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Assessment of biotic condition of Atlantic Rain Forest streams: A fish-based multimetric approach

Bianca de Freitas Terra[a,*], Robert M. Hughes[b], Marcio Rocha Francelino[c], Francisco Gerson Araújo[a]

[a] Laboratório de Ecologia de Peixes, Departamento de Biologia Animal, Instituto de Biologia, Universidade Federal Rural do Rio de Janeiro, Rodovia 465, km 7, CEP 23897-000, Seropédica, Rio de Janeiro, Brazil
[b] Amnis Opes Institute and Department of Fisheries & Wildlife, Oregon State University, 200 SW 35th Street, Corvallis, OR 97333, USA
[c] Laboratório de Geoprocessamento Ambiental, Departamenteo de Silvicultura, Instituto de Florestas, Universidade Federal Rural do Rio de Janeiro, Rodovia 465, km 7, CEP 23897-000, Seropédica, Rio de Janeiro, Brazil

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ABSTRACT

We developed a preliminary fish-based multimetric index (MMI) to assess biotic condition of Atlantic Rain Forest streams in Southeastern Brazil. We used least-disturbed sites as proxies of reference conditions for metric development. To determine the disturbance gradient we used an Integrated Disturbance Index (IDI) that summarized the multiple disturbances measured at local/regional catchment scales in a single index, describing the totality of exposure of the streams to human pressures. For our 48 sites, nine were least-disturbed (IDI < 0.25), five were most-disturbed (IDI > 1.35) and 34 were intermediate. Initially, we considered 41 candidate metrics selected primarily from previous studies. We screened this pool of candidate metrics using a series of tests: range test, signal-to-noise test, correlation with natural gradients, responsiveness test, and redundancy test. After screening, we selected six metrics for the MMI: % Characiform individuals, % water column native individuals, % benthic invertivorous individuals, % tolerant species, % intolerant species, and % detritivorous individuals. Metrics such as diversity, dominance, species richness and biomass that have been historically used for assessing ecosystem condition failed one or more screening tests. We conclude that an IDI and rigorous metric screening are critical to the MMI development process and for meaningful assessments of stream condition.

1. Introduction

Multimetric indices (MMIs) have been applied as an integral part of water quality monitoring programs worldwide (Furse et al., 2006; Yagow et al., 2006; Marchant et al., 2006; Borja et al., 2008). First proposed by Karr (1981), many countries have adopted one or more fish MMIs as official tools for monitoring aquatic systems (Hughes and Oberdorff, 1999; Roset et al., 2007). In the United States, MMIs have been widely adopted by water management agencies as the primary tool for assessing the biological condition of streams and lakes (Karr and Chu, 1999; USEPA, 2002) and for making national stream and river assessments (USEPA, 2013). In Europe, following the Water Framework Directive (European Commission, 2000), implementation of biological monitoring became mandatory and MMIs help guide restoration activities and management of aquatic ecosystems (Hering et al., 2006; Pont et al., 2006). However, in developing countries such as Brazil, the use of MMIs are not required in monitoring programs, although several studies aiming to develop such tools have been conducted in recent years (Ferreira and Casatti, 2006a; Pinto et al., 2006; Pinto and Araújo, 2007; Baptista et al., 2007; Mugnai et al., 2008; Casatti et al., 2009; Oliveira et al., 2011; Terra and Araújo, 2011).

The lack of a biomonitoring program reflects the absence of an integrated view of the ecological condition of the water bodies, which hinders creation of rational recovery and protection plans (Hughes et al., 2000). On the other hand, high tropical taxonomic diversity and the lack of reliable information about physical and chemical disturbance thresholds hinder MMI development. It is important to overcome these challenges in countries such as Brazil by developing an MMI that efficiently uses available biological information, as well as known disturbance gradients. For example, using an Integrated Disturbance Index (IDI; Ligeiro et al., 2013) for the identifying reference sites is feasible because it does not depend on previous classifications based on physical or chemical thresholds. The IDI combines disturbance at local and regional scales in a single index describing the total disturbance of the sites by human pressures.

* Corresponding author. Tel.: +55 02137873983.
E-mail addresses: biancafterra@gmail.com, biancafterra@yahoo.com.br (B.d.F. Terra).

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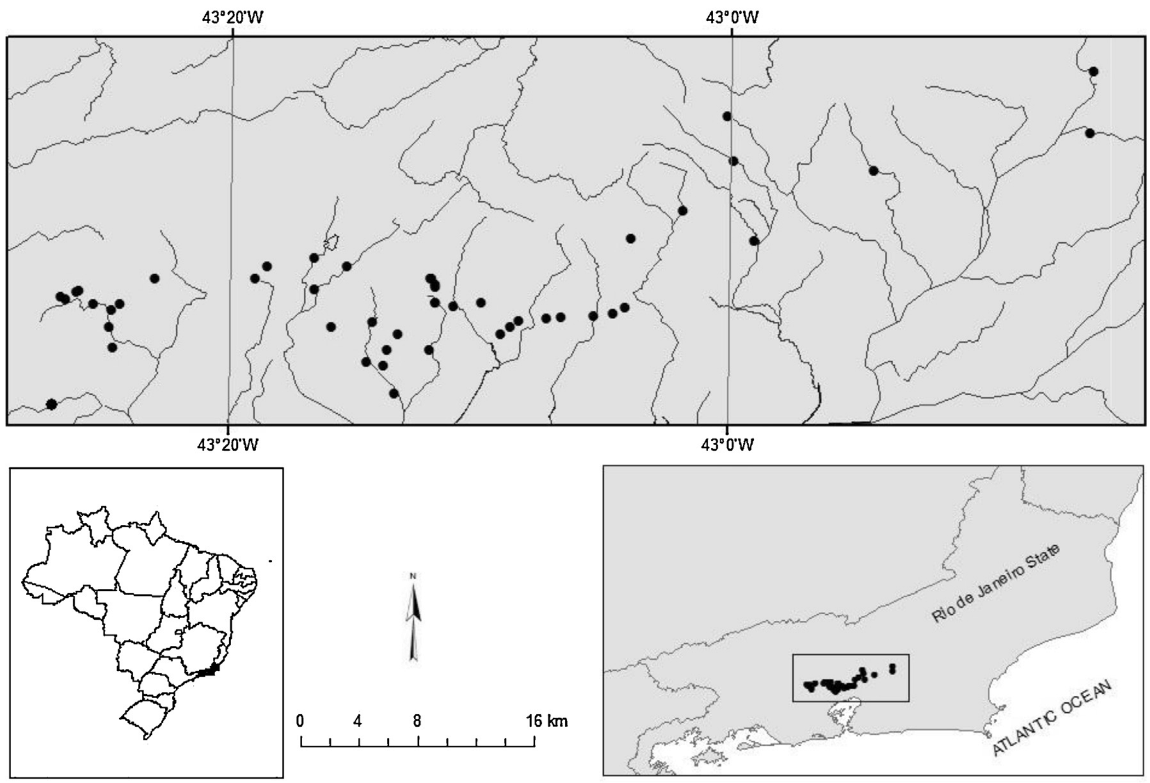

Fig. 1. Locations and distribution of the 48 sites sampled in five basins that drain to Guanabara Bay, Rio de Janeiro, Brazil.

The Atlantic Rain Forest is one of the most threatened biomes of the world, with an estimated 11.4–16% remaining of the 150 million ha originally in Brazil (Ribeiro et al., 2009). The remaining biome covers approximately 157,000 km^2 along the Brazilian coast (Ribeiro et al., 2009), and its rivers and streams support a highly diverse and endemic fish fauna (Bizerril, 1994). The most important reason for that diversity and endemicity is the great number of independent coastal drainages (or groups of basins), and the isolating effect of mountain ranges and seawater among coastal rivers (Bizerril, 1994; Menezes et al., 2007). According to Abilhoa et al. (2011), 70% of the freshwater fishes in the Atlantic Forest can be considered exclusive to the coastal drainages of this biome. However, the high water demands, untreated wastewater disposal, and intensive land uses conflict with conservation policies for Atlantic Rain Forest streams. Consequently, these systems need to be monitored and assessed, and biotic indices have been shown to be useful tools for doing so elsewhere in Brazil (e.g., Bozzetti and Schulz, 2004; Ferreira and Casatti, 2006a; Casatti et al., 2009).

In this study, our goal was to assess the ecological quality of Atlantic Rain Forest streams through use of an MMI. To do so we (a) determined a disturbance gradient, (b) selected fish-based ecological indicators (metrics) capable of distinguishing fish assemblages along that gradient, (c) combined those metrics into an MMI, and (d) assessed the performance of that MMI statistically.

2. Materials and methods

2.1. Study area

We conducted this study in five basins, all of which drain to Guanabara Bay in the Atlantic Rain Forest biome, in the State of Rio de Janeiro, southeastern Brazil. The study area included four Conservation Units (Tinguá Biological Reserve, Petrópolis Environmental Protection Area, Serra dos Órgãos National Park, and Três Picos State Park). This area is bounded by the Serra do Mar, with altitudes between 800 and 1800 m a.s.l. The climate is warm and humid, with two well-defined seasons: a wet season from October to March, and a dry season from April to September, an average annual temperature of 22 °C, and mean annual precipitation near 1700 mm (SEMADS, 2001).

2.2. Site selection

We studied 48 sites distributed in five river basins: Estrela (23 sites), Suruí (5), Roncador (2), Iguaçu (12) and Guapimirim (6). The sites were randomly chosen within first to fourth-order streams, with mean stream width ranging from 1.0 to 16.0 m (Fig. 1; Table 1). We also ensured that the sites had distinctly different channel slopes, substrates, and anthropogenic pressures (urbanization, sewage discharges, deforestation).

2.3. Sampling design

We sampled fish during the dry season from May to October (2010 and 2011) to standardize the seasonal context. Although our goal was to assess the influence of human actions, not natural assemblage variation through time, we re-sampled seven of the sites during the wet season from February 2010 to March 2011 (Table 1) to measure the repeatability or precision of the candidate metrics.

Following the USEPA's national protocol (Peck et al., 2006), at each random point, a site was extended upstream for 40 times the mean wetted channel width, or a minimum of 100 m. In each stream site, 11 equidistant cross-section transects were marked, defining 10 sections of the same length. In the middle of each section another

Table 1
Physical characteristics of 48 Atlantic Rain Forest stream sites, southeastern Brazil. Total length sampled is the product of the number of channel widths sampled (40) and the mean width of the channel, with a site length minimum of 100 m. Season column indicates the seasons that sites were sampled. IDI = Integrated Disturbance Index; LD = least-disturbed; MD = most-disturbed.

Code	Season		Mean width (m)	Channel length sampled (m)	Elevation (m)	IDI	Disturbance
	Dry	Wet					
1			6	240	21	0.28	
2			4	160	24	1.13	
3			4	160	50	0.15	LD
4			3	120	15	0.46	
5			8	320	56	0.82	
6			6	240	88	0.36	
7			5	200	19	1.35	MD
8			3	120	103	0.64	
9			2.5	100	42	0.25	
10			6	240	32	0.95	
11			1	100	26	0.60	
12			1	100	43	0.40	
13			8	320	20	1.11	
14			8	320	35	1.16	
15			1.5	100	18	0.78	
16			1.5	100	14	0.62	
17			0.5	100	43	0.98	
18			4	160	12	1.01	
19			4	160	43	0.50	
20			3	120	82	0.03	LD
21			6	240	101	0.63	
22			2	100	64	0.48	
23			2	100	55	0.50	
24			4	160	33	0.23	LD
25			2	100	12	0.65	
26			2	100	12	0.62	
27			7	280	207	0.33	
28			5	200	261	0.00	LD
29			1	100	12	1.45	MD
30			3.5	140	11	1.35	MD
31			5	200	8	1.51	MD
39			4	160	228	0.10	LD
40			4	160	217	0.20	LD
41			1.5	100	152	0.50	
42			4	240	144	0.25	LD
43			3	120	35	0.96	
44			15	500	23	1.36	MD
45			9	360	54	0.78	
46			5	200	113	0.20	LD
47			5	200	33	0.85	
48			4	160	22	0.42	
49			7	280	43	1.00	
50			16	500	18	0.50	
51			15	160	862	0.33	
52			8	160	340	0.00	LD
53			7	280	40	0.70	
54			9	360	43	0.60	
55			5	200	26	1.19	

transect was marked to complement habitat and physico-chemical measurements. Thus, a total of 21 transects were sampled along each site.

2.4. Habitat measurements

At five equidistant points in each of the 21 transects, we measured depth, substrate size (silt: <0.06 mm; sand: 0.06–2.0 mm; small gravel: 2.0–16 mm; large gravel: 60–250 mm; cobble: 250–1000 mm; and boulder: 1000–4000 mm), and current velocity (measured 5 cm above the bottom). At 10 transects in each section we measured: the width, riparian structure (e.g., mid-channel and margin shading), pH, conductivity, dissolved oxygen, temperature, and turbidity, following Peck et al. (2006). At each transect in the channel and riparian zone, respectively, we visually estimate the percent area of selected habitat characteristics (e.g., flow type, large wood, aquatic macrophytes) and human disturbance (e.g., pasture, crops, pipes, trash, erosion, dam, sewer, building).

2.5. Fish sampling

We electrofished via alternating current (2500 W, 110/220 V) with two hoop-shaped (440 mm × 300 mm) anodes supporting a net (3 mm mesh). Two people, each with an anode, fished from one edge to the other of each quadrat removing all fishes detected in the electric field. We used only electrofishing for three reasons: (1) it is recognized as being a widely applicable tool for monitoring fish assemblages elsewhere (Vaux et al., 2000; CEN, 2003; Hughes et al., 2002; Hughes and Peck, 2008; Rabeni et al., 2009), (2) most sites had high slopes, small intertices and boulder substrates that hindered the use of nets (Terra et al., 2013), and (3) it facilitated effort standardization (CEN, 2003; Rabeni et al., 2009). All the fish we collected were identified to species, counted, weighed (g), and measured for total length (mm).

2.6. Determination of disturbance gradient

We used least-disturbed sites to define reference conditions (Hughes, 1995; Stoddard et al., 2006; Whittier et al., 2007a) for metric development. To determine the disturbance gradient, we used the Integrated Disturbance Index (IDI) following Ligeiro et al. (2013), who combined the multiple disturbances measured at local (Local Disturbance Index – LDI) and regional (Catchment Disturbance Index – CDI) scales into a single index, describing the total disturbance of the sites by human pressures. The LDI was calculated from the W1_hall metric, as described in Kaufmann et al. (1999), which sums human disturbances observed in-channel and in the riparian zone. In this study, we calculated the metric using eight types of disturbance: trash, sewer, building, domestic animals, agriculture, pasture, erosion, and dams along the ten sections demarked at the site. The values were weighted according to impact and the distance of the disturbance from the stream channel: trash (1.5), sewer (2.0), building (1.0), domestic animals (1.0), agriculture (1.0), pasture (1.0), erosion (1.5), and dam (1.0).

The CDI was based on human land uses in the catchments, and was calculated as the sum of those uses, each one weighted differently: % urban (4.0), % agricultural (2.0), and % pasture (1.0) (Ligeiro et al., 2013). Each land use area was estimated for the catchment above the site through use of 1:25,000 scale orthophotos, freely available from the Instituto Brasileiro de Geografia e Estatística (IBGE). Land uses were determined by visual interpretation and vectorization directly on the computer screen using ArcGis 10 software packages (ESRI, 2007).

We used Pearson correlations to evaluate the collinearity between both indices and the scores were weak ($r < 0.51$). Such low correlations confirm that potential impacts from different scale disturbances are weakly associated, but that use of local and catchment indexes together may help avoid misleading and partial interpretation of biological response to human pressures. Because both indices do not share the same numerical scale, we followed Ligeiro et al. (2013) and rescaled the raw values of each index by dividing by 75% of the maximum value that each can theoretically achieve (LDI = 3; CDI = 300). We then calculated the IDI by applying the Pythagorean Theorem following Ligeiro et al. (2013): $\mathrm{IDI} = ((\mathrm{LDI}/3)^2 + (\mathrm{CDI}/300)^2)^{1/2}$.

To validate the IDI, we used physical-chemical variables and habitat metrics calculated from the raw field data. With those metrics, we aimed to represent key aspects of the habitats of the sites, such as morphology (mean depth, mean width × mean depth), habitat heterogeneity (% pool, log of mean substrate diameter, % riparian canopy and % macrophyte cover) and water quality (temperature, dissolved oxygen, conductivity, and turbidity). We performed Principal Components Analysis (PCA) on those environmental variables (Table 2) to determine the position of samples along the main environmental gradients. After that, we regressed PCA axis 1 against the IDI. We expected that sites classified by the IDI as most-disturbed would also have poor water quality and considerable macrohabitat modification.

2.7. Fish-based metrics

Initially, we considered 41 candidate metrics selected primarily from previous studies of fish responses to anthropogenic pressures and descriptions of stream fish assemblages (Araújo et al., 2003; Bozzetti and Schulz, 2004; Ferreira and Casatti, 2006a; Pinto and Araújo, 2007; Magalhães et al., 2008; Casatti et al., 2009) (Table 3). Those metrics represent a range of structural and functional fish assemblage characteristics including diversity, composition, and habitat, trophic and tolerance guilds. Proportional metrics were employed because they were found to be more precise and stable between season than total number metrics (Terra et al., 2013). Metrics that considered occurrence of common species were based on those that contributed >1% of total abundance at a site. Tolerant species were those classified as such by others (Araújo, 1998; Casatti et al., 2009; Terra and Araújo, 2011) in Atlantic rain forest aquatic systems. Those species tend to increase with disturbance (Hughes and Oberdorff, 1999; Pont et al., 2006) and are common across sites with low dissolved oxygen, heavy organic pollution, high levels of turbidity, or severely modified habitat (Lyons et al., 2000). Intolerant species disappear early in the degradation sequence associated with agriculture and urban development (Ganasan and Hughes, 1998) and they were classified based on expert judgment (Appendix A). Although an empirical classification for those intolerant species does not yet exist, metrics based on general intolerance to stressors have been widely used in multimetric index development (Pont et al., 2006; Zhu and Chang, 2008; Kanno et al., 2010; Delgado et al., 2012; Lyons, 2012). All fish species were allocated to their ecological and functional guilds based on previous classifications by Araújo (1998), Ferreira and Casatti (2006b), Mazzoni and Costa (2007), Pinto and Araújo (2007), and Gomiero et al. (2008); personal observations updated with available literature; and FishBase online database information (Froese and Pauly, 2012).

2.8. Metric selection

The methodology used for metric selection was derived from Hering et al. (2006), Whittier et al. (2007a), and Henriques et al. (2013). We screened this pool of candidate metrics using a series of tests: (a) range; (b) signal-to-noise (among-site variability versus temporal variability); (c) correlation with natural gradients; (d) responsiveness (sensitivity in separating most-disturbed and least-disturbed sites); and (e) redundancy.

Metrics with a range <10% were eliminated as proposed by Klemm et al. (2003). The temporal variability in the metrics (between dry and wet season) was tested with signal-to-noise (S:N) test, a measure of the repeatability or precision of the metric values (Kaufmann et al., 1999; Stoddard et al., 2008). We used this test by calculating S:N ratios of variance among dry season sites sampled (signal) to the mean of variances of pairs of sites sampled in the dry and wet season (noise). A S:N value ≤ 1 indicates that a metric has as much or more variability within a site (over time) as it does across different sites and thus does not distinguish well among sites. We rejected only very noisy metrics, that is, those with S:N values ≤ 3 as proposed by Whittier et al. (2007a).

The correlation of the metrics with natural gradients (altitude, catchment area) was tested by regressing metric values from the least-disturbed sites against those natural gradients. We used only least-disturbed sites values because we wanted to evaluate those relationships without the often covarying effect of human disturbance. We considered that there was a strong relationship if the slope was different from zero, and if this difference was significant ($p < 0.05$). For metrics that showed a relationship with a natural gradient we produced a natural-gradient corrected metric by substituting the original value of the metrics with the residuals from the regression equation for all sites. We evaluated those relationships between metrics and natural gradients because such relationships might obscure potential stressor relationships (Whittier et al., 2007a).

We used one-way univariate analysis of variance using permutations (PERMANOVA; Anderson et al., 2008) to evaluate metric responsiveness, or the ability of metrics to distinguish between least-disturbed and most-disturbed sites (one fixed factor: disturbed IDI classification; 2 levels: least-disturbed and

Table 2
Abiotic characterization of 48 Atlantic Rain Forest stream sites, southeastern Brazil, classified by the Integrated Disturbance Index (IDI) in three classes: least-disturbed, intermediate and most-disturbed. SD = standard deviation.

	Code	Least-disturbed		Intermediate		Most-disturbed	
		Mean	SD	Mean	SD	Mean	SD
Temperature	PHYXTEM	18.34	0.99	19.72	1.42	21.57	0.92
Dissolved oxygen (mg L^{-1})	PHYXOD	8.56	1.11	8.70	1.30	6.63	0.77
Conductivity (mS/cm)	PHYXCOND	3.27	2.40	4.43	2.06	13.18	8.54
Turbidity (NTU)	PHYXTUR	0.82	0.84	2.40	2.89	6.85	4.84
Depth (m)	Depth	0.21	0.08	0.18	0.08	0.23	0.03
Mean width × mean depth	XWXD	1.35	1.04	1.10	1.02	1.21	0.75
% Pool	PERPOOL	43.01	11.78	40.22	27.34	51.80	34.05
Log of mean substrate diameter	Sub DMM	98.84	135.22	24.10	32.34	3.12	3.34
% Riparian canopy	XCANOPY	64.65	21.88	50.85	31.58	14.23	16.20
% Macrophyte cover	SHELXMACRF	0.78	2.20	6.66	14.65	17.10	13.65

Table 3
Candidate metrics used to characterize the fish assemblage response to anthropogenic pressures. Metric class: I = Diversity/Composition; II = Habitat association; III = Trophic structure; IV = Tolerance. Screening tests: 1 = Range, 2 = Signal: Noise, 3 = Correlation with natural gradient, 4 = Responsiveness, 5 = Redundancy, and MMI = Multimetric Index (X = shows in which test the metric failed; √ = shows which metric was selected for the MMI).

Metric	Class	1	2	3	4	5	MMI
Shannon diversity	I				X		
Dominance	I		X				
Biomass by square meter	I		X				
Biomass of native species by square meter	I		X				
% Common species	I		X				
% Common species individuals	I	X					
% Native species	I	X					
% Native individuals	I		X				
% Characiform species	I		X				
% Characiform individuals	I						√
% Siluriform species	I	X					
% Siluriform individuals	I				X		
% Characiform & Siluriform species	I				X		
% Abundance of Characiforms & Siluriforms	I	X					
% Water column native species	II		X				
% Water column native individuals	II			X			√
% Benthic species	II				X		
% Benthic individuals	II			X	X		
% Invertivorous species	III				X		
% Invertivorous individuals	III				X		
% Benthic invertivorous species	III					X	
% Benthic invertivorous individuals	III						√
% Piscivorous species	III					X	
% Piscivorous individuals	III		X				
% Native piscivorous species	III					X	
% Native piscivorous individuals	III		X				
% Omnivorous species	III		X				
% Omnivorous individuals	III				X		
% Detritivorous species	III				X		
% Detritivorous individuals	III						√
% *Poecilia reticulata* individuals	III		X				
% Tolerant species	IV						√
% Tolerant individuals	IV					X	
% Intolerant species	IV						√
% Intolerant individuals	IV			X	X		
% Non-tolerant native species	IV					X	
% Non-tolerant native individuals	IV					X	
% Non-tolerant piscivorous species	IV			X		X	
% Non-tolerant piscivorous individuals	IV	X					
% Non-tolerant native piscivorous individuals	IV	X					
% Non-tolerant native piscivorous species	IV	X					

most-disturbed). Redundancy among metrics was tested using Spearman correlation and we considered metrics redundant if their correlation coefficients were $\geq|0.70|$. For redundant metric pairs, we excluded the one with the higher overall mean correlation with multiple metrics. Finally, we produced box plots of the metric values for the least-disturbed, intermediate, and most-disturbed sites to determine overlap across disturbance classes. To further interpret metric differences, we performed a SIMPER (Similarity Percentage) routine to identify the species that contributed most to dissimilarities between least- and most-disturbed sites.

2.9. Metric and index scoring

Each metric was scored on a continuous scale from 0 (poor) to 10 (good) using lower and upper expectation limits (Hughes et al., 1998; McCormick et al., 2001; Bramblett et al., 2005). We used the 5th and 95th percentiles of raw values to exclude the effects of extreme values that may impair metric interpretation. Metrics that were believed to decrease with environmental degradation received the highest scores (10), corresponding to the 95th percentile of observed raw values, and lowest scores (0), based on the 5th percentile of observed raw values. For metrics believed to increase with environmental degradation, a score of 10 corresponded to the 5th percentile of raw values, and a 0 corresponded to the 95th percentile of raw values.

Calculation of the final MMI score followed Klemm et al. (2003), with the score for each site calculated as the sum of individual metric scores divided by the total number of metrics. Thus, the final MMI score ranged between 0 and 10. The final MMI scores were assigned to three different quality classes. According to Ganasan and Hughes (1998), many classes/categories can confound interpretation and, consequently, decisions by environmental managers. For assessing relationship between classes of IDI and MMI we followed Delgado et al. (2012) that join two matrices in a single file, one labeled by IDI classes and the other labeled by MMI classes. Then we run an ANOSIM (Analysis of Similarity) procedure based on a Bray–Curtis similarity distance measure that compares all pair-wise combination (classes of IDI and MMI) giving a *R*- and *p*-values for each comparison. Following this procedure we can assess relationship between classes of IDI and MMI.

2.10. Assemblage similarity and environmental responses

We conducted additional analyses to assess assemblage similarities and responses to environmental conditions through use of the raw assemblage data. This helped us assess whether sites could be meaningfully grouped based on the relative similarities of their fish assemblages. First we used MDS on Bray–Curtis similarity matrices and log-transformed data (Clarke and Warwick, 2001). Then, to help visualize the influence of biological and environmental variables on the fish MDS, we created bubble plots for the MMI, IDI, altitude, forest, richness, and biomass.

2.11. Data analyses

The simple regression, residual calculations, and Pearson and Spearman correlations were conducted with STATISTICA 7.1 software. Analysis of similarity (ANOSIM) was conducted in PAST (Hammer et al., 2001) based on Bray–Curtis similarity matrices. All the other analyses were performed in PRIMER 6 with PERMANOVA+ software. Before Principal Components Analysis (PCA), variables were normalized by subtracting the mean and dividing by the standard deviation to place all variables on a comparable measurement scale. Prior to Non-metric Multi-Dimensional Scaling (MDS), Similarity Percentage analysis (SIMPER) and, One-way multivariate analysis of variance using permutation (PERMANOVA), data were log-transformed ($x+1$). The resemblance matrix based on Bray Curtis similarity was constructed to perform MDS and SIMPER, however the PERMANOVA was based on a Euclidean distance matrix, in which *p*-Values were calculated using 999 permutations and the sum of squares was type III.

3. Results

A total of 19,293 fish specimens representing 68 species distributed across 13 families were collected during the course of the study (Appendix A). Richness per site varied from 0 to 30 species. *Astyanax taeniatus*, *Poecilia reticulata*, *Rineloricaria* sp.1, *Scleromystax barbatus*, *Characidium vidali*, and *Trichomycterus* cf. *zonatus* constituted over 50% of the total abundance across study sites.

3.1. Disturbance gradient

For our 48 sites, nine were classified as least-disturbed ($IDI < 0.25$), five as most-disturbed ($IDI > 1.35$) and 34 as intermediate (Table 1). The PCA ordination of the environmental variables showed that the first component (PC1) accounted for 26.5% of the variability of the data (Fig. 2). Sites classified as least-disturbed were characterized by high dissolved oxygen concentration, whereas sites previously classified as most-disturbed were clearly separated from the least-disturbed sites by higher temperatures, turbidities, and conductivities. Thus, PC1 was used as a second disturbance gradient. When the IDI values were regressed against PCA axis 1, a clear separation of the IDI classes was evident with the least-disturbed sites having low IDI values in the lower right of the graph and the most-disturbed sites in the upper left of the graph ($F = 17.24$, $R^2 = 0.282$, $p = 0.0001$; Fig. 3).

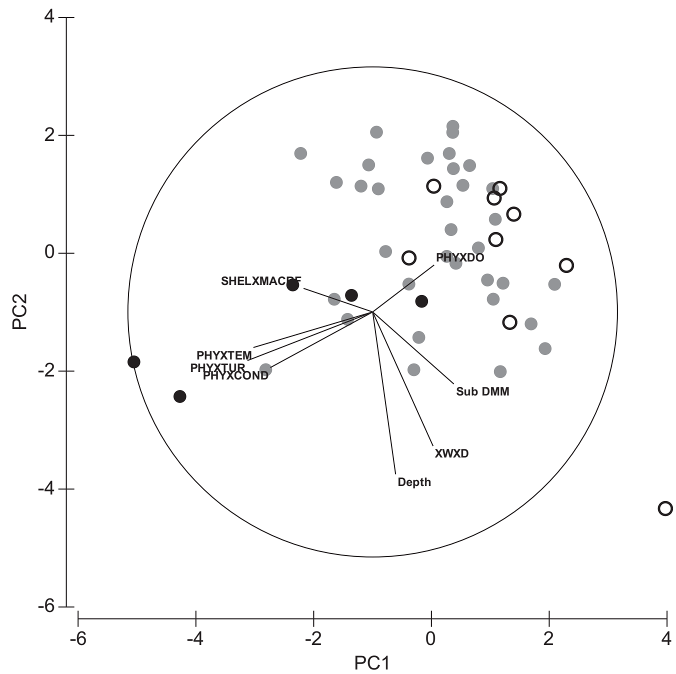

Fig. 2. Principal Component Analysis (PCA) plot based on the environmental variables (Codes in Table 2) measured in each site grouped by IDI (Integrated Disturbance Index) classification (black circle = most-disturbed; gray circle = intermediate; white circle = least-disturbed sites) (PC1 = 26.5%; PC2 = 17.9%).

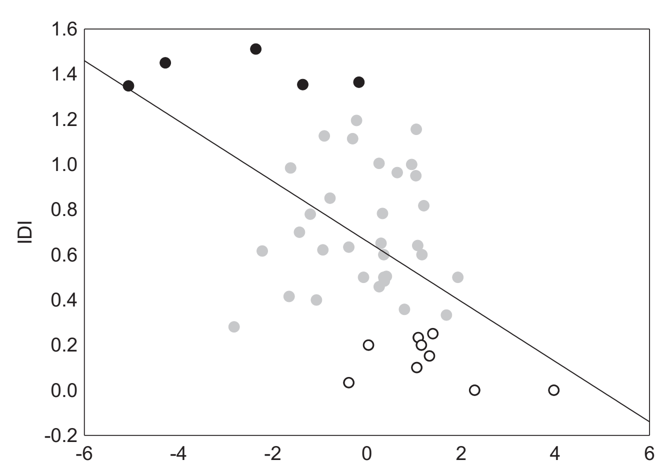

Fig. 3. Relationship between the IDI (Integrated Disturbance Index) score per sampling site (0 = best condition; 1.5 = worst ecological condition) and a physical and chemical gradient, expressed as the sample derived from PCA (Principal Component Analysis) axis 1. White circles = least-disturbed sites, gray circles = intermediate sites, and black circles = most-disturbed sites.

Table 4
Final MMI metrics for Atlantic Rain Forest streams. ER = expected response to disturbance, S/N = signal-noise, Min = minimum value observed, Max = maximum value observed, *R* = the regression between metrics and natural gradients (altitude and catchment area = CA), *F* = test for least-disturbed vs. most-disturbed sites.

Metrics	ER	S/N	Min	Max	Altitude (*R*)	CA (*R*)	Pseudo-*F*
% Characiform individuals	–	8.46	0	88	0.23	0.00	5.60*
% Water column native individuals	–	3.07	1	89	0.67*	0.02	9.70*
% Benthic invertivorous individuals	–	15.15	0	100	0.49	0.27	6.98*
% Tolerant species	+	10.63	0	50	0.28	0.50	14.38*
% Intolerant species	–	27.37	0	100	0.47	0.35	11.77*
% Detritivorous individuals	+	9.40	0	100	0.09	0.45	19.36*

* $p < 0.05$.

3.2. Metric selection and scoring

Our metric screening process efficiently reduced the set of candidate metrics (Table 3). From the initial 41 metrics, seven failed the range test and, 11 of the remaining 34 metrics had S:N ratios < 3.0 and were rejected. We calibrated three metrics for altitude (% water column native individuals, % benthic individuals, % intolerant individuals) and one for catchment area (% non-tolerant piscivorous species). Among the remaining 23 metrics, nine metrics did not significantly differ between least-disturbed and most-disturbed sites.

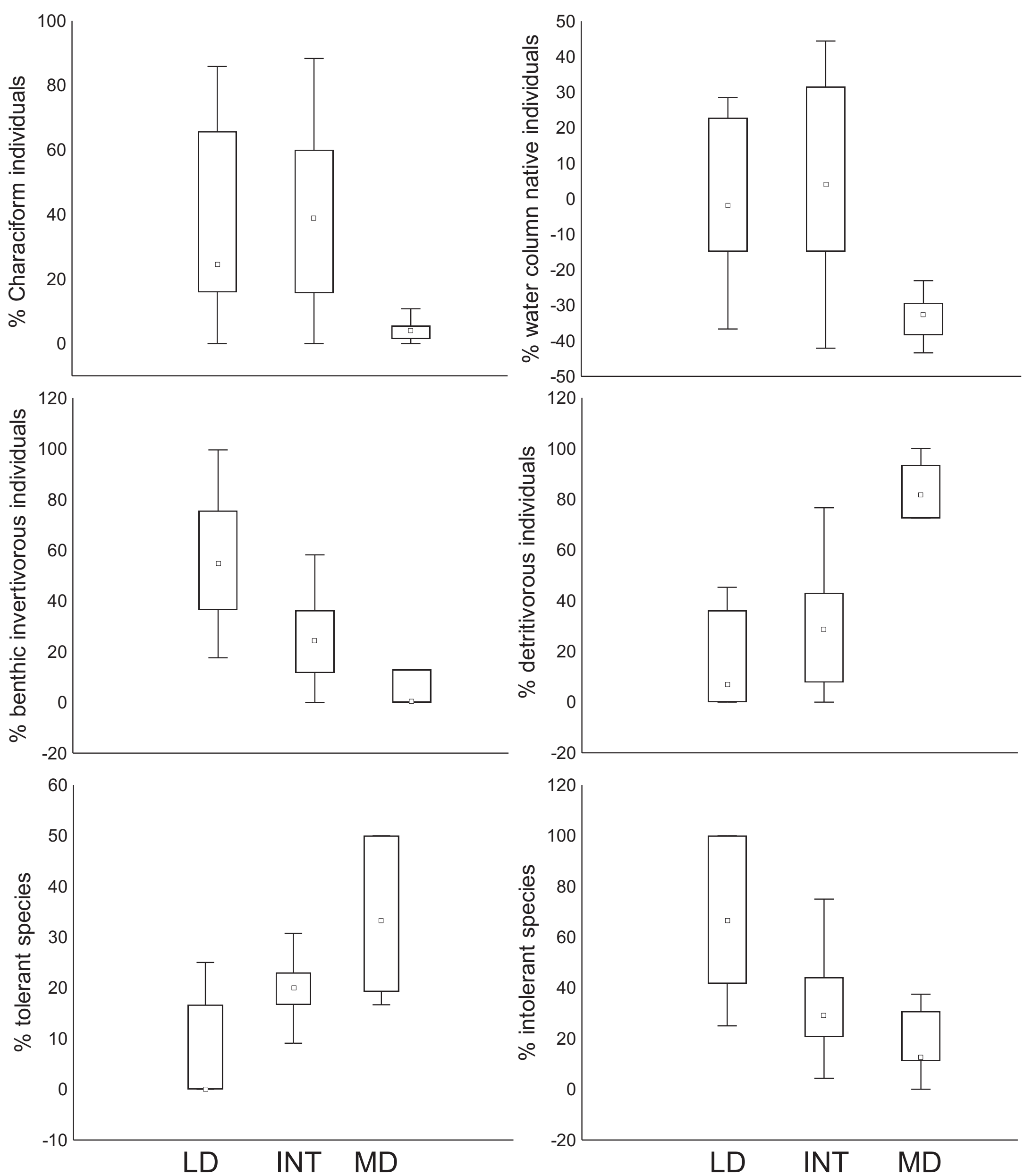

Fig. 4. Final fish-based multimetric index metrics for Atlantic Rain Forest streams. Rectangles delineate the 1st and 3rd quartiles, small squares are medians, bars are maxima and minima. LD = least-disturbed; INT = intermediate; MD = most-disturbed.

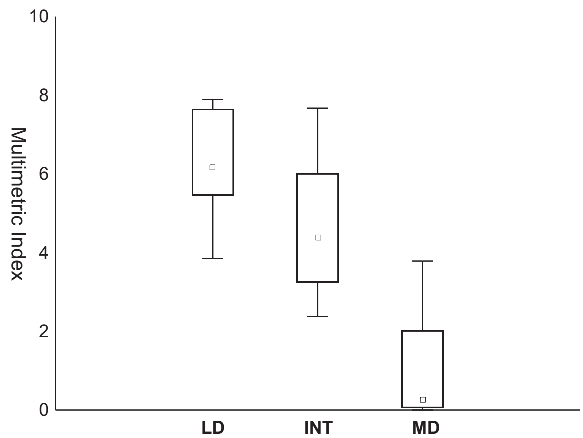

Fig. 5. Relationship of the fish-based MMI scores of Atlantic Rain Forest sites to IDI (Integrated Disturbance Index) disturbance classes. LD = least-disturbed, INT = intermediate, MD = most-disturbed.

Table 5
R values of ANOSIM (pair-wise comparisons between sample groups: IDI and MMI classes) using Bray–Curtis similarities of species composition (*R* global = 0.1784; *p* = 0.0001). LD = least-disturbed, INT = intermediate and MD = most-disturbed.

	Good	Moderate	Poor
LD	−0.015	0.134	0.513*
INT	0.178*	−0.002	0.034
MD	0.536*	0.351*	0.048

* $p \leq 0.05$.

Eight of the remaining 14 metrics were highly correlated with each other. The final index included six metrics (Table 4; Fig. 4).

3.3. Final index and environmental responses

We assigned three different quality classes based on index scores: least-disturbed (>6.0), intermediate (3.0–5.0), and most-disturbed (<3.0). The intermediate sites, which were not used in metric selection or index development, received scores intermediate between those of the least-disturbed and most-disturbed sites (Fig. 5) based on the final multimetric index values. ANOSIM indicated that no significant differences existed between the MMI and IDI corresponding quality classes, Least disturbed–Good, Intermediate–Moderate, Most disturbed–Poor (Table 5). On the other hand, the highest significant differences were found between the classes Least-disturbed (MMI) and Poor (IDI) and between the classes most-disturbed (MMI) and Good (IDI). The species with the greatest contribution for each disturbance class were *R. transfasciatus*, *T.* cf. *zonatus*, *B. ornaticeps*, and *C. vidali* for least-disturbed sites, and *P. reticulata*, *Rhamdia quelen*, *Geophagus brasiliensis*, and *Hypostomus affinis* in most-disturbed sites (Table 6).

Table 6
Percent contribution of the most abundant taxa in each disturbance class (SIMPER). LD = least-disturbed, INT = intermediate and MD = most-disturbed.

Species	Groups		
	LD	INT	MD
Average similarity (%)	28.6	31.01	28.41
R. transfasciatus	34.6		
T. cf. *zonatus*	21.18		
B. ornaticeps	13.69		
C. vidali	13.03		
A. taeniatus		7.45	
S. barbatus		6.98	
A. leptus		6.23	
P. reticulata		8.2	39.13
R. quelen		8.11	6.98
G. brasiliensis			14.72
H. affinis			5.58
	Groups		
	LD × INT	LD × MD	INT × MD
Average dissimilarity (%)	80.32	89.96	71.64

The MDS ordinations identified gradients in fish assemblages among disturbance classes (stress = 0.17; Fig. 6A). The MMI and IDI show two differing groups (Fig. 6C, D). Bubble plots overlaying altitude on the fish species MDS indicate that the least-disturbed sites occurred at higher altitudes (Fig. 6E). Richness and biomass (Fig. 6G, H), were greatest in the intermediate sites. However, catchment area and percentage of forest did not distinguish disturbance classes clearly (Fig. 6B, F).

4. Discussion

4.1. Metric selection

We developed a fish-based MMI that (a) gave a significant negative linear response to a gradient of human disturbances, and (b) remained consistent whatever the natural environmental conditions. For this we used six metrics (one from the diversity/composition category, one from habitat association, two from trophic structure, and two from tolerance) representing fish assemblage structure and function (Table 4).

Percentage of Characiform individuals was the metric from the diversity/composition category included in the final multimetric index. According to Casatti et al. (2009), degraded environments are often dominated by more tolerant Perciform and Cyprinodontiform species, modifying the expected predominance of Characiform and Siluriform species. The Characiforms are one of the most abundant fish orders in tropical streams (Lévêque et al., 2008), representing more than 30% of the Atlantic Rain Forest stream richness (Abilhoa et al., 2011). Among them, there is a large range of trophic guilds (invertivores, herbivores, piscivores, omnivores), but most of these species occupy water column habitats, associated with their fusiform body shape and high mobility. Riffle elimination by low-head dams, decreased allochthonous food and increased erosion resulting from deforestation, and increased turbidity and decreased dissolved oxygen from untreated sewage discharge can reduce Characiform richness and abundance by decreasing habitat diversity, reducing food availability, and degrading water quality (Pinto and Araújo, 2007).

Our habitat metric, percentage of water column native individuals, also responded to the above disturbances. Although this metric is closely related to percentage of Characiform species, it includes two additional important components: (a) it considers only native individuals, excluding the strong negative non-native species influences in these assemblages (Vitule et al., 2009) and (b) it considers other water column species that are common components of Atlantic Rain Forest streams, such as Gymnotiforms and non-tolerant Perciforms (i.e. *Crenicichla*, *Australoheros*).

The tolerance category was represented by two metrics at opposite ends of the disturbance gradient: percentage of tolerant species and percentage of intolerant species. The tolerant species were abundant in disturbed sites; generalist species that dominated those sites, but where other common species did not persist, included *G. brasiliensis*, *H. affinis*, *P. reticulata*, and *R. quelen* (Table 6). These metrics support the specialization-disturbance hypothesis (Vázquez and Simberloff, 2002), which states that specialists are more affected by habitat disturbance than generalists. Moreover, intolerant species are those that first disappear in case of disturbance, and are not commonly found in intermediate sites either (see

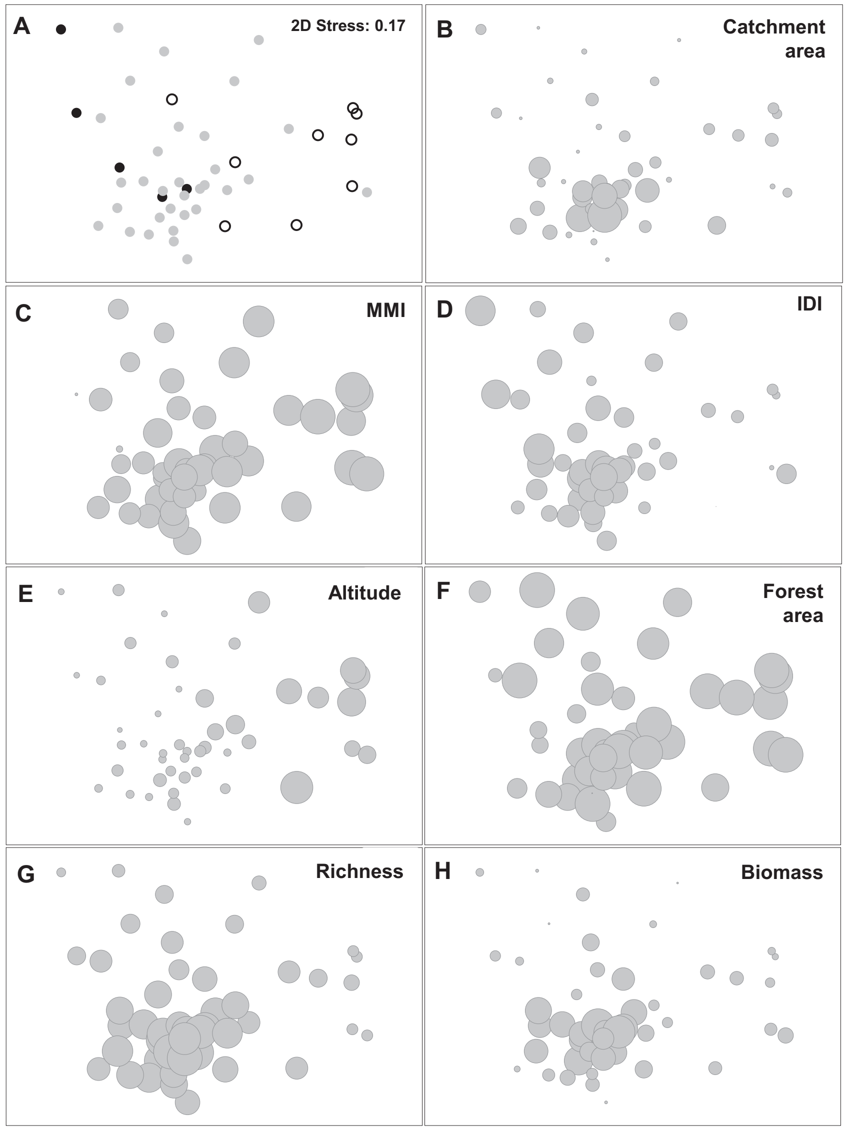

Fig. 6. MDS plots of fish assemblages at each of the 48 Atlantic Rain Forest sites based on Bray–Curtis similarity measures and depicting different variables. A – black circles = most-disturbed, gray = intermediate, open = least-disturbed. Scale of bubbles: B – 600–6000; C – 0.8–8.0; D – 0.2–2.0; E – 40–400; F – 0–100; G – 3–30; H – 900–730.

Section 2). *R. transfasciatus*, *T.* cf. *zonatus*, *B. ornaticeps*, and *C. vidali* responded positively to least-disturbed sites (Table 6). This can be associated with different factors such as the availability of diverse habitats (e.g., patches of pools and riffles, and boulder and cobble cover) and the availability of riparian food inputs.

The trophic category was represented by the percentage of benthic invertivorous individuals and the percentage of detritivorous individuals. The preceding four intolerant species also are classified as invertivores or omnivores with a tendency to be invertivorous (in the *C. vidali* case) (Brasil-Sousa et al., 2009; Rondineli et al., 2009; Rezende et al., 2011). Invertivores are affected by decreased invertebrate prey resulting from riparian vegetation removal and/or sewage discharge. In addition, all four species except *B. ornaticeps* are benthic species; having low mobility and high dependence on the substrate, they are likely limited by sewage discharge and erosion that increase turbidity and siltation. Although *H. affinis* and *G. brasiliensis* also are benthic species, they are detritivores (Delariva and Agostinho, 2001) and were most abundant in the disturbed sites. In their case, an increase of detrital food, mainly from the discharge of organic matter and sewage, aided their persistence. It has been demonstrated that detritivores benefit from sewage plumes (Henriques et al., 2013). The same thing occurs with *P. reticulata*, a detritivorous generalist species (Ferreira and Casatti, 2006b). Being a topminnow, living near the clearer and aerated surface layer of streams, it is frequently associated with polluted systems that have low oxygen concentrations and high turbidity elsewhere in the water column (Pinto and Araújo, 2007; Casatti et al., 2009).

4.2. Multimetric index advantages

One positive aspect of our MMI was the way human impact was defined, following Ligeiro et al. (2013). This approach is an alternative to that employing only site physical and chemical threshold to indicate disturbance and develop MMIs for a large number of sites (Ligeiro et al., 2013; Stoddard et al., 2008). Contrary to established bioassessment programs (like those applied in the USA and Europe), in South America, we lack reliable information about physical and chemical thresholds that precisely indicate disturbance. Instead, a single index summarizing overall anthropogenic pressures, although never perfect, is a quick and practical way to describe the pressure on individual sites and the relative pressure of a site in comparison to others (Wang et al., 2008; Ligeiro et al., 2013).

An important characteristic of our MMI is the calibration of metrics, eliminating the effect of natural size and altitude gradients, and enabling application of the same index regardless of basin area or site altitude. Partitioning the effects of natural factors from the effects of anthropogenic stressors has been reported as a critical component of nearly all bioassessment programs (Herlihy et al., 2008; Pont et al., 2009). Employing metrics that are correlated with the natural environment as well as the level of disturbance will reduce the sensitivity of the index to anthropogenic disturbance (McCormick et al., 2001; Whittier et al., 2007a). So, the use of residuals (the deviation between the observed and the predicted value of a metric) instead of the initial metric value was an important alternative to maintain the metric in the selection process. A similar approach has been used in predictive model MMIs for benthic macroinvertebrate assemblages (Moya et al., 2007, 2011), and fish assemblages (Oberdorff et al., 2002; Pont et al., 2006, 2009; Tejerina-Garro et al., 2006).

4.3. Multimetric index limitations

Our MMI suffers two limitations: (a) the absence of pristine reference sites and (b) the lack of functional and biological information about many fish species. Ideally, we should have used a large number of reference sites to validate the capacity of our index in predicting metric trends in the absence of human disturbance. This was unfortunately not possible due to the lack of pristine sites in this region (Bozzetti and Schulz, 2004) and difficulties in sampling minimally disturbed sites. Although this is one of the most diverse biomes in the world, a large part or its area has been devastated. The Atlantic Rain Forest has been almost completely replaced by urban areas, sugar cane, coffee, eucalyptus, pines trees, soy beans and pasture land, and the riparian vegetation of most river headwaters is not preserved (Barletta et al., 2010). In addition, the two largest urban regions of the country occur in the Atlantic Rain Forest biome which provides ecosystem services to >70% of the Brazilian population. Remnant areas preserved as conservation units, which contain least-disturbed streams, are far from roads and difficult to access. As highlighted by Bozzetti and Schulz (2004) and Herlihy et al. (2008), the choice of least-disturbed sites situated only in headwaters is problematic because of natural longitudinal changes in stream processes and fish faunas (Vannote et al., 1980). So, unlike most fish-based MMIs developed in tropical regions that used a posteriori reference methods, presuming that 'the least-impacted" condition would emerge from the dataset, (Bozzetti and Schulz, 2004; Pinto and Araújo, 2007; Esteves and Alexandre, 2011; Terra and Araújo, 2011), we adopted the a priori least-disturbed site approach to define reference conditions for metric development (Stoddard et al., 2006; Whittier et al., 2007a). Least-disturbed condition is found in conjunction with the best available physical, chemical, and biological habitat conditions given today's state of the landscape. Such least-disturbed sites are selected according to a set of explicit criteria defining what is "best" or least-disturbed by human activities (Stoddard et al., 2006; Herlihy et al., 2008; Whittier et al., 2007b).

Although there are large numbers of Atlantic Rain Forest fish species and endemic species, available information concerning their habitat, trophic, reproduction, and life history guilds is scarce (Barletta et al., 2010). The limited guild information hinders MMI creation because functional guild metrics are an important component of a robust and precise index, given that they tend to suffer smaller natural variations and respond more predictably to stress (Elliott et al., 2007; Marzin et al., 2012; Pont et al., 2006). This shortage of appropriate information about ecology, biology, and taxonomy is one of the most important challenges for Brazilian fish biologists in the coming years, especially given the large numbers of Brazilian species identified and yet to be identified.

4.4. Assemblage descriptors and disturbance

We developed an MMI for assessing the biological disturbance generated by human activities. Sites classified by our MMI as most-disturbed were those with degraded riparian vegetation, sewage discharge, trash, and human habitation in the margin, consequently those with the highest IDI scores as well. Those sites had the poorest assemblage condition, with more tolerant species and few individuals. On the other hand, the least-disturbed sites were those that had more instream habitat structure and complexity, dense riparian vegetation, and no or insignificant sewage discharge, trash, and near-stream human habitation. In those sites, species most closely associated with habitat complexity to realize biological functions (reproducing, feeding, hiding, migrating) were most abundant. However, when we analyzed classic assemblage variables like richness, biomass, and abundance, the highest values of those variables were consistently found in the intermediate-disturbance sites (Fig. 6G, H). Those sites, if evaluated only through use of the classic assemblage variables, would suggest better condition than our least-disturbed sites. Such a pattern is explained by the Intermediate Disturbance Hypothesis, which states that local species diversity is maximized when ecological disturbance is neither too rare nor too frequent (Connell, 1978). McCormick et al. (2001) reported a similar pattern for number of native cyprinid species and number of native benthic species in response to riparian disturbance of Appalachian Highlands (USA) streams. For these reasons, Whittier et al. (2007a) did not select species richness, biomass, nor abundance metrics for the MMIs that they developed for western USA streams; nor did Pont et al. (2006) do so for their European MMI.

Our MMI indicated that the least-disturbed sites occurred in the highest altitudes (Fig. 6E). Obviously, such sites are more difficult to access by humans, which is likely the main reason for their good ecological condition. However, the large forest areas in their catchments did not guarantee good ecological condition; even with >50% forest in their catchments, site classifications ranged between most-disturbed and least-disturbed (Fig. 6F). This disturbance range indicates that other human activities, independent of forest cover, may influence stream ecological condition strongly (Ligeiro et al., 2013).

5. Conclusions

Our MMI is one of the few indices to assess ecological quality of Atlantic Rain Forest streams. The results obtained here highlight the importance of employing a disturbance gradient index when developing an MMI to ensure that the gradient of perturbation will be detected by the index. This is especially important in regions where pristine systems are scarce or difficult to access. The use of an extensive group of screening steps in metric selection, including natural gradient calibrations, also was important to ensure a robust and discriminating index. In this paper, we produced a cost-effective alternative to physico-chemical assessment, because we have shown that the fish-based MMI responded efficiently to multiple human pressures and aided assessment of ecological status. There is a pressing need to arm environmental managers with appropriate indices to ensure that freshwater ecosystems are protected and where necessary rehabilitated effectively to good ecological condition (European Commission, 2000). However, it would be premature to reach a final conclusion regarding the use of this MMI in all Atlantic Rain Forest streams without further testing, and this study is but a starting point for the successful use of an MMI in monitoring programs.

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Appendix A.

List of species collected in 48 Atlantic Rain Forest stream sites, southeastern Brazil.

Order	Family	Species
Characiforms	Crenuchidae	*Characidium interruptum* Pellegrin, 1909[‡]
		Characidium vidali Travassos, 1967[‡]
	Characidae	*Astyanax bimaculatus* (Linnaeus, 1758)[†]
		Astyanax giton Eigenmann, 1908
		Astyanax hastatus Myers, 1928
		Astyanax intermedius Eigenmann, 1908
		Astyanax janeiroensis Eigenmann, 1908
		Astyanax parahybae Eigenmann, 1908
		Astyanax sp.
		Astyanax sp.1
		Astyanax sp.2
		Astyanax taeniatus (Jenyns, 1842)[†]
		Brycon opalinus (Cuvier, 1819) [‡]
		Bryconamericus microcephalus (Miranda Ribeiro, 1908) [‡]
		Bryconamericus ornaticeps Bizerril & Perez-Neto, 1995[‡]
		Bryconamericus tenuis Bizerril & Auraujo, 1992[‡]
		Deuterodon parahybae Eigenmann, 1908
		Deuterodon sp.
		Deuterodon sp.2
		Hyphessobrycon reticulatus Ellis, 1911[‡]
		Mimagoniates microlepis (Steindachner, 1877)[‡]
		Oligosarcus hepsetus (Cuvier, 1829)
	Erythrinidae	*Hoplerythrinus unitaeniatus* (Spix & Agassiz, 1829)
		Hoplias malabaricus (Bloch, 1794)
Siluriforms	Callichthyidae	*Callichthys callichthys* (Linnaeus, 1758)
		Corydoras nattereri Steindachner, 1876
		Hoplosternum littorale (Hancock, 1828)
		Scleromystax barbatus (Quoy & Gaimard, 1824)
	Loricariidae	*Ancistrus multispinis* (Regan, 1912)[‡]
		Hemipsilichthys gobio (Lütken, 1874)[‡]
		Hisonotus notatus Eigenmann & Eigenmann, 1889[‡]
		Hypostomus affinis (Steindachner, 1877)[†]
		Hypostomus sp.
		Kronichthys heylandi (Boulenger, 1900)[‡]
		Loricariichthys castaneus (Castelnau, 1855)
		Neoplecostomus microps (Steindachner, 1877)[‡]
		Parotocinclus maculicauda (Steindachner, 1877)
		Parotocinclus sp.
		Pseudotothyris obtusa (Miranda Ribeiro, 1911)[‡]
		Rineloricaria sp.1
		Rineloricaria sp.2
		Schizolecis guntheri (Miranda Ribeiro, 1918)[‡]
	Heptapteridae	*Acentronichthys leptos* Eigenmann & Eigenmann, 1889[‡]
		Heptapterus sp. [‡]
		Pimelodella lateristriga (Lichtenstein, 1823)
		Rhamdia quelen (Quoy & Gaimard, 1824)[†]
		Rhamdioglanis transfasciatus Miranda Ribeiro, 1908[‡]
	Trichomycteridae	*Homodiaetus passarellii* (Miranda Ribeiro, 1944)[‡]
		Listrura nematopteryx de Pinna, 1988[‡]
		Trichomycterus cf. *paquequerense*[‡]
		Trichomycterus sp.
		Trichomycterus cf. *zonatus*[‡]
Gymnotiforms	Gymnotidae	*Gymnotus sylvius* Albert & Fernandes-Matioli, 1999
		Gymnotus pantherinus (Steindachner, 1908)[‡]
Cyprinodontiforms	Rivulidae	*Kryptolebias brasiliensis* (Valenciennes, 1821)[‡]
	Poeciliidae	*Phalloceros* aff. *anisophalos*

		Phalloceros harpagos Lucinda, 2008
		Poecilia reticulata Peters, 1859†
		Poecilia vivipara Bloch & Schneider, 1801
		Xiphophorus sp.*
Synbranchiforms	Synbranchidae	*Synbranchus marmoratus* Bloch, 1795
Perciforms	Cichlidae	*Cichla kelberi* Kullander & Ferreira, 2006*
		Cichlasoma sp.*
		Crenicichla lacustris (Castelnau, 1855)
		Crenicichla cf. *lepidota*
		Geophagus brasiliensis (Quoy & Gaimard, 1824)†
		Oreochromis niloticus (Linnaeus, 1758)*
	Gobiidae	*Awaous tajasica* (Lichtenstein, 1822)

* Alien species.
† Tolerant species.
‡ Intolerant species.

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