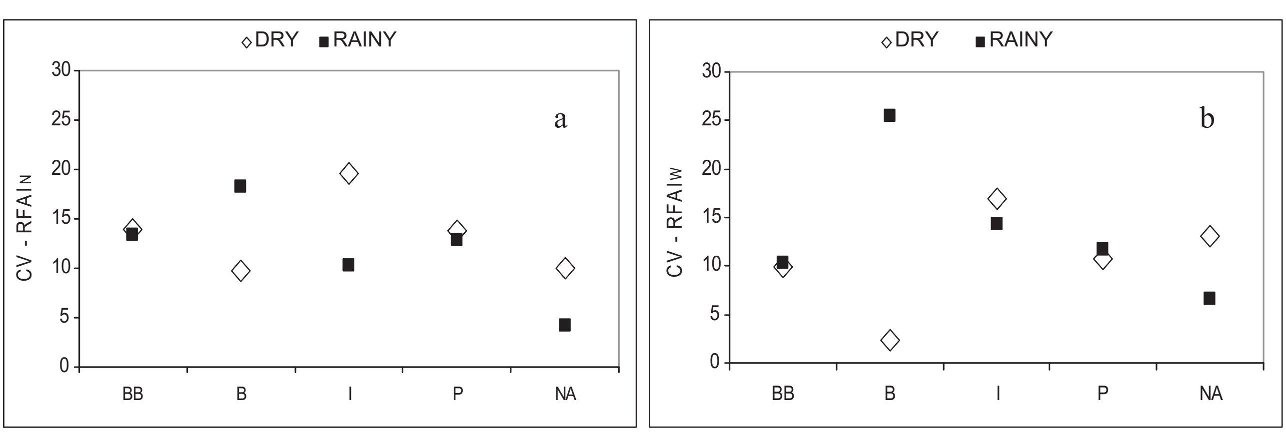

Fig. 4. Coefficients of variability (CV) for reservoirs RFAIs scores and sampling periods. Reservoir codes: BB = Barra Bonita; B = Bariri; I = Ibitinga; P = Promissão; NA = Nova Avanhandava.

Table 5
Spearman rank correlation showing the responsiveness of the selected metrics and of the final RFAIs scores with environmental variables, and the first two PCA axes derived from physicochemical and habitat variables registered in the field (only showed correlation coefficients significant for $p < 0.05$).

	Var5	Var11	Var9	Var6	Var16	Var18	Var20	Var22	Var23	Var12	Var17	Var21	Var19	Var10	$RFAI_N$	$RFAI_W$
Depth (m)	0.33				−0.28		0.29					0.28	−0.26			
Water Temperature (ºC)		0.49	0.29	−0.25		0.44		0.25	0.27	0.48			0.39	0.45	0.28	0.42
pH		0.31				0.41	−0.31	0.39		0.29		−0.33	0.43			0.27
Transparency (m)	−0.38					0.27							0.28			
Conductivity (μS/cm)	0.48							−0.35					−0.30		−0.29	−0.25
O_2 (mg/L)	−0.45					0.57	−0.43	0.42				−0.47	0.64			0.42
Riparian forest (%)				−0.34												
Emergent macrophytes (%)											−0.26					
Floating macrophytes (%)					−0.32				−0.28		−0.29			−0.25		
Submerged macrophytes (%)	−0.61					0.64	−0.66	0.58				−0.63	0.60			0.33
I PCA	−0.62					0.69	−0.61	0.57				−0.60	0.69		0.29	0.40
II PCA													−0.31			
$RFAI_N$	−0.37	–	–	−0.41	–	0.36	–	–	0.29							0.86
$RFAI_W$	−0.53			−0.36				–	–	–	–	−0.27	0.57	–	0.86	

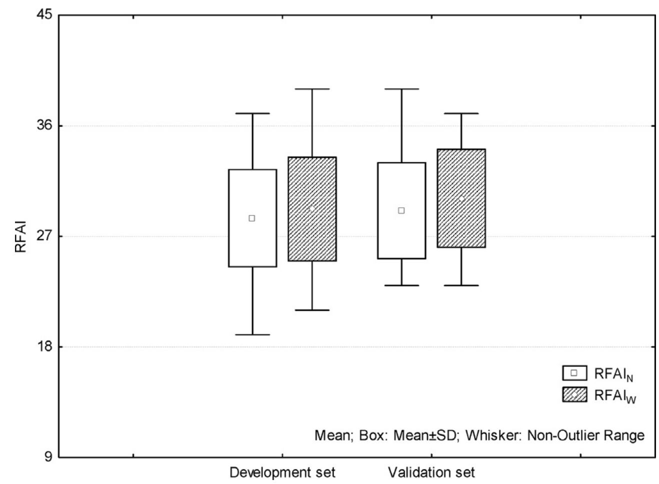

Fig. 5. Comparison between RFAIs scores of the Development and Validation data sets.

responsive to the following: Number of tolerant species (Var5), Percent of dominance (Var6), Percent of top carnivores by number (Var18) and Number of migratory species (Var23). $RFAI_W$ to was responsive to the following: Number of tolerant specie (Var5), Percent of dominance (Var6), Percent of iliophagous by weight (Var21) and Number of top carnivores by weight (Var19) (Table 5).

Finally, the first two axes of the PCA, related to the physicochemical and habitat variables of the original data set ($n = 96$), explained 39.86% of the total variance (I PCA: 23.59; II PCA: 16.26). The first axis, showed the separation of Barra Bonita and Bariri from Ibitinga, Promissão and Nova Avanhandava (Fig. 6). In general, Barra Bonita and Bariri were deeper, with higher water conductivity and abundant floating and emergent macrophytes. In contrast, the Ibitinga, Promissão and Nova Avanhandava reservoirs, presented high values of pH, dissolved oxygen and transparency with high percentages of riparian forest and submerged macrophytes. The first PCA axis can be considered representative of the environmental improvement along the longitudinal gradient in the cascade system, and there was a significantly correlation to the RFAIs ($RFAI_N$: $r_S = 0.29$, and $RFAI_W$: $r_S = 0.40$, $n = 96$; $p < 0.01$) (Table 5). The second PCA axis was representative of the temporal variability, showing the separation between the dry and rainy periods, but neither of the RFAIs was correlated with this axis.

4. Discussion

The multimetric approach for the biological assessment of reservoirs or other habitats heavily altered by humans represents a fundamental step in monitoring evolution and in providing maintenance/mitigation actions to safeguard the anthropogenic services that depend on the ecological functioning of aquatic ecosystems.

The biological components of the cascade reservoir system of the Tietê River are subject to multiple and known categories of stressors represented by (i) untreated industrial and domestic sewage from the metropolitan region of São Paulo capital city, (ii) nutrients and pesticides input from intensive agriculture uses, and (iii) the deeply altered river hydraulic flood regime solely managed to maximize electric production. In this context, we are unable to clearly separate the effects of each stressor from the naturals ones as the overall catchment area of the cascade system is heavily altered by human concentration and uses. Thus, it is more useful to assess the environmental conditions of the system by analyzing the "response" of the organisms living in this stressed environment using a "response-oriented" approach (Abbasi and Abbasi, 2012).

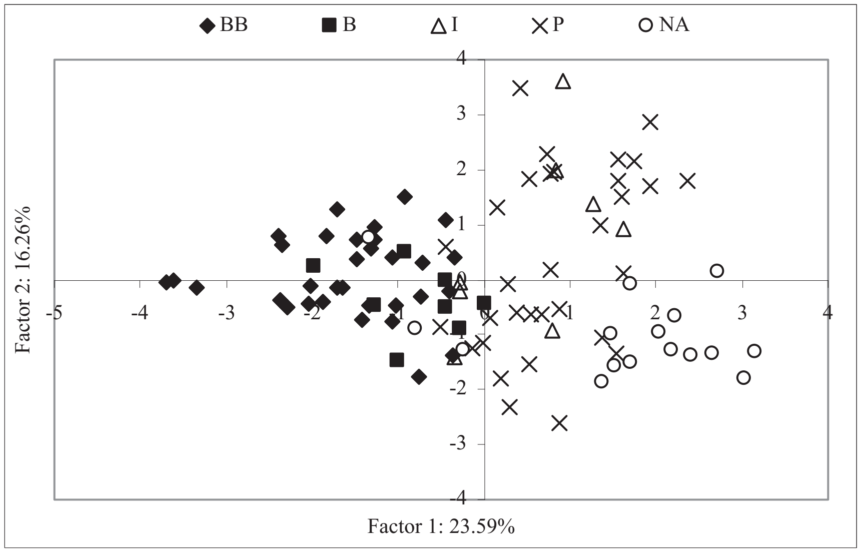

Fig. 6. PCA from physicochemical and habitat variables from the total data set (n = 96). Reservoir codes: BB = Barra Bonita; B = Bariri; I = Ibitinga; P = Promissão; NA = Nova Avanhandava.

For this, we recorded the basic water quality and environmental variables that are important for fish life and selected those ecological attributes of fish assemblages that are more responsive to the longitudinal environmental gradient clearly observed in the Tietê cascade system, which showed the upstream reservoirs (Barra Bonita and Bariri reservoirs) in a worse condition in comparison with the downstream reservoirs of Promissão and Nova Avanhandava. This aspect was reported by Barbosa et al. (1999) who first documented an "inverted water quality gradient" in this cascade system that was principally attributed to untreated sewage from São Paulo city. Additionally, Barrella and Petrere (2003), affirmed that this reservoir cascade acts as a quasi efficient sewage water treatment plant, contributing to the improvement of the water quality in the lower section of the river gradient.

We developed our indices by excluding the central habitat of reservoirs, as this habitat is characterized by low fish species composition and abundances. This aspect is related to the simplified structure of central reservoir habitat and to the more restrictive environmental conditions typical of the reservoirs' hypolimnion, especially in the rainy (summer) period. In agreement with Sutela et al. (2013), we suggest that monitoring and ecological status assessment of reservoirs should focus on littoral biota at the reservoir shoreline and tributary mouth habitats.

The final indices represent a refinement and optimization of an index preliminarily adapted only to the Barra Bonita reservoir by Petesse et al. (2007). The extension of the multimetric approach to five out of six reservoirs of the cascade improved the scoring, criterion that was based on the best (maximum or minimum depending on the metrics) observed conditions (Jennings et al., 1995) along the longitudinal gradient of disturbance observed in the sequence of the studied reservoirs.

In this study, five out of nine metrics (Number of tolerant species, Percent of dominance, Percent of omnivores individuals, Percent of carnivores individuals, Percent of individuals with lesion or anomalies) were common to those selected in other studies in the USA (Jennings et al., 1995; McDonough and Hickman, 1999), Brazil (Terra and Araújo, 2011) and France (Launois et al., 2011), evidencing the suitability of these ecological attributes for the bio-assessment of reservoirs. The selection of the other metrics (percentage of iliophagous, number of migratory species and abundance/biomass of native and non-native species) appears to reflect local factors such as species composition and the productivity of each water body.

The metrics "abundance and weight of iliophagous" and "abundance and weight of top carnivores" showed a clear longitudinal gradient in agreement with the improvement in water quality and trophic classification resulting from the PCA analyses and CETESB annual monitoring (CETESB, 2008). In particular, the highest iliophagous metric values were observed in the upstream reservoirs of Barra Bonita and Bariri, and this finding can be associated with the organic enrichment originating from cultural eutrophication. Conversely, the carnivores showed maximum values in the downstream reservoirs Promissão and Nova Avanhandava, which are characterized by mesotrophic and oligotrophic conditions, respectively.

The $RFAI_N$ and $RFAI_W$ indices are highly correlated and both showed slightly better scores in the rainy season. The better scores observed in this period may be associated with the following: (i) the rainy season triggers reproduction in many tropical species (Vazzoler and Menezes, 1992), increasing their activities and catchability; (ii) in tropical environments, the rainy season is frequently associated with fish kills due to the high summer temperatures, lower dissolved oxygen content, resuspension of toxic elements from the substrata, and toxic algae blooms (Barrella and Petrere, 2003). In large and deep reservoirs, as in the case of the Tietê cascade system, this aspect particularly affects the central and deeper areas. Under these conditions, the fish assemblages concentrate along the shoreline or display short migratory behaviors by moving to reservoir tributaries looking for better environmental conditions or suitable reproductive substrata (Agostinho et al., 2007).

In the dry season, the environmental conditions became less limiting in comparison to the rainy season, increasing the water volume available for the fish, especially in the Bariri, Ibitinga and Nova Avanhandava reservoirs, where the water level is roughly stable year-round (Table 2). In the dry season, the Barra Bonita and Promissão reservoirs experience the maximum water storage (April–July), increasing the connectivity with the fluvial plain and the availability of some food resources, nursery and shelter habitats for fish assemblages.

Concerning spatial variability, the $RFAI_W$ index appears to be more effective in detecting differences among reservoirs. Furthermore, it appears to be more responsive to environmental variables than $RFAI_N$. This result can be justified by considering that many species are small-sized Characidae, which are characterized by high feeding plasticity and reproductive compensation (*r*-strategists) (Agostinho et al., 2007). These species are adapted to impairment conditions, as in the case of the upstream reservoirs, but due to their small body size, little contributes to the biomass of the system.

These findings, allow us to affirm that the dry season is the better period for the development of bio-assessment sampling protocols in this system. In fact, this season exhibits more homogeneous species distributions and stable environmental conditions. This agrees with Pinto and Araujo (2007) and Terra and Araújo (2011), who suggested the dry season as the preferred sampling period for tropical fluvial and river-reservoir environments, respectively. We justified this choice by taking into account that the Bariri, Ibitinga and Nova Avanhandava reservoirs are the smallest in the cascade system and that, with the worsening of the water quality during the rainy season, the fish assemblages concentrate along the fish assemblages concentrated in the shoreline or migrated into tributary habitats migrate into tributary habitats and thus bias the fish sampling.

We based our analyses on a site-specific approach and this should preclude the utilization of the presented indices out of this study area. However, according with our objectives, this approach was found to be a valid model for the development of a multimetric assessment tool in other cascade systems. At the catchment scale, considering that cascade systems are “unique” in their typology, our approach may be considered advantageous especially for defining management actions. In fact, from the observed status, it will be possible to define specific objectives of management similar to the “Best Attainable Condition” (BAC) proposed by Stoddard et al. (2006). In this case, BAC refers to an expected condition that can be achieved by taking into account the best management practices and the application of economic resources to manage for the best condition in the face of human disturbance. This objective can be calibrated on different spatial (local or catchment scale) and temporal scales (objectives at short, medium and long terms) depending of the importance of the intervention. New assessments every 3–5 years can be realized to verify the progress of the management objectives, allowing the refinement of the metrics, and the better calibration of the scoring criteria.

Further studies should include cascade reservoirs from other river systems to allow the selection of metrics at the regional or eco-regional level and the recording of more specific stressors variables. In this case, the data set will be representative of a wide physicochemical and environmental variability, thus widening the applicability of the indices.

5. Conclusion

The final RFAIs and the selected metrics were able to explain the observed biological gradient in the Tietê cascade reservoir system. They indicated that the fish multimetric approach is a suitable tool for the assessment of reservoir biological conditions. Based on the RFAIs scores, our findings showed that the Tietê River cascade reservoir system should be divided into two groups: (i) Barra Bonita and Bariri, with fish assemblages in poor condition, and (ii) Ibitinga, Promissão and Nova Avanhandava with fish assemblages in reasonable condition. This finding is in agreement with the longitudinal water quality and habitat improvement detected by physicochemical analyses and the habitat variables collected in the present study.

Finally, we recommend utilizing weight metrics in reservoir index development and, focusing the sampling in littoral areas, and we suggest that the dry season be considered as the preferable sampling season for bio-assessment programs in tropical areas.

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