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The protected areas system in Brazil as a baseline condition for wetlands management and fish conservancy: the example of the Pantanal National Park

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Considering the need for the Brazilian government to develop tools for environmental monitoring for biodiversity conservancy purposes in the national protected areas system, this paper focuses on determining reference site metrics (or baselines) for adapting the Index of Biotic Integrity (IBI) based on the fish assemblages in the Pantanal National Park (PNP). The habitats in the PNP were grouped into four categories: main rivers, *corixos* (channels connecting the floodplain), permanent bays, and temporary bays. Fish samplings were performed at 12 points during the dry season (Oct-Nov 2010 and 2011). 146 fish species were identified from the total 18,954 individuals collected with standardized fishing gear. There was no association between the structure of the fish assemblage and categories, suggesting a theory on homogeneity of habitats. The final IBI_{PNP} consists of nine metrics, most of them were framed in *excellent* class, some in *fair*, and none in *poor*. There was no significant difference in IBI_{PNP} scores between the two sampled years. This approach provides a direct application for wetland management purposes.

Keywords: Conservation unit, Fish assemblages, Floodplain, Index of biotic integrity, Multimetric indices.

É iminente que o Brasil desenvolva ferramentas para monitorar o sistema de áreas protegidas do país, formado especialmente pelas Unidades de Conservação e tendo como foco a proteção efetiva da biodiversidade. Nesse contexto, esse trabalho discute métricas de referência (ou linhas de base) para compor um Índice de Integridade Biótica (IIB) baseado nas comunidades de peixes, tendo como área de estudo o Parque Nacional do Pantanal Matogrossense. Os hábitats encontrados no Parque foram agrupados em quatro estratos ambientais: rios, corixos (cursos d´água que se conectam à planície de inundação) e baías permanentes e temporárias. As coletas de peixes foram realizadas em 12 pontos amostrais durante o período seco (out/nov de 2010 e 2011). Foram identificadas 146 espécies de peixes, totalizando 18.954 exemplares coletados em amostragens padronizadas. Não houve associação entre a estrutura da comunidade e os estratos ambientais, sugerindo homogeneidade de hábitats. O IIB final foi composto por nove métricas, a maior parte alocada na classe excelente de integridade biótica, poucas na classe regular e nenhuma na classe pobre. Não houve diferença significativa no IIB entre os dois anos consecutivos. Esses resultados oferecem subsídios técnicos para a tomada de decisão sobre a gestão das áreas alagáveis no Pantanal brasileiro.

Palavras-chave: Assembleia de peixes, Índice de integridade biótica, Índices multimétricos, Planície de inundação, Unidade de conservação.

Introduction

Human disturbances interact in a complex way with aquatic ecosystems, and their effects can rarely be evaluated using only physical or chemical variables as indirect measures of environmental integrity. Assessing the response of the aquatic biota (*e.g.*, freshwater fish) often provides a more integrated view of environmental impacts (Fausch *et al*., 1990). These are the principles of the original idea of the Index of Biotic Integrity (IBI) (Karr, 1981; Karr *et al*., 1986) that combined 12 metrics (or attributes) of fish assemblages to measure and predict the outcomes of anthropogenic environmental disturbance through a given environmental gradient.

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The applications of the IBI in the U.S. and Europe demonstrated the effectiveness of the method and highlighted the need to modify or adapt the index to particularities of regions and systems under study (Hughes, Oberdorff, 1999; Simon, Sanders, 1999; Roset *et al*., 2007).

In Brazil, the first adaptation of the IBI based on fish assemblages was carried out in 1998 (Araújo *et al*., 2003) in one of the most critical segments of the highly polluted Paraíba do Sul River, which runs through the main industrial region of the country. In the Upper Paraná River, small streams (Ferreira, Casatti, 2006; Casatti *et al*., 2009) and reservoirs (Petesse *et al*., 2007; Terra *et al*., 2010) were also investigated by adapting the IBI to these systems.

According to the original proposition (Karr, 1981; Karr *et al*., 1986), the intensity of environmental degradation should be evaluated by comparing the modified habitats with pristine environments in the same or a nearby region (such as a protected area) taken as reference conditions or baselines. However, pristine sites are frequently not found, explained by an environmental and cultural "illness" known as SBS - Shifting Baseline Syndrome (Pauly, 1995). In those cases, some authors have considered the site with the lowest degree of modification to represent reference conditions (Hughes *et al*., 1986; McDonough, Hickman, 1999; Pont *et al*., 2006). From this moment on, the baseline becomes shifted in a contagious and probably irreversible process. One generation later (25-30 yrs.), there will last no environmental collective memory of the prior baseline.

In the face of rapid degradation of aquatic environments around the world, it is argued that adaptations of IBI should also be undertaken for the few environments where human modifications are still not apparent. For these environments, reference conditions can and should be established before human modification becomes evident and irreversible. This idea is, again, in line with the SBS concept: after an anthropogenic impacts are disseminated, there are no reference points that could be used as a base for future evaluation of ongoing stresses on ecosystem functionality (Pauly, 1995). The problem arises because there are few situations in which a monitoring tool is implemented before the impact is present. Nonetheless, efforts should be made to change this scenario, at least for the few ecosystems that still have pristine conditions, so that the reference point could drive policy makers in proposing environmental and conservation programs.

Thus, the present study aims to adapt the IBI for the fish community of the Pantanal National Park, a large wetland area in Brazil. The most remarkable characteristic of wetlands of the Pantanal is seasonal variation in water levels, which controls ecosystem dynamics. The Pantanal is a Biosphere Reserve by UNESCO and can be considered a biodiversity refuge among Brazilian wetlands.

Material and Methods

Study area. The Pantanal National Park (PNP) is located in the extreme southwest of the Mato Grosso State in the municipality of Poconé at the junction of the Paraguay and Cuiabá rivers (Fig. 1). This unique Park retains a considerable sample of the original lowland with a total area of 1,350 km^2, equivalent to 1% of the Brazilian Pantanal biome (Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis, 2003). Founded in September 1981, this national protected area (Ministério do Meio Ambiente, 2000) has been managed since 2007 by the Instituto Chico Mendes de Conservação da Biodiversidade, an agency of the Brazilian government.

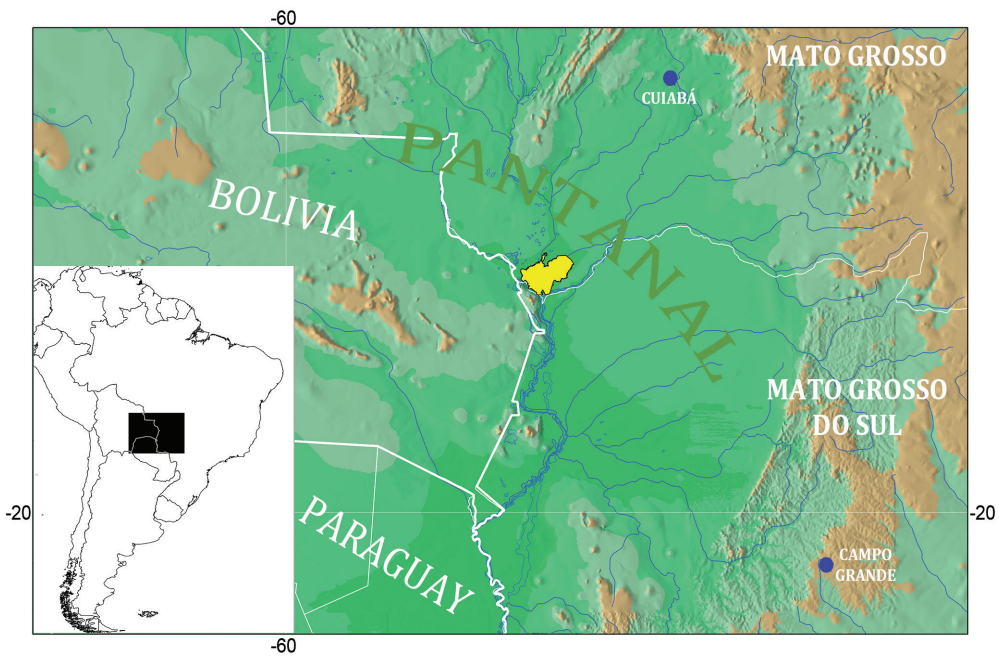

Fig. 1. Map of study area locating the Pantanal National Park in the centered or lighter spot (17°50'46.60"S, 57°24'10.40"W) of the Upper Paraguay River basin, Brazil.

Sampling design and fish collection. Based on cartographic charts of habitat distribution and photographic records, four regions, totaling 12 sampling sites (named P1 through P12), were pre-established and visited in the field for confirmation. Strata were defined based on water flow (lotic or lentic) and connectivity with main river channel (permanent or temporary connection). Category A (lotic environments represented by P4 and P6) was characterized by two major rivers, Cuiabá and Paraguay, in the Pantanal floodplain. These rivers are permanent water bodies with well-defined channels, and, henceforth, this category is designated as main channel sites. Category B (lentic environments; P3, P7, and P8) is composed of floodplain lakes with permanent connection to the main river channel throughout the hydrological cycle (Burro, Três Bocas, and Morro bays). This category is identified as permanent bays. With the exception of Três Bocas Bay, which is deeper, the other two are shallower and are about 50 cm deep during the dry season. Category C (lentic environments; P5, P10, and P11) is represented by smaller bays (Nove, Morro, and Biguerinho river bays), called *baiotas*, with intermittent connection with the main river and average depths ranging from 1 to 2 meters during the high-water season. These river bays can dry out completely in the low water season (except in years with higher flows) and, hereinafter, are referred to as temporary bays. Lastly, category D (lotic environments; P1, P2, P9, and P12) is represented by connected channels linking the main rivers and other elements of the floodplain, such as bays, and are referred locally as *corixos* (Boca do Ricardo, Morro do Caracara, Caracarazinho do Morro river, and Biguerinho river). As they link the main rivers to the floodplain, the current is weaker than in the main channel, and they have no delimited headwater and mouth. However, they may exhibit very well defined channels. When the channels are not well defined, they are called *vazante* (ebb), where the water simply overflows. This type of body is identified as corixos.

In a preliminary recognition campaign in the PNP area (Sep-2009), these 12 sampling points were selected out of 40 other potential locations based on: a) typology of habitat structure, b) cartographic analysis, and c) access by boat, considering that the core area of the PNP is unreachable. The samples were taken during the dry season of 2010 and 2011 because, during wet season, the four categories are indistinguishable and fish capture is less efficient due to high water level.

The sampling effort was characterized by 30 sequential hauls, separated by a minimum distance of 3 m along the marginal vegetation. Medium to large fish were collected with two cast nets (with 2 and 8 cm between opposite knots and with 15 hauls of each cast net in each sampling point) and gillnets (set of 7 nets with mesh ranging from 4 to 16 cm between opposite knots that were set for 2 hours in the water during sunlight). The sequence of nets was set at random. The small-sized fish species, usually associated with macrophytes, were collected with sieves built from net shading (nylon 1.5 mm opposite knots mesh size) that had dimensions of 1.5 m x 1.5 m and 0.7 m depth.

Metrics selection and description. The IBI_{PNP} metrics were adapted from Karr *et al*. (1986), Ganasan, Hughes (1998), Araújo *et al*. (2003), Bozzetti, Schulz (2004), Petesse *et al*. (2007), and Ferreira (2011). 34 candidate metrics were selected and grouped into four categories (Tab. 1) considering the following traits of the fish community structure: (i) richness and species assemblages (8 metrics); (ii) fish abundance (8 metrics); (iii) composition of feeding groups (10 metrics) and; (iv) indicators of environmental health at community level (8 metrics). All metrics were calculated in numbers of fish, as the measurement of biomass is difficult to obtain in the field due to the amount of fish collected. With exception of metrics 32 (number of introduced species) and 33 (number of hybrid species) only native species of the Pantanal ecosystem were considered.

In order to select appropriate metrics to compose the IBI_{PNP}, 3 tests were conducted (i) a range test to assess the magnitude and variability of the metrics in the sampling sites, (ii) a responsiveness test to evaluate the response of each metric to habitat modification, and (iii) a redundancy test to exclude highly correlated metrics of modification (Stoddard *et al.*, 2008; Petesse *et al*., 2014). Metrics with ranges equal or smaller than 4 or with 75% of identical values (range test) were excluded, since they would be unable to detect differences among the sampling locations (Whittier *et al.*, 2007) and therefore would have low discriminatory power. The metric responses to environmental modification (responsiveness test) were verified by the Spearman correlation coefficient (r_S) between each remaining metric and the following abiotic characteristics: macrophyte cover-MC, macrophyte integrity-MI, presence of communal bird nests-PN, air temperature-AT, water temperature-WT, water transparency-WTp, depth-D, electrical conductivity-EC, dissolved oxygen-DO, and oxidation/reduction potential-ORP (Polaz, 2013). Metrics that showed significant correlation ($p<0.05$) passed the test. The Spearman correlation was also calculated to identify pairs of redundant metrics (redundancy test). For pairs of metrics that had a correlation coefficient above 0.7 (Whittier *et al*., 2007), the ones that exhibited the lowest number of significant correlations in the responsiveness test were excluded (McCormick *et al*., 2001; Whittier *et al*., 2007).

Criteria for scoring and classes of integrity. As in most applications of IBI, ordinal scores were used to standardize metrics in different scales considering the categories: impacted biota (1), fair or intermediate (3), and not impaired (5).

The choice of a scale with discrete values (1, 3, and 5) is mainly justified by facilitating the communication of results to non-specialists that is a desirable feature for the management of protected areas open to the public.

Tab. 1. Candidate metrics, expected responses to disturbance and results of criteria tests (1, 2 and 3) to compose the IBI_{PNP}. The sign (-) indicates that the measure did not pass the test.

Metrics	Response to disturbance	Test 1 - Range test	Test 2 - Responsiveness	Test 3 - Redundancy
Richness and species composition (8 metrics)				
1. Total number of native species (S)	Decrease	Passed the test	-	-
2. Total number of native fish orders (Ord)	Decrease	-	-	-
3. Total number of native fish family (Fam)	Decrease	Passed the test	Passed the test	Passed the test
4. Total number of rare fish species (Rar)	Decrease	Passed the test	-	-
5. Total number of Characiformes species (SCh)	Decrease	Passed the test	-	-
6. Total number of Siluriformes species (SSi)	Decrease	Passed the test	Passed the test	Passed the test
7. Total number of Gymnotiformes species (SGy)	Decrease	Passed the test	-	-
8. Total number of Cichlidae species (SCi)	Decrease	Passed the test	Passed the test	Passed the test
Abundance (8 metrics)				
9. Total number of individuals (N)	Decrease	Passed the test	Passed the test	Passed the test
10. Number of species comprising 80% of abundance (N80)	Decrease	Passed the test	Passed the test	Passed the test
11. Percent abundance of Characiformes (NCh)	Decrease	Passed the test	Passed the test	Passed the test
12. Percent abundance of Siluriformes (NSi)	Decrease	Passed the test	Passed the test	-
13. Percent abundance of Gymnotiformes (NGy)	Decrease	Passed the test	Passed the test	Passed the test
14. Percent abundance of Cichlidae (NCi)	Decrease	Passed the test	-	-
15. Percent abundance of individuals larger than 30 cm (L30)	Decrease	Passed the test	-	-
16. Percent abundance of large migratory fish species (Mig)	Decrease	Passed the test	-	-
Feeding groups (10 metrics)				
17. Number of *carnivorous* species (SCar)	Decrease	Passed the test	-	-
18. Percent abundance of carnivores (NCar)	Decrease	Passed the test	-	-
19. Number of *omnivorous* species (SOni)	Increase	Passed the test	-	-
20. Percent abundance of omnivores (NOni)	Increase	Passed the test	-	-
21. Number of *detritivorous* species (SDet)	Decrease	Passed the test	-	-
22. Percent abundance of scavengers (NDet)	Decrease	Passed the test	-	-
23. Number of *insectivorous/invertivorous* species (SIns)	Decrease	Passed the test	-	-
24. Percent abundance of insectivores/invertivores (NIns)	Decrease	Passed the test	-	-
25. Number of *herbivorous* species (SHer)	Decrease	Passed the test	Passed the test	Passed the test
26. Percent abundance of herbivores (NHer)	Decrease	Passed the test	-	-
Assemblage indicators and environmental health (8 metrics)				
27. Shannon diversity índex (H´)	Decrease	Passed the test	-	-
28. Pielou´s evenness index (J)	Decrease	Passed the test	Passed the test	Passed the test
29. Percent abundance of *Abramites hypselonotus* (A.hyp)	Decrease	Passed the test	-	-
30. Percent abundance of the genus *Eigenmannia* (Eig)	Decrease	Passed the test	-	-
31. Percent abundance of the genera *Pygocentrus* and *Serrasalmus*	Increase	Passed the test	-	-
32. Total number of introduced species (aliens/exotic/aloctonous) (SInt)	Increase	-	-	-
33. Total number of hybrid species (NHib)	Increase	-	-	-
34. Percent abundance of fish with anomalies (damage/deformation) (NAno)	Increase	-	-	-

In order to exclude the effect of outliers, only the interval between the 5th and 95th percentiles were considered. For the remaining metrics, the 25th percentile of the distribution as the inferior limit was assumed. Values above the 25th percentile were considered unimpaired (score 5), and values below the 25th percentile were bisected. Those in the top half received a score of 3, and those in the bottom half received a score of 1. Gerritsen *et al*. (2003) recommended this procedure for scoring when most sites are undisturbed. This score attribution was inverted for metrics that increase with disturbance intensity such as the number of introduced species. Finally, the scores were summed to obtain the final IBI, which was classified as "poor", "fair", or "excellent" classes of biotic integrity. A two-way Analysis of Variance was used to test the effects of water body categorization and year of sampling on the final IBI_{PNP} (Zar, 2010).

Results

Fish assemblage composition. 18,954 individuals were collected and distributed in nine orders for 31 families and 146 species of freshwater fish (Britski *et al*., 2007; Oliveira *et al*., 2011; Eschmeyer *et al.*, 2017) (Tab. 2). Some of these species are listed in Polaz *et al*. (2014). The phylogenetic orders comprising the highest percentage of the richness were Characiformes (69 species; 47.3%) and Siluriformes (50 species; 34.2%), followed by Perciformes (11 species; 7.5%), and Gymnotiformes (11 species; 7.5%). These four orders represented 96.5% of the total fish assemblages. The highest number of species was within the Characidae family (25.3%), followed by Loricariidae (10.3%) and Cichlidae (6.8%).

Tab. 2. Taxonomic list of sampled species according to Eschmeyer, Fricke (2017) and species abundance, feeding and migratory guilds in the region sampled in the Pantanal National Park, during the dry season (Oct-Nov, 2010 and 2011). PB - permanent bays, TB - temporary bays, CR - "corixos", MR - main rivers. TG - trophic guilds; C - carnivores; I - insectivores/ invertivores; O - omnivores; D - detritivores; H - herbivores; ND - not determined trophic guild; LdM - long-distance migratory species.

ID	SPECIES	PB	TB	CR	MR	Total	TG	LdM
	ACTINOPTERYGII							
	BELONIFORMES							
	Belonidae							
1	*Potamorhaphis eigenmanni* Miranda Ribeiro, 1915	0	1	1	0	2	C	
	CHARACIFORMES							
	Acestrorhynchidae							
2	*Acestrorhynchus pantaneiro* Menezes, 1992	0	1	4	0	5	C	
	Anostomidae							
3	*Abramites hypselonotus* (Günther, 1868)	44	9	24	13	90	H	
4	*Leporinus friderici* Bloch, 1794	1	0	6	0	7	O	
5	*Leporinus lacustris* Amaral Campos, 1945	9	22	16	6	53	O	
6	*Leporinus striatus* Kner, 1858	13	1	8	11	33	O	
7	*Megaleporinus macrocephalus* (Garavello & Britski, 1988)	1	0	7	1	9	O	Yes
8	*Megaleporinus obtusidens* (Valenciennes, 1837)	0	1	2	0	3	O	
9	*Schizodon borellii* (Boulenger, 1900)	8	12	42	7	69	H	
10	*Schizodon isognathus* Kner, 1858	2	0	1	0	3	H	
	Bryconidae							
11	*Brycon hilarii* (Valenciennes, 1850)	0	1	1	0	2	O	Yes
	Characidae							
12	*Aphyocharax anisitsi* Eigenmann & Kennedy, 1903	0	12	0	0	12	I	
13	*Aphyocharax dentatus* Eigenmann & Kennedy, 1903	4	27	16	2	49	O	
14	*Aphyocharax nattereri* (Steindachner, 1882)	18	108	45	2	173	I	
15	*Aphyocharax rathbuni* Eigenmann, 1907	0	34	3	0	37	I	
16	*Astyanax abramis* (Jenyns, 1842)	0	68	0	1	69	I	
17	*Astyanax asuncionensis* Géry, 1972	1	3	2	0	6	O	
18	*Bryconamericus exodon* Eigenmann, 1907	15	0	21	63	99	I	
19	*Bryconamericus stramineus* Eigenmann, 1908	25	16	47	4	92	I	
20	*Charax leticiae* Lucena, 1987	1	20	1	1	23	C	
21	*Ctenobrycon alleni* (Eigenmann & Kennedy, 1907)	18	55	45	1	119	O	
22	*Cynopotamus kincaidi* (Schultz, 1950)	1	0	1	0	2	C	
23	*Galeocharax humeralis* (Valenciennes, 1834)	6	8	2	0	16	C	
24	*Gymnocorymbus ternetzi* (Boulenger, 1895)	1	1	0	0	2	O	
25	*Hemigrammus lunatus* Durbin, 1918	331	284	114	19	748	O	
26	*Hemigrammus marginatus* Ellis, 1911	0	34	5	0	39	I	
27	*Hyphessobrycon eques* (Steindachner, 1882)	50	294	116	23	483	I	
28	*Hyphessobrycon megalopterus* (Eigenmann, 1915)	4	74	50	0	128	O	
29	*Jupiaba acanthogaster* (Eigenmann, 1911)	218	205	80	30	533	O	
30	*Moenkhausia bonita* Benine, Castro & Sabino, 2004	1	14	5	0	20	O	
31	*Moenkhausia dichroura* (Kner, 1858)	38	17	37	20	112	O	

Tab. 2. (continued)

ID	SPECIES	PB	TB	CR	MR	Total	TG	LdM
32	*Moenkhausia forestii* Benine, Mariguela & Oliveira, 2009	46	190	45	10	291	O	
33	*Moenkhausia oligolepis* (Günther, 1864)	1	0	0	0	1	O	
34	*Odontostilbe paraguayensis* Eigenmann & Kennedy, 1903	102	0	30	0	132	O	
35	*Odontostilbe pequira* (Steindachner, 1882)	224	71	83	46	424	O	
36	*Poptella paraguayensis* (Eigenmann, 1907)	5	3	5	0	13	I	
37	*Prionobrama paraguayensis* (Eigenmann, 1914)	298	854	336	22	1510	O	
38	*Psellogrammus kennedyi* (Eigenmann, 1903)	7	16	105	10	138	O	
39	*Roeboides descalvadensis* Fowler, 1932	0	14	5	3	22	C	
40	*Roeboides microlepis* (Reinhardt, 1851)	1	1	5	7	14	C	
41	*Serrapinnus calliurus* (Boulenger, 1900)	403	323	108	55	889	O	
42	*Serrapinnus kriegi* (Schindler, 1937)	0	0	24	0	24	O	
43	*Serrapinnus microdon* (Eigenmann, 1915)	53	1005	395	0	1450	O	
44	*Tetragonopterus argenteus* Cuvier, 1816	3	3	10	0	16	O	
	Crenuchidae							
45	*Characidium laterale* (Boulenger, 1895)	150	487	306	72	1015	I	
46	*Characidium* aff. *zebra* Eigenmann, 1909	8	0	2	27	37	I	
	Curimatidae							
47	*Curimatella dorsalis* (Eigenmann & Eigenmann, 1889)	0	5	0	0	5	D	
48	*Curimatopsis myersi* Vari, 1982	0	96	2	0	98	D	
49	*Cyphocharax gillii* (Eigenmann & Kennedy, 1903)	0	6	2	4	12	D	
50	*Potamorhina squamoralevis* (Braga & Azpelicueta, 1983)	27	37	57	20	141	D	
51	*Psectrogaster curviventris* Eigenmann & Kennedy, 1903	3	20	63	1	87	D	
	Cynodontidae							
52	*Rhaphiodon vulpinus* Spix & Agassiz, 1829	6	0	1	2	9	C	
	Erythrinidae							
53	*Hoplerythrinus unitaeniatus* (Spix & Agassiz, 1829)	2	0	1	0	3	O	
54	*Hoplias* aff. *malabaricus* (Bloch, 1794)	12	5	16	9	42	C	
	Gasteropelecidae							
55	*Gasteropelecus sternicla* (Linnaeus, 1758)	7	7	50	1	65	I	
56	*Thoracocharax stellatus* (Kner, 1858)	0	0	0	5	5	I	
	Hemiodontidae							
57	*Hemiodus orthonops* Eigenmann & Kennedy, 1903	1	0	0	1	2	O	
	Iguanodectidae							
58	*Piabucus melanostoma* Holmberg, 1891	10	0	5	0	15	H	
	Lebiasinidae							
59	*Pyrrhulina australis* Eigenmann & Kennedy, 1903	45	187	86	17	335	I	
	Prochilodontidae							
60	*Prochilodus lineatus* (Valenciennes, 1837)	26	8	1	2	37	D	Yes
	Serrassalmidae							
61	*Metynnis maculatus* (Kner, 1858)	0	2	0	0	2	H	
62	*Myleus levis* Eigenmann & McAtee, 1907	0	4	11	1	16	H	
63	*Mylossoma duriventre* (Cuvier, 1818)	6	7	25	11	49	H	
64	*Piaractus mesopotamicus* (Holmberg, 1887)	4	1	9	2	16	H	Yes
65	*Pygocentrus nattereri* Kner, 1858	62	36	65	17	180	C	
66	*Serrasalmus maculatus* Kner, 1858	9	56	25	4	94	C	
67	*Serrasalmus marginatus* Valenciennes, 1837	57	10	39	3	109	C	
	Triportheidae							
68	*Engraulisoma taeniatum* Castro, 1981	0	0	2	0	2	I	
69	*Triportheus nematurus* (Kner, 1858)	9	7	23	6	45	I	
70	*Triportheus pantanensis* Malabarba, 2004	6	3	27	1	37	I	

Tab. 2. (continued)

ID	SPECIES	PB	TB	CR	MR	Total	TG	LdM
	CLUPEIFORMES							
	Engraulidae							
71	*Anchoviella* sp.	0	20	4	0	24	ND	
	ORDER CYPRINODONTIFORMES							
	Poeciliidae							
72	*Pamphorichthys hasemani* (Henn, 1916)	0	0	1	0	1	I	
	Cynolebiidae							
73	*Melanorivulus punctatus* (Boulenger, 1895)	0	0	4	0	4	I	
	ORDER GYMNOTIFORMES							
	Apteronotidae							
74	*Apteronotus albifrons* (Linnaeus, 1766)	7	3	4	0	14	I	
75	*Apteronotus caudimaculosus* de Santana, 2003	8	15	18	8	49	O	
76	*Sternarchorhynchus curvirostris* (Boulenger, 1887)	0	0	2	0	2	I	
	Gymnotidae							
77	*Gymnotus inaequilabiatus* (Valenciennes, 1839)	7	7	5	0	19	I	
78	*Gymnotus paraguensis* Albert & Crampton, 2003	0	1	6	1	8	I	
	Hypopomidae							
79	*Brachyhypopomus* spp.	60	96	53	25	234	ND	
	Ramphichthyidae							
80	*Gymnorhamphichthys britskii* Carvalho, Ramos & Albert, 2011	2	5	1	2	10	I	
81	*Rhamphichthys hahni* (Meiken, 1937)	0	1	0	1	2	I	
	Sternopygidae							
82	*Eigenmannia trilineata* López & Castello, 1966	960	638	180	323	2101	I	
83	*Eigenmannia virescens* (Valenciennes, 1836)	22	29	126	9	186	I	
84	*Sternopygus macrurus* (Bloch & Schneider, 1801)	83	68	61	36	248	I	
	ORDER PERCIFORMES							
	Cichlidae							
85	*Aequidens plagiozonatus* Kullander, 1984	5	0	10	0	15	O	
86	*Apistogramma borellii* (Regan, 1906)	20	9	45	0	74	I	
87	*Apistogramma commbrae* (Regan, 1906)	3	4	3	2	12	I	
88	*Apistogramma trifasciata* (Eigenmann & Kennedy, 1903)	82	116	141	30	369	I	
89	*Astronotus crassipinnis* (Heckel, 1840)	0	0	2	0	2	I	
90	*Cichlasoma dimerus* (Heckel, 1840)	11	14	22	10	57	D	
91	*Crenicichla lepidota* Heckel, 1840	39	52	65	24	180	C	
92	*Crenicichla vittata* Heckel, 1840	1	0	0	0	1	O	
93	*Laetacara dorsigera* (Heckel, 1840)	1	0	0	0	1	O	
94	*Mesonauta festivus* (Heckel, 1840)	0	4	2	3	9	H	
	Sciaenidae							
95	*Plagioscion ternetzi* Boulenger, 1895	6	0	1	2	9	C	
	ORDER SILURIFORMES							
	Aspredinidae							
96	*Amaralia oviraptor* Friel & Carvalho, 2016	1	0	0	0	1	C	
97	*Pseudobunocephalus rugosus* (Eigenmann & Kennedy, 1903)	3	1	0	0	4	D	
	Auchenipteridae							
98	*Auchenipterus nigripinnis* (Boulenger, 1895)	2	0	0	0	2	C	
99	*Auchenipterus osteomystax* (Miranda Ribeiro, 1918)	3	0	1	0	4	C	
100	*Entomocorus benjamini* Eigenmann, 1917	3	2	0	0	5	I	
101	*Tatia neivai* (Ihering, 1930)	0	0	1	0	1	I	
102	*Trachelyopterus coriaceus* Valenciennes, 1840	1	0	0	0	1	O	
103	*Trachelyopterus galeatus* (Linnaeus, 1766)	11	4	14	11	40	O	
104	*Trachelyopterus striatulus* (Steindachner, 1877)	0	0	3	3	6	O	

Tab. 2. (continued)

ID	SPECIES	PB	TB	CR	MR	Total	TG	LdM
	Callichthydae							
105	*Callichthys callichthys* (Linnaeus, 1758)	7	0	3	0	10	O	
106	*Corydoras hastatus* Eigenmann & Eigenmann, 1888	0	110	144	3	257	I	
107	*Hoplosternum littorale* (Hancock, 1828)	0	0	2	0	2	I	
	Doradidae							
108	*Anadoras weddellii* (Castelnau, 1855)	0	1	3	0	4	O	
109	*Ossancora eigenmanni* (Boulenger, 1895)	3	7	15	14	39	I	
110	*Oxydoras kneri* Bleeker, 1862	13	0	12	2	27	O	
111	*Platydoras armatulus* (Valenciennes, 1840)	5	1	2	5	13	O	
112	*Pterodoras granulosus* (Valenciennes, 1821)	1	0	8	2	11	O	
	Heptapteridae							
113	*Imparfinis* spp.	12	20	77	4	113	ND	
114	*Phenacorhamdia hoehnei* (Miranda Ribeiro, 1914)	0	0	4	0	4	I	
115	*Pimelodella gracilis* (Valenciennes, 1835)	0	7	1	15	23	O	
116	*Pimelodella mucosa* Eigenmann & Ward, 1907	0	0	1	0	1	I	
117	*Pimelodella notomelas* Eigenmann, 1917	1	0	0	0	1	I	
118	*Rhamdia* aff. *quelen* (Quoy & Gaimard, 1824)	3	1	1	2	7	O	
	Loricariidae							
119	*Ancistrus* spp.	3	0	0	0	3	ND	
120	*Farlowella paraguayensis* Retzer & Page, 1997	0	0	2	8	10	D	
121	*Hemiodontichthys acipenserinus* (Kner, 1853)	0	0	0	1	1	I	
122	*Hypoptopoma inexspectatum* (Holmberg, 1893)	433	314	474	120	1341	D	
123	*Hypostomus cochliodon* Kner, 1854	18	43	28	22	111	D	
124	*Hypostomus latifrons* Weber, 1986	32	3	23	0	58	D	
125	*Hypostomus* spp.	83	55	69	44	251	D	
126	*Loricaria* spp.	3	0	0	0	3	D	
127	*Loricariichthys labialis* (Boulenger, 1895)	1	0	0	0	1	D	
128	*Loricariichthys platymetopon* Isbrücker & Nijssen, 1979	3	0	0	0	3	D	
129	*Otocinclus vittatus* Regan, 1904	488	462	347	116	1413	H	
130	*Pterygoplichthys ambrosettii* (Holmberg, 1893)	3	8	7	30	48	D	
131	*Rineloricaria parva* (Boulenger, 1895)	409	236	214	166	1025	D	
132	*Spatuloricaria evansii* (Boulenger, 1892)	1	0	0	0	1	D	
133	*Sturisoma barbatum* (Kner, 1853)	0	0	5	0	5	D	
	Pimelodidae							
134	*Hemisorubim platyrhynchos* (Valenciennes, 1840)	0	0	1	1	2	C	Yes
135	*Hypophthalmus edentatus* Spix & Agassiz, 1829	0	1	0	0	1	O	
136	*Pimelodus argenteus* Perugia, 1891	5	4	2	0	11	O	
137	*Pimelodus pantaneiro* Souza-Filho & Shibatta, 2007	1	1	4	1	7	O	
138	*Pseudoplatystoma corruscans* (Spix & Agassiz, 1829)	1	0	0	0	1	C	Yes
139	*Pseudoplatystoma reticulatum* Eigenmann & Eigenmann, 1889	1	1	4	1	7	C	Yes
140	*Sorubim lima* (Bloch & Schneider, 1801)	1	0	1	1	3	C	Yes
	Scoloplacidae							
141	*Scoloplax distolothrix* Schaefer, Weitzman & Britski, 1989	2	0	0	8	10	I	
142	*Scoloplax empousa* Schaefer, Weitzman & Britski, 1989	1	2	0	0	3	I	
	Trichomycteridae							
143	*Ituglanis eichorniarum* (Miranda Ribeiro, 1912)	1	0	0	1	2	I	
144	*Ituglanis herberti* (Miranda Ribeiro, 1940)	1	0	0	0	1	I	
145	*Trichomycterus johnsoni* (Fowler, 1932)	0	1	0	0	1	I	
	SYNBRANCHIFORMES							
	Synbranchidae							
146	*Synbranchus marmoratus* Bloch, 1795	0	1	2	1	4	I	
		5280	7154	4902	1621	18954		

Five feeding groups were recognized with the most common being insectivory/invertivory (47 species; 32.2%), followed by omnivory with 44 species (30.1%), carnivory/piscivory (21 species, 14.4%), detritivory (20 species, 13.7%), and herbivory represented by 10 species (6.9%). Four entities were considered undetermined (2.7%): *Anchoviella* sp., *Ancistrus* spp., *Brachyhypopomus* spp., and *Imparfinis* spp. Additional information on species list and categorization into feeding and migratory guilds could be also consulted in Polaz (2013, chapter 2, p.106-8).

Metrics selection. Nine of the 34 candidate metrics passed the criteria established by the range, responsiveness, and redundancy tests (Tab. 1). The number of native orders (metric 2) ranged from 3 to 6, and, therefore, they did not pass the range test (range < 4). Introduced species or hybrids were not sampled (metrics 32 and 33, respectively), and only 4 of the 24 samples exhibited had single individual anomaly (metric 34). In all these cases, the metrics did not meet the first criteria, as more than 75% of the values were zero.

Ten of the remaining 30 metrics (3, 6, 8, 9, 10, 11, 12, 13, 25, and 28) passed the second test (responsiveness), as they were correlated with at least one environmental variable. Most metrics (3, 8, 9, 25, and 28) showed a significant correlation with only one of the abiotic factors, one metric (10) with two factors, three metrics (6, 12, and 13) with three factors, and one metric (11) with four factors. Among the abiotic variables, the macrophyte cover (MC) and the oxidation-reduction potential (ORP) showed the highest number of metrics correlation. In particular, four metrics were correlated with MC, while there were four others with ORP. A test was proposed for the communal bird nests (locally known as *ninhais*) as a metrics because, especially in the reproductive season, they can provide a relevant feeding microhabitat for the fish community. Nonetheless, this factor and water depth were not correlated with any metrics.

From the 10 metrics accepted by the second test, the pairwise correlation matrix was calculated using the Spearman correlation coefficient (see more in supplementary material), resulting in only one redundant pair (metrics 11 and 12, r_S = -0.863). It was decided to keep metric 11 as it had the greater number of significant correlations (four correlations) with the environmental variables (responsiveness test). Therefore, only metric 12 did not pass in the test of redundancy.

Metrics scoring and IBI_{PNP} calculation. All nine selected metrics are expected to decrease their values with increasing intensities of disturbance. Therefore, lower scores indicate worse conditions, and the highest scores indicate the best conditions. As there are nine metrics, the final IBI_{PNP} for a site could range from 9 (the worst condition in which all of the metrics are given the value 1) to 45 (the best condition in which all metrics are given the value 5) (Tab. 3).

Tab. 3. Score intervals and ranges of values of the metrics for calculating the IBI_{PNP}.

Metrics	1	3	5	Minimum	Maximum
3. Total number of native fish family (Fam)	< 13	13 - 14	> 14	11	23
6. Total number of Siluriformes species (SSi)	< 8	8 - 10	> 10	6	18
8. Total number of Cichlidae species (SCi)	< 1	1 - 2	>2	0	6
9. Total number of individuals (N)	< 443	443 - 769	>769	105	2519
10. Number of species comprising 80% of abundance (N80)	< 7	7 - 10	>10	3	28
11. Percent abundance of Characiformes (NCh)	< 16,05	16,05 - 27,25	>27,25	4,44	87,37
13. Percent abundance of Gymnotiformes (NGy)	< 9,42	9,42-15,44	>15,44	3,18	47,77
25. Number of *herbivorous* species (SHer)	< 1	1 - 2	>2	0	7
28. Pielou´s evenness index (J)	<0,56	0,56 - 0,61	>0,61	0,51	0,86

Sites with excellent biotic integrity (ranging from 35 to 45) represented the best-observed situations. The community composition includes the most intolerant forms in all ranges of age category and sex ratio. They have a diverse and balanced trophic structure, the presence of large species and migratory fish, and the expected fish diversity for the biome. Fair conditions (ranging from 22 to 34) are those sites in which the number of species is close the expected richness, having a relatively balanced trophic structure but with some species decreasing their relative abundance and size below the values expected for the best condition. This fish community begins to show some signs of stress and needs to be monitored. The poor sites (ranging from 9 to 21) have low diversity, simplified trophic structure, dominance of tolerant and introduced species (non-native and/or exotic ones), and may show a high percentage of individuals with anomalies and parasites.

The IBI_{PNP} ranged from 27 to 45. No sites were classified as poor, 19 were excellent ($IBI_{PNP} \geq 35$), and only 5 were classified as fair (IBI_{PNP} between 22 and 34). In 2010, with the exception of P10 ("fair"), all sites fit in the best class of integrity ("excellent"), and in 2011, while P10 increased from "fair" to "excellent", four points (P3, P4, P5, and P9) had their scores decreased (from "excellent" to "fair") (Tab. 4).

However, there were no significant effects of water body categorization ($F_{3,16}$ = 1.48, p = 0.257), year ($F_{2,16}$ = 0.39, p = 0.543), or interaction ($F_{3,16}$ = 1.84, p = 0.180) on the mean values for the IBI_{PNP} (Figs. 2-3).

Tab. 4. Scores of selected metrics for IBI_{PNP} in 24 sampling points in the Pantanal National Park during the dry period (Oct-Nov) of 2010 and 2011. (*) Metrics that had different classes of integrity in 2010 and 2011.

Year	Localities	Stratum	Fam	SSi	SCi	N	80%	NCh	NGy	SHer	J	Total	IBI_{PNP}
2010	P1	CR	5	3	5	5	3	5	1	5	3	35	Excellent
2010	P2	CR	5	5	5	3	1	5	3	5	5	37	Excellent
2010	P3	PB	5	5	5	5	5	5	5	5	5	45	Excellent
2010	P4	MR	5	5	5	3	5	5	5	5	5	43	Excellent
2010	P5	TB	5	5	5	1	5	5	5	5	1	37	Excellent
2010	P6	MR	5	5	5	5	5	5	5	5	5	45	Excellent
2010	P7	PB	5	5	5	3	5	5	3	5	5	41	Excellent
2010	P8	PB	3	5	3	5	5	5	1	3	5	35	Excellent
2010	P9	CR	5	1	5	1	5	5	3	5	5	35	Excellent
2010	P10	TB	3	1	5	1	1	5	1	5	5	27	Fair
2010	P11	TB	5	5	5	1	3	5	1	5	5	35	Excellent
2010	P12	CR	5	5	5	3	3	5	1	5	5	37	Excellent
2011	P1	CR	3	1	5	5	5	5	5	5	5	39	Excellent
2011	P2	CR	5	5	5	3	3	5	1	5	5	37	Excellent
2011	P3	PB	5	3	3	3	3	1	5	3	5	31	Fair*
2011	P4	MR	1	1	1	1	3	5	5	5	5	27	Fair*
2011	P5	TB	3	3	5	5	5	1	5	1	5	33	Fair*
2011	P6	MR	5	3	5	3	5	5	5	5	5	41	Excellent
2011	P7	PB	5	5	5	3	5	5	5	3	5	41	Excellent
2011	P8	PB	5	5	5	3	3	5	5	5	5	41	Excellent
2011	P9	CR	5	1	5	1	3	5	3	5	5	33	Fair*
2011	P10	TB	5	3	5	5	3	5	1	5	5	37	Excellent*
2011	P11	TB	3	1	5	3	5	5	3	5	5	35	Excellent
2011	P12	CR	5	5	5	5	5	5	3	5	5	43	Excellent

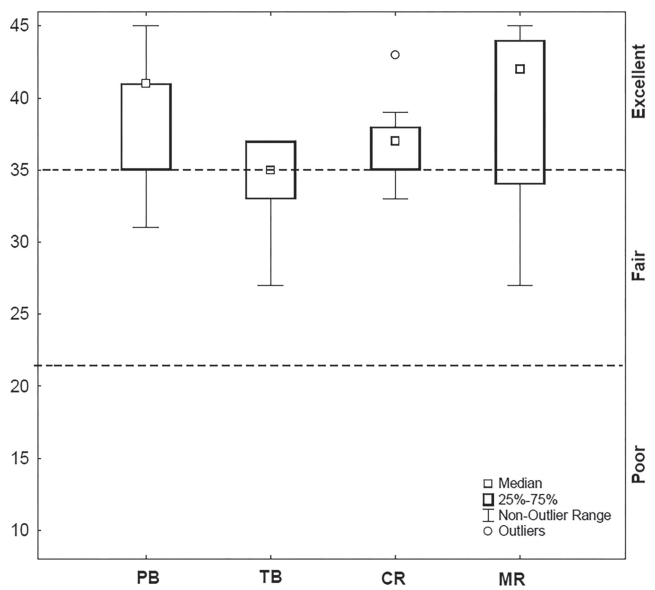

Fig. 2. Distribution of the IBI_{PNP} in the strata sampled for the Pantanal National Park during the dry season (Oct-Nov, 2010 and 2011). PB - permanent bays, TB - temporary bays, CR - corixos, MR - main rivers.

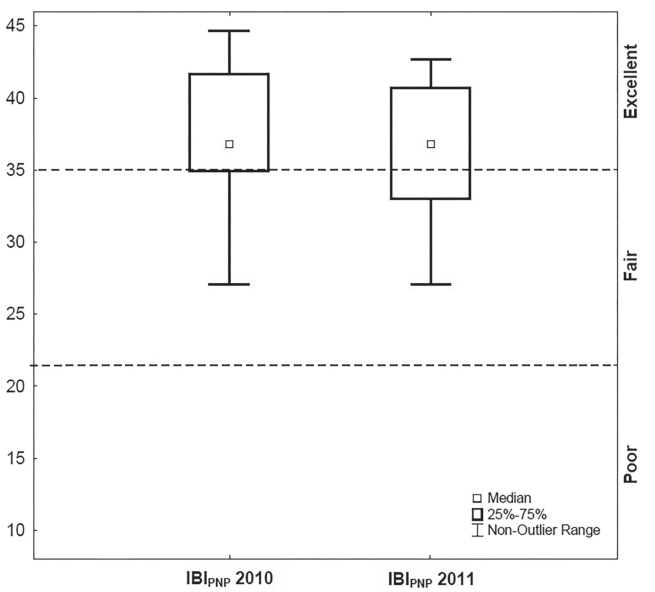

Fig. 3. Distribution of the IBI_{PNP} for the years of 2010 and 2011.

Discussion

The PNP is a large area within the Pantanal biome representing the wetlands of Central-West Brazil. This national park is closed for public visitation as of this publication. However, tourism will soon be allowed, which may result in additional sources of environmental impact if this activity is poorly managed. The building of small reservoirs in the headwaters of its main rivers may also lead to changes in the hydrological cycle and consequently modify the ecosystem dynamics. The multimetric indices are best suited to assess the impact on aquatic environments from multiple sources, as they take into account different ecological attributes of communities (Seegert, 2000). Thus, this attempt to conduct the first implementation of an index of biotic integrity to PNP is aimed to provide a monitoring tool and a reference condition for assessing future environmental impairment within the park and its surroundings.

Applying the IBI to a preserved area has the unique possibility of gathering information with confidence on the distribution of reference values. As the metrics are measured before the beginning of disturbance processes and virtually all sites can be considered in good or excellent biotic integrity, there is no need to model sample values for baseline conditions in this case. A logical difficulty, however, is that in the absence of human impacts, the IBI_{PNP} values become difficulty to validate. Considering scenarios of strong disturbance, some responses are easily predicted, such as the reduction of species richness, migratory fishes, and intolerant species. On the other hand, predicting the responses in advance of these impacts may be difficult and challenging when the disturbances are subtle. Because the PNP is a conservation unit not severely impacted, small values for a metric distribution were not expected. Score 1 should represent clear evidence of human impact, but within the range of observations on hand, sites classified as 1 are considered as far as possible from the best potential condition (score 5) for a determined metric. This is expected due to natural variation, even when analyzing pristine environments (Gerritsen *et al*., 2003). The challenge of adapting an IBI to an undisturbed area is just as difficult as adapting it to a heavily disturbed one, where natural conditions are no longer available. In both cases, not all classes of disturbance-integrity are present, and the researchers have only a partial view of the range of possibilities for the metrics distribution.

Due to this limitation, the choice for candidate metrics on IBI's adapted for other environments was investigated and the appropriate metrics were selected based on the three established criteria: range test, responsiveness, and redundancy (Whittier *et al*. 2007, Petesse *et al*., 2014). The second criteria, for example, attempted to identify metrics that were sensitive to environmental conditions, expecting that these responses will exacerbate in the presence of anthropogenic impacts. In addition, following Gerritsen *et al*. (2003), the choice for the kind of metric scoring reflected the fact that the observed values will act as reference for future impacts within the region.

Significant differences among the types of water bodies and between years on the mean values of IBI_{PNP} were not detected. This suggests that despite being clearly heterogeneous, the categories of water bodies analyzed have similar integrities, and these patterns do not change significantly from one year to another. In other applications of the IBI, temporal variability have been generally less important than spatial variability (Petesse *et al*., 2007; Casatti *et al*., 2009; Terra, Araújo, 2010; Ferreira, 2011), being the reason why Casatti *et al*. (2009) reinforced the importance of investing in the spatial variability instead of seasonality in early adaptations of multimetric indices. Terra, Araújo (2010), for instance, reported that seasonality had no effect on Reservoir Fish Assemblage Index (RFAI; an adaptation of IBI for reservoirs) in a transitional river-dam stretch of the Paraíba do Sul River that covers the Funil reservoir.

Considering this question, the current temporal study and sampling did not investigate the variation during the hydrological cycle (drought, flood, ebb, and flow), which l is the most important factor controlling community dynamics in the Pantanal. It was expected that large changes in fish communities during hydrological cycle would occur, but the similarity found in the IBI_{PNP} between the dry seasons of 2010 and 2011 suggests that the fish populations are adapted to such phenomena. When year to year variability is not important, Karr *et al*. (1986) recommended that fish sampling should be conducted in a season that minimizes variation in fish community, due to discrete events such as fish recruitment. Several factors contribute to variations in the observed values for the IBI of a particular place and time because the index is entirely dependent on the composition and abundance of the collected fish population. Some pattern of movements and the collectors experience, for example, interfere decisively in the species and individuals sampled (*e.g*., larger or smaller) (Fore *et al*., 1994). This makes the multimetric indices behave as a random variable, adding a stochastic component upon the estimate of biotic integrity. In the Pantanal, recruitment takes place mainly during flood periods. As such, more variation in the community composition during flow and flood periods due to fish migrations and recruitment can be expected. Fish sampling is also less efficient during these seasons due to the higher water volume. In the dry season, the aquatic environments are more constrained, and sampling is more efficient, yielding a better characterization of fish communities (Junk *et al*., 1989; Junk, 2001). These facts provide us a better picture of how fish community structures are organized, which is a practical concern if the IBI_{PNP} is established as a monitoring tool.

Nine out of the 34 candidate metrics that were selected were related to fish richness and composition (3 metrics), abundance (4 metrics), composition of feeding

groups (1 metric), and indicators at the community levels (1 metric). Four metrics handle with the patterns observed at higher taxonomic levels such as order (total number of Siluriformes species or abundance of Characiformes and Gymnotiformes) or family (total number of Cichlidae species). As an advantage, these taxonomic levels are easily recognized by non-specialists. Other metrics, such as total number of individuals or number of species comprising 80% of total abundance, are easily calculated. For example, there is no need to determine each species when counting how many species comprise 80% of total population. In addition, Petesse *et al*. (2007) concluded that calculating the index in terms of fish biomass or fish numbers generates similar results in a previous application of a multimetric index to reservoirs. If the same applies for the IBI_{PNP}, the application in numbers would require less field effort because it would be impracticable to weigh the high numbers of fish captured in the field (mainly the small ones).

The IBI_{PNP} values are subject to the environmental characteristics observed within the park. In this case, it should be considered that the type of gear employed tended to catch the fish living in shallow habitats (<2m depth). Thus, there is a need to standardize the sampling method for future comparisons.

By combining indicators into an overall multimetric index to summarize biological condition, it is reinforced that the presented results in metrics combination always carried to the same baseline: PNP sites can be considered as references. Although the IBI_{PNP} can be used as a reference for future adaptations, further studies are needed on the biology of each species and how they interact with their habitats. It is also suggested that wetland areas outside of the park with evident signs of anthropogenic degradation be selected to validate our results and to provide new opportunities to test the IBI_{PNP}.

In addition, research that has the opportunity to investigate pristine areas should be encouraged and supported by governments and international organizations because information in these areas, even if already available, cannot be easily transferred to multimetric indices. Given that budget and time to conduct additional studies is always a limitation, in addition to considering the degree of human impact on aquatic environments, such information will be of great value to substantiate management decisions.

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