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A Fish-Based Index of Biotic Integrity for Neotropical Rainforest Sandy Soil Streams—Southern Brazil

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Abstract: Multimetric indices are considered a low-cost and rapid means of assessing ecological integrity in streams. This study aimed to develop a fish-based Index of Biotic Integrity (N3S-IBI) in an agricultural region within the domains of the Atlantic rainforest in Brazil. We sampled 23 first-order streams and used large-scale land use and a local physical condition index to choose reference sites and to classify sites according to the disturbance level. N3S-IBI resulted in six metrics (Simpson's dominance; the numbers of Characiformes and non-native individuals (*Poecilia reticulata*); and the percentages of Characidae species, intolerant insectivorous individuals, and tolerant species), contemplating tolerance, composition, abundance, richness, trophic habits, and origin. The low number of metrics contributes to a quick and easy biomonitoring process. N3S-IBI showed an excellent performance to separate least and most disturbed sites in our study area and can provide additional knowledge about anthropogenic effects within this impacted region. In fact, this tool could be utilized by managers to direct restoration actions for the most disturbed sites and to strengthen the preservation of the least disturbed sites.

Keywords: ecological quality; freshwater ecosystems; biomonitoring; Characiformes; Cyprinodontiformes; *Poecilia*; sugarcane; IBI

1. Introduction

Surface freshwaters are among the most disturbed ecosystems on the planet and in recent decades have suffered faster rates of decline in biodiversity than those observed in terrestrial ecosystems [1,2]. The conversion of natural landscapes for human use is among the main drivers of the impact on these systems [3–6]. In fact, agricultural practices are the most widespread cause of stream degradation, degrading riparian areas, decreasing bank stability, modifying natural hydrological patterns, increasing chemical and sediments inputs (including pesticides, heavy metals, and herbicides), and altering natural habitats and biological communities [1,3,7–12]. Indeed, many studies have shown that agriculture and the alterations derived from its practices present strong influence on the integrity of fish communities, affecting ecological traits related to feeding and reproduction and promoting the increase in the abundance of non-native and tolerant species [10,12–15].

The Southern region of Brazil has also experienced an increase in crop areas in the last decades, mainly sugarcane, corn, wheat, and soybean plantations, with direct impacts on the watersheds [16,17].

Common endpoints are the replacement of fish species that are habitat and feeding specialists by widespread generalists and the increasing of species that are tolerant to a wide range of physicochemical conditions [10,18,19]. The predominance of Characiformes and Siluriformes species in preserved streams of the Neotropical region is well known, being constantly near to 80% in headwaters [20–23]. However, in disturbed conditions there may be changes in this composition, including the increase of more tolerant species of Perciformes and Cyprinodontiformes [24–26]. For example, the high representativeness of non-native *Poecilia reticulata* Peters, 1859, and native *Phalloceros harpagos* Lucinda, 2008, both Cyprinodontiformes, seems to be common in disturbed first-order streams of the upper Paraná river basin [20,27–29].

Given the degradation that aquatic resources in these regions are experiencing, the development of tools to assess their ecological status is a major challenge in the management of freshwater ecosystems. One of the most used methods for assessing the ecological integrity of streams and rivers is the Index of Biotic Integrity (IBI). This method was first presented by Karr [30] and was later adapted to a wide range of conditions worldwide including Temperate [31,32], Mediterranean [33,34], and Neotropical streams [10,18,35,36]. The IBI is based on the principle that biological communities respond to human changes in a predictable and quantifiable manner, representing an integration of the physical, chemical, and biological conditions of the system [37]. This type of tools is composed by a set of metrics related to the species composition and functional attributes of biological assemblages, such as taxa richness, trophic and habitat niche, and abundance. Application of this multimetric approach often involves the adaptation of metrics for each river type, i.e., to the specific biota and environmental conditions under study, and the subsequent use of specific methods for their selection [38]. The selected metrics should be sensitive to different types of degradation and respond in a predictive way to the pressure gradients. The value of the index at a site is compared with the expected value on the same river type representing the least disturbed conditions (reference conditions), with the stream biotic integrity varying according to the degree of this deviation. Neotropical streams encompass a variety of reference conditions, fish communities, and types of pressures that need to be addressed on a regional basis.

Giving the challenges of using biotic indicators in environmental management and regulations in Brazil [39], there is an urgent need to provide managers and decision makers with user-friendly tools based on the biotic component of the aquatic systems. In this paper, our goal was to develop a fish-based IBI for an agricultural dominated Neotropical region within the Atlantic rainforest domain, being the first one focused only on sandy soil first-order streams. Thus, we expected to detect changes in fish assemblages according to the environmental conditions of these types of streams. This sandstone region has peculiar drainage characteristics regarding soil and sediment transport—for example, removal of forests to give place to other land use types increases siltation in a more pronounced manner than in other nearby areas [40]. Headwater streams represent the maximum interface with the landscape [41] and therefore are extremely vulnerable to human pressures, including the ecological effects on its native fish assemblages [42,43]. Although research has highlighted the consequences of anthropic impacts on stream biotic integrity on some agricultural areas of the Atlantic rainforest region [10,19,26,35], no studies were conducted to exclusively reveal those effects on fish assemblages in headwater streams of a single geomorphological unit. Moreover, our work presents an unusually large number of statistically tested metrics, aiming at greater robustness of the IBI, including testing with combined guilds.

2. Materials and Methods

2.1. Study Area and Site Selection

We selected 23 streams that drain small catchments in four sub-basins of the upper Paraná river basin (Ivaí, Paraná, Paranapanema, and Pirapó) (Figure 1). The study reaches were similar in catchment size and channel dimensions and represented small headwater streams. The upper Paraná river region is mainly comprised of sandy soils with homogeneous grain sizes, originated from Caiuá Sandstone geological formation, and within the domains of the semi-deciduous forest, originating from the Atlantic rainforest [44–47]. This biome is characterized by a tropical climate with an average annual rainfall of 1626 mm year−1. The average annual temperature and relative humidity are 27.9 °C and 68.8%, respectively [48]. Although study sites presented differences in land use at the catchment scale, the land cover of the region is dominated by agricultural uses (e.g., sugarcane, corn, peanut, orange, and soy). The selection of the study sites followed Cionek [49] and was primarily based on land-use at the catchment level in order to provide a wide range of environmental conditions that could represent distinct degradation status of the streams. The selection was conducted in ArcGis software® (ESRI, Redlands, CA, USA) through the delimitation of headwater watersheds. We used SRTM images to create and Landsat 8 OLI images to verify the stream basins. For each first order watershed, we calculated land-use percentage of agriculture (e.g., sugarcane, corn, peanut, orange, soy), pasture (grassland without use or used for extensive livestock), forest (natural forest, excluding eucalypt plantation), urban and exposed soil areas (usually temporarily exposed, before the next plantation—Table S1). All classifications were confirmed in the field. We included in our study the streams that were in watersheds with the largest proportion of each land use typology. In-stream and riparian environmental conditions were assessed by means of both a Rapid Assessment Protocol (described below) and limnological parameters (i.e., conductivity, pH, water temperature, turbidity, water velocity—Table S2).

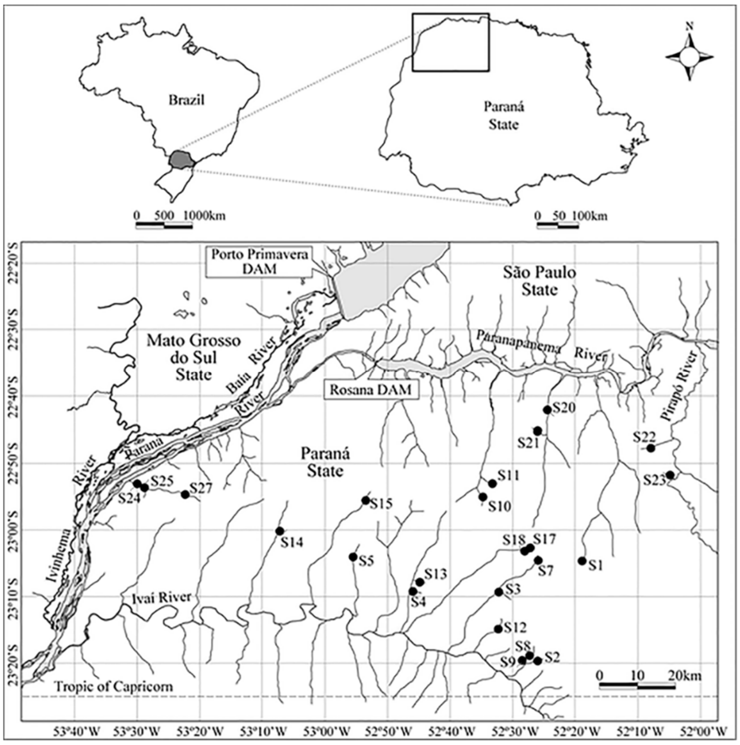

Figure 1. Location of the sampled stream sites.

2.2. *Measuring Anthropogenic Disturbance at Local-Scale*

A Rapid Assessment Protocol (RAP) especially designed for the study region [50], based on that proposed by Barbour et al. [51], was used to quantify local effects of anthropogenic disturbance at the sites during sampling visits (Table S3). RAP consists of nine parameters that are visually evaluated and scored as poor (scores 0 to 5), regular (scores 6 to 10), good (scores 11 to 15), and optimal (scores 16 to 20). The mean value of all parameters is used to determinate the stream physical condition. RAP cannot be applied without previous training, in order to properly identify natural features and provide an accurate evaluation. We always evaluate 30 m stretches—a feasible length to be visually assessed. RAP quantifies the underwater available cover, underwater habitat complexity, velocity/depth combinations, channel sinuosity, water level amplitude, channel integrity, bank stability, bank vegetative protection, and vegetation conservation on the riparian area.

2.3. Reference Conditions and Site Classification

The reference conditions were defined as the best conditions given today's state of the landscape, i.e., the least disturbed sites [50,52]. Based on the criteria used by other authors for the selection of reference sites [53–55] and considering the particular conditions of our sandy soil streams, we proposed pressure variables at two spatial scales. Regarding the catchment level, we considered the following land cover classes (see description of each type of land-use in Section 2.1): agriculture, urban (these two types of land-use are by far the most impactful in our study area) [56]; pasture (mostly, significantly less impacted areas than those in the previously two land uses), and forests. Thus, according to the RAP scores (i.e., local characterization) and predominant land use type (i.e., catchment characterization), and following the procedures proposed by Whittier et al. [31], we used assessment scores as least disturbed sites (LD), intermediate sites (ID) and most disturbed sites (MD). We defined LD sites as those meeting three criteria: (i) to feature the lowest percentage of agriculture at the catchment level (less than 35%, including sugarcane crops); (ii) absence of urbanized areas in the watershed; and (iii) classified as excellent or good, according the RAP. The MD sites were defined as those (i) presenting the highest percentage of agriculture + urban at the catchment level (more than 35%) and (ii) classified as poor or regular, according the RAP. The ID sites were those presenting (i) low agricultural + urban land use at the catchment level but degraded local habitat conditions (poor or regular) or (ii) low disturbed habitats (excellent or good) but high agricultural + urban land use. Similarly to Santos and Esteves [10], in a highly agricultural area in southern Brazil, we could expect non-pristine reference sites (including areas with low natural forest cover). However, we believe that these criteria assured us the selection of a group of sites that were significantly less altered than those clearly impacted by agriculture and urban land use.

2.4. Fish Sampling

Fish assemblages were sampled in April 2015, which represents the beginning of the drought period, to minimize the effect of rainfall on the habitat structure. The streams do not dry out completely in the low water season. We used daytime electrofishing with three consecutive efforts applied to each 80-m stream reach [57]; the sites were seine-blocked to prevent fishes from escaping. All individuals were counted and screened on the field. Up to 10 individuals of each morphospecies (whenever possible) were anesthetized with benzocaine [58] and fixed in 10% formalin, while the remainder were returned alive to nature. From each individual, we obtained total weight (g), standard length (cm), and identification based on specialized literature [59,60]. Specimens from all species were deposited in the Ichthyology Collection of NUPELIA (Nucleus of Research in Limnology, Ichthyology and Aquaculture) from the State University of Maringá (UEM), Brazil.

2.5. Metrics Evaluation and IBI Construction

Biological characteristics of fish species were determined from the literature and according to Pereira [61] (Table S4). The Index of Biotic Integrity for Neotropical Sandy Soil Streams (N3S-IBI) was developed following Whittier et al. and Krause et al. [31,55]. First, a list of 227 candidate metrics (Table S5) were compiled from the literature and organized into the following groups: diversity/abundance, composition (individuals), composition (family/order), origin (native/non-native), habitat guilds, reproductive guilds, tolerance, and trophic guilds. Metrics were calculated using standardized measures (1000 m^2 for every candidate metric) of number, biomass and proportion of taxa or individuals. The candidate metrics that passed the following four criteria were selected: (a) *range of homogeneity test*, that checks the distribution of metric values across all of the available data and allows for the elimination of those with very small ranges; (b) *responsiveness to disturbances*, performed with a

Kruskal–Wallis test ($p < 0.1$) to verify the capacity of the metrics to distinguish between the least and most disturbed sites; (c) *redundancy*, performed with a Spearman correlation test to choose metrics without redundant information with other metrics ($r > 0.70$), and (d) *range test for metric values*, based on the analyses of box plots of the metric scores (medians) for the LD and MD sites, to check if there was no overlap between most values of the two groups.

For metric scoring and calculation of final N3S-IBI, we used the 75th percentile of metric values of the LD sites for each positive metric (i.e., those that are highest in the least disturbed sites) as the scoring ceiling, to reduce the restriction of LD, and the 5th percentile of the distribution of values at all sites as the scoring floor (score = 0). Metric scores between the 5th and 75th percentile were interpolated linearly [31]. For negative metrics, we reversed the floor and ceiling values. The scored metrics were summed and divided by the number of metrics, and the index was scaled to a range of 0 to 1, where 0 corresponds to the worst and 1 to the best quality of each stream [62]. The quality is expressed within one of five quality classes (excellent, good, regular, poor, and very poor), thereby facilitating the communication of results to non-specialists.

A comparison between the disturbance categories of sites (i.e., least, intermediate, and most disturbed) was made using the Kruskal–Wallis test ($p < 0.05$) to verify the ability of IBI to classify environmental quality. In addition, we checked the correlation between the final index scores of the N3S-IBI and RAP and land use values for all sites using Spearman's test. We used R software for all statistical analysis [63].

3. Results

3.1. Fish Assemblages

The selected method for establishing reference conditions returned to us 7 LD, 9 ID, and 7 MD sites where we collected 5166 individuals comprised of 32 fish species, 13 families, and 6 orders (Table S4). For all sites combined, Cyprinodontiformes was the most abundant order (63.3%), followed by Siluriformes (26.7%) and Characiformes (8.7%) (Table 1). The Cyprinodontiform non-native species *P. reticulata* (Poeciliidae) was the most abundant species (58.0%), especially in MD sites where it represented 70.7% of the captures, while the native *P. harpagos* (Poeciliidae) was the most captured Cyprinodontiform species in the LD sites (27.0%). The Siluriform *Hypostomus ancistroides* (Ihering, 1911) (Loricariidae) presented the highest frequency of occurrence, being captured in 16 out of 23 streams.

Table 1. Abundance of sampled fish orders (%) according to the disturbance class. LD = least disturbed sites, ID = intermediate sites, and MD = most disturbed sites.

Order	Abundance at Sites (%)			
	LD	ID	MD	TOTAL
Siluriformes	30.2	26.4	26.4	26.7
Characiformes	39.0	45.8	0.3	8.7
Cyprinodontiformes	27.5	20.0	73.1	63.3
Native (*P. harpagos*)	27.0	8.0	2.3	5.4
Non-native (*P. reticulata*)	0.6	12.0	70.7	58.0

3.2. N3S-IBI Data Development

Of all candidate metrics, only 6 metrics were approved in all tests to compose the final N3S-IBI (Table 2). Most of the metrics (119) were eliminated during the responsiveness test, and only six out of 13 metrics passed the final range test (Figure 2). The chosen metrics were Simpson's dominance (diversity), number of Characiformes individuals (composition (individuals)), percentage of Characidae species (composition (family/order)), number of non-native individuals (origin), percentage of tolerant species (tolerance), and percentage of intolerant insectivorous (individuals) (trophic guild).

Table 2. Metrics evaluation test by class and number of remaining metrics to build the Index of Biotic Integrity (N3S-IBI).

Metric Class	Start	Metric Evaluation Test			
		Range	Responsiveness	Redundancy	Final Range Test
Diversity/Abundance	5	5	5	1	1
Composition (individuals)	126	126	18	5	1
Composition (family/order)	27	9	2	1	1
Origin	6	6	4	2	1
Habitat guild	12	12	3	0	0
Reproductive guild	15	15	6	2	0
Tolerance	12	10	3	1	1
Trophic guild	24	24	5	1	1
TOTAL	227	207	108	13	6

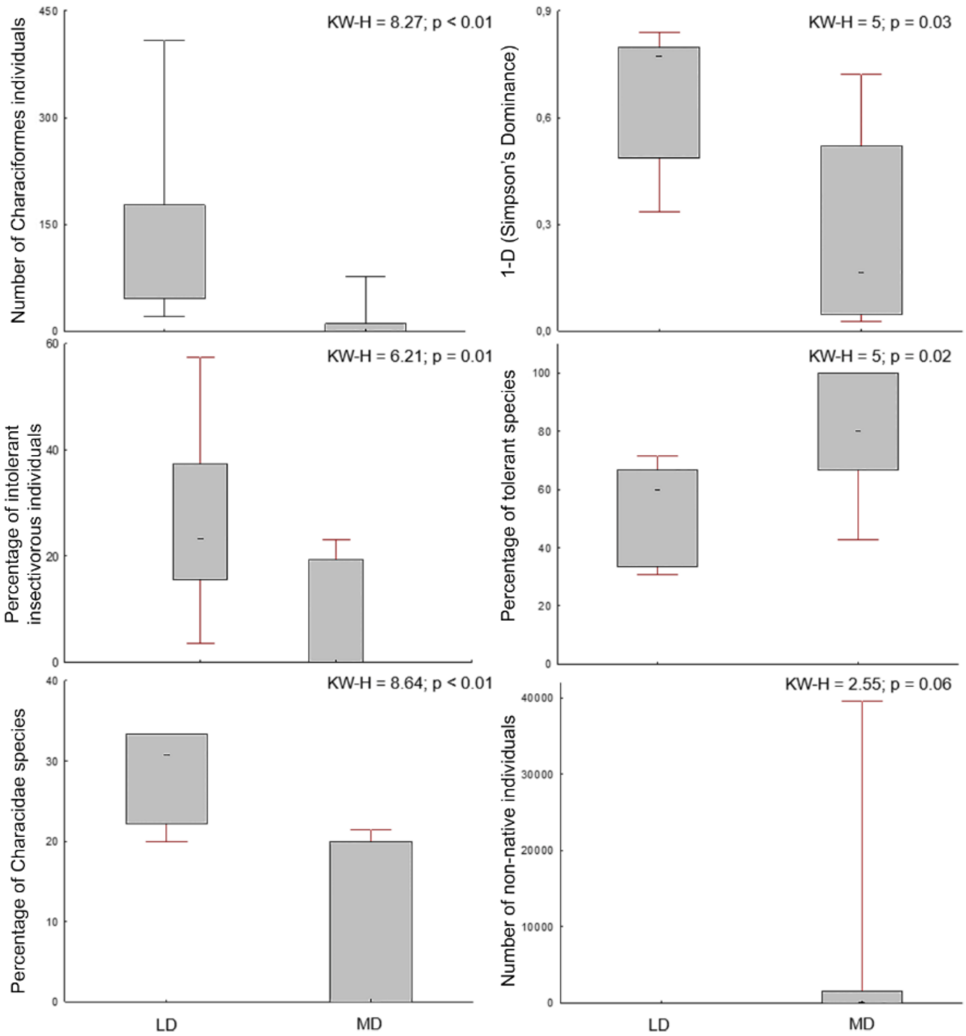

Figure 2. Distribution of metric values from the final range test for the least disturbed (LD) and most disturbed (MD) sites, and results from the Kruskal–Wallis test ($n = 14$; $p < 0.05$). Metrics passed this final range test if a majority of the sites did not have the same metric score regardless of disturbance class.

Final scores of the N3S-IBI, RAP values (see also Tables S6 and S7), and land use are shown in Table 3. Positive Spearman's correlation between N3S-IBI and RAP for all sites was marginally significant ($\varrho = 0.41$; $p = 0.055$), while the negative correlation between N3S-IBI and agricultural + urban land use (ϱ = −0.31, p = 0.15) was not statistically significant although it was ecologically relevant [64]. There was a significant difference in N3S-IBI values between LD and MD sites (Kruskal–Wallis test: H = 9.52; p = 0.009; n = 23) (Figure 3).

Table 3. N3S-IBI scores, RAP values, land use, and disturbance for the twenty-three Neotropical sandy soil streams; ALU = agricultural land use; ULU = urban land use; LD = least disturbed; ID = intermediate; MD = most disturbed.

Stream	IBI	RAP	Land Use	Disturbance	Watershed River
s20	1.00 (Excellent)	160 (Excellent)	33% ALU <3% ULU	LD	Paranapanema
s04	0.99 (Excellent)	150 (Excellent)	60% ALU <3% ULU	ID	Ivaí
s23	0.97 (Excellent)	91 (Good)	73% ALU <3% ULU	ID	Pirapó
s15	0.84 (Excellent)	86 (Good)	5% ALU <3% ULU	LD	Ivaí
s08	0.83 (Excellent)	97 (Good)	62% ALU <3% ULU	ID	Ivaí
s27	0.82 (Excellent)	117 (Good)	12% ALU <3% ULU	LD	Paraná
s25	0.76 (Good)	86 (Good)	33% ALU <3% ULU	LD	Paraná
s05	0.74 (Good)	108 (Good)	24% ALU <3% ULU	LD	Ivaí
s02	0.72 (Good)	135 (Excellent)	67% ALU <3% ULU	ID	Ivaí
s24	0.70 (Good)	86 (Good)	11% ALU <3% ULU	LD	Paraná
s14	0.54 (Regular)	115 (Good)	17% ALU <3% ULU	LD	Ivaí
s03	0.54 (Regular)	61 (Regular)	26% ALU 3% ULU	MD	Ivaí
s13	0.54 (Regular)	148 (Excellent)	77% ALU <3% ULU	ID	Ivaí
s09	0.40 (Poor)	37 (Regular)	69% ALU 4% ULU	MD	Ivaí
s22	0.36 (Poor)	116 (Good)	90% ALU <3% ULU	ID	Pirapó
s11	0.36 (Poor)	79 (Regular)	77% ALU <3% ULU	MD	Paranapanema
s10	0.30 (Poor)	115 (Good)	62% ALU <3% ULU	ID	Paranapanema
s17	0.27 (Poor)	59 (Regular)	20% ALU 18% ULU	MD	Ivaí
s12	0.17 (Very Poor)	58 (Regular)	71% ALU <3% ULU	MD	Ivaí
s21	0.17 (Very Poor)	162 (Excellent)	22% ALU <3% ULU	ID	Paranapanema
s01	0.16 (Very Poor)	94 (Good)	39% ALU <3% ULU	ID	Paranapanema
s07	0.09 (Very Poor)	76 (Regular)	26% ALU 14% ULU	MD	Ivaí
s18	0.00 (Very Poor)	60 (Regular)	9% ALU 87% ULU	MD	Ivaí

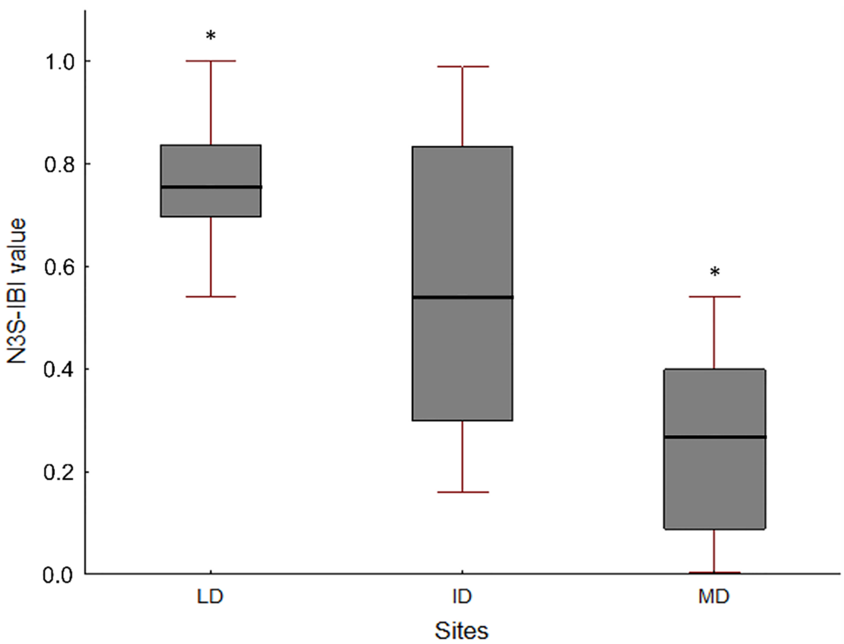

Figure 3. Distribution of N3S-IBI scores across disturbance classes; LD = least disturbed; ID = intermediate; MD = most disturbed. * represents significant difference (Kruskal–Wallis: H = 9.52; p = 0.009; n = 23).

The three streams from Paraná River basin presented IBI values between good and excellent (Table 3). These sites occur within or nearby an Environmental Protection Area (in Portuguese: Área de Proteção Ambiental das Ilhas e Várzeas do Rio Paraná). Streams from the Paranapanema river basin presented regular to excellent local habitat (RAP scores > 79) but only one showed an excellent biotic integrity, while the others were scored as poor or very poor. On the other hand, the streams of the Ivaí River basin presented a wide range of IBI values, across the gradient of very poor to excellent.

4. Discussion

The IBI for Neotropical sandy soil streams was composed of six metrics: Simpson's dominance, number of Characiformes individuals, percentage of Characidae species, percentage of intolerant insectivorous individuals, number of non-native individuals, and percentage of tolerant species. Our hypothesis was not rejected, and the N3S-IBI showed an excellent performance to separate least and most disturbed sites in our study area. We agree with Karr and Chu [65] that the selection of appropriate metrics is the key step in the proper functioning of this type of index. In fact, appropriate statistical criteria and sufficient expertise about the studied region are essential to the choice of metrics, and we believe that those selected for our N3S-IBI were properly representative of the sandy bottom streams of the upper Paraná river basin.

Some characteristics of our metrics were: (i) Simpson's dominance: it is a metric used in some IBIs, in which disturbed streams tend to show dominance of few species and present low overall species richness, whereas more preserved streams exhibit few dominant species and more rare species [10,66,67]; (ii) number of Characiformes individuals: the abundance of individuals from this order tends to decrease in disturbed streams, giving place to the occupancy of more disturbance-tolerant species, such as *P. reticulata* [66]; this same pattern may also be expected for the (iii) percentage of Characidae species, although both metrics were not redundant; (iv) percentage of intolerant insectivorous individuals, which tend to be replaced in disturbed sites by tolerant species showing generalist feeding behaviors [30,37]; (v) percentage of tolerant species: tolerance is a frequent metric for biomonitoring tools and was also important for distinguishing our streams [18,26,66]; (vi) number of non-native individuals: some authors have pointed out the relevance of non-native fishes as indicators of disturbance intensity [68–70], and in our study sites the non-native species are represented by *P. reticulata*, a very tolerant species to environmental degradation [71].

The low abundances and/or the absence of Characiformes/Characidae in the most degraded streams represent a good predicting metric for the studied systems. We found a strong relationship between stream degradation and the decrease of both Characiform and Characid species and individuals. Our degraded sites were frequently depleted of riparian vegetation, and their streams presented eroded channels, low potential cover for fish, high habitat homogeneity, and substrates revealing a negligible presence of elements other than sand. Although these species represent a large range of trophic guilds, most of them are water column feeders and intolerant to habitat destruction and persistent water quality degradation [35]. Other Neotropical IBIs, that somehow followed robust metric selection processes, have found this relation [35,39], although Santos and Esteves [10] had not selected these metrics for an agricultural dominated region of São Paulo state. We believe that the higher number of Characid species in our study, all intolerant to degradation, could have strengthened the response of this metric.

A decrease of insectivores typically reflects a degradation of the invertebrate food base of lotic systems and turned out to be a good predictor of stream integrity [37]. In our study, the impoverishment of the invertebrate fauna might be directly associated to the absence of riparian protection in the most disturbed sites. In fact, the prevalence of a few riparian plant species, with dominant grass species along the immediate margin of streams, tends to homogenize the invertebrate community, which represents an essential food resource to intolerant insectivorous fish [35,72–74]. Additionally, land-use and water quality degradation (including substances as pesticides and heavy metals) may have reduced the diversity and richness of macroinvertebrates in the most disturbed sites, decreasing the food availability for these species [52,75,76].

The occurrence of Cyprinodontiformes is quite common in tropical streams [8,27–29,77,78], especially because they present ovoviviparity as a reproductive strategy and a wide variety of feeding habits [29,79–81], characteristics only common to these species in the sampled sites. Our results showed that the native Cyprinodontiform (*P. harpagos*) is more abundant in the least-disturbed sites, while in the most-disturbed sites it was replaced by the non-native *P. reticulata*. Such a change in the identity of the Cyprinodontiform species occurrence between LD and MD sites contributed to the good performance of the N3S-IBI for stream quality assessment. The ability of non-native fishes to thrive in degraded conditions and consequently to impact natural fish assemblages has also been detected worldwide [68–70] and represents one of the main causes of decline in biotic integrity. Moreover, while studying Neotropical streams, Ferreira and Casatti [66] used the percentage of *P. reticulata* as a metric, since the presence of this species is one of the most prominent indicators of degraded condition to streams on the South of Brazil. However, *P. harpagos* was also used as an indicator of stream degradation on another IBI for Neotropical streams [10], although it was not statistically significant for this approach in our study. This is an example of the importance of developing different IBIs of regional scale in multi-diverse regions. Ruaro et al. [71] proposed a multimetric index to another Neotropical basin and analyzed the influence of non-native species on the loss of ecological integrity. Our study brings new outcomes for Neotropical streams from a nearby region that drains through Caiuá Sandstone geological formation, helping to fill a knowledge gap in one of the most impacted regions by monocultures, providing more information about these anthropogenic impacts.

Biotic indices can be developed with distinct biotic communities, such as periphyton, macroinvertebrates, and fishes. Each presents particular pros and cons regarding its application [82]. However, larger-sized organisms (like fish) are readily identified and sorted in the field, compared to the time required for screening and identification of macroinvertebrates [37]. This is particularly relevant for the IBIs, which intend to be expeditious tools of environmental quality assessment. Moreover, within our study region, fishes are amongst the most popular and easily recognizable organisms to raise awareness about results provided by the N3S-IBI. This index was composed of six metrics, which makes a quick and easy biomonitoring tool. Therefore, like Ruaro and Gubiani [83], we also believe that a simple and fast index is efficient for monitoring aquatic environments. It is practically impossible to apply a single monitoring tool to an entire country when it has the continental proportions and the heterogeneity of biomes of Brazil. However, we support proposals such as that of Stoddard et al. [84], with the development of local and regional tools, interconnected in a single monitoring system. Although there is no standardization in the collection methodologies for studies of fish-based IBIs in Brazil, there are some papers developing this tool in many states, especially in the South-Central region [10,35,39,85–87]. However, we suggest the adoption of an electrofishing protocol as an initial step for a single regional tool that would facilitate the use of fish data collected by colleagues throughout all localities, similar to the one used by Oliveira et al. [88] in Portugal.

The establishment of reference sites is one of the first steps in assessing the ecological status of streams using IBIs [78,89] and defined as being those with least anthropic influence. Our reference and disturbed sites did not overlap in the results. In fact, the MD and LD sites were totally separated by the IBI values, and the ID sites were equally distributed among them. We understand that the same did not occur with the RAP classification because it is a specific habitat tool, and the visual evaluation does not allow for the identification of larger-scale and limnological influences; however, it was useful to determine our reference sites, and this should be applied as a complementary tool. Although some authors did not find a relation between land use and biotic integrity [90,91], we also believe that N3S-IBI was significantly affected by land use at a larger scale, which proved to be a useful tool to classify our reference sites, clearly identifying the degree of disturbance in the sampled streams. Fish assemblages supply an integrated response to environmental changes that can be accounted for by means of community composition alterations. Variation of physical or chemical indices can be temporary or not large enough to allow the proper detection of the multiple human impacts on freshwater ecosystems [37]. In fact, the biological indices represent an integration of the physical, chemical, hydrological, and biological conditions of the system; ultimately, it is not possible to measure all factors that impact biotic integrity [37]. However, the characterization of any environmental factor affecting the aquatic biota, as the habitat quality (e.g., provided by the RAP) can provide an initial profiling of the ecosystems and improve our ability to interpret biological responses [92]. Additionally, it can show us that biological responses may not vary until a sufficient alteration has occurred within the systems (i.e., resilience) [93].

Often public management agencies collect information restricted only to monitoring fluviometric levels, physical and chemical parameters, and quantification of specific bacteria [94,95], possibly due to the volume of work and costs. Water is a vital resource for all living beings, so it is understood that its monitoring must go beyond these parameters and include the biotic integrity of the aquatic communities. Initiatives to conserve freshwater systems in a large country such as Brazil go through the development of regional tools. In this way, although it needs to be validated, N3S-IBI is a well-developed tool for freshwater systems in a region of approximately 3 million ha [95]. On the other hand, it is imperative to improve the monitoring system. Thus, all actions concerning the collection of biological integrity data from aquatic systems are more than welcome, whether if they are from a public institution or non-governmental groups. Environmental researchers have been neglected in Brazil over the years, but the political role of their contributions [85] becomes clearer when tools like N3S-IBI are an accessible facilitator in the engagement of people inside and outside the control agencies and the academic environment. In fact, this tool could be utilized by managers to direct restoration actions for the most disturbed sites and to strengthen the preservation of the least disturbed ones.

Supplementary Materials: The following are available online at http://www.mdpi.com/2073-4441/12/4/1215/s1, Table S1: Abiotic variables at the catchment scale., Table S2: Local abiotic variables., Table S3: Rapid Assessment Protocol adapted to the study region., Table S4: Classification of sampled species according to their biological characteristics., Table S5: Candidate metrics for the N3S-IBI., Table S6: RAP metrics., Table S7: Rapid Assessment Protocol—Field Spreadsheet.

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analysis and supervised all manuscript preparation steps. G.G., E.B., V.d.M.C., M.T.F and J.M.O. commented on the manuscript. G.G., E.B., V.d.M.C., M.T.F and J.M.O. have read and agreed to the published version of the manuscript. All authors have read and agreed to the published version of the manuscript.

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