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ASSESSMENT OF STREAM ENVIRONMENTAL CONDITION USING FISH-BASED METRICS IN A PROTECTED AREA AND ITS DISTURBED BUFFER ZONE, NORTHEASTERN ATLANTIC RAINFOREST

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Abstract: The susceptibility of streams to anthropogenic interference raises the need for continuous assessment of their environmental condition. From early studies to recent approaches, metrics derived from fish assemblages have proven to be fundamental tools in evaluating the ecological condition of watercourses. We assessed the environmental degradation of streams inside and surrounding the Córrego Grande Biological Reserve, state of Espírito Santo, Brazil, to develop and apply a biotic index for this region based on fish metrics. We performed samplings in 19 stream sites along 2012, 2018 and 2019, and collected 24 species belonging to five orders, 11 families, and 21 genera. The sites were classified as least-disturbed (N = 9, all within the protected area), intermediate (N = 8), and most-disturbed (N = 2). Ordination analyses distinctly separated the sites according to their disturbance classes, revealing that the percentage of native vegetation cover in 100 m buffers and type of riparian vegetation played an important role in the environmental quality of these sites. From the initial 38 metrics, three were able to distinguish between disturbance classes: percentage of Siluriformes and Characiformes species, percentage of nektonic species, and percentage of omnivorous individuals. Our results show that these metrics are significant factors to be considered in monitoring the environmental degradation of Atlantic Forest streams.

Keywords: bioindicators, conservation, freshwater, multimetric index.

INTRODUCTION

Streams are highly susceptible to anthropogenic impacts such as pollution, alterations in the riparian zone, and landscape fragmentation (Araújo 1998, Barletta *et al.* 2010, Ávila *et al.* 2018), which supports the need for continuous approaches to assess the conservation status of these environments. According to Casatti *et al.* (2009b), the assessment of aquatic environments should incorporate attributes able to integrate the behavior of elements and biological processes that express anthropogenic interference with aquatic communities. Karr (1981) developed a pioneer biotic integrity index that considered the tolerance of fish species, trophic and population structure, among other factors, providing reasons why fish might be excellent indicators of environmental degradation. This index was mirrored and improved by several authors in Brazil (*e.g.* Araújo 1998, Ferreira & Casatti 2006, Casatti *et al.* 2009b, Terra *et al.* 2013, Carvalho *et al.* 2017). These authors developed or adapted environmental assessment methods that consider functional aspects of the species, quality of the hydric systems, riparian environment, the presence of certain species and orders, and chemical and physical variables of the water. Multimetric indices (MMI), applied in water quality monitoring programs in several countries (*e.g.*, USA, France, Spain) are not commonly used in developing countries like Brazil, because of the absence of (i) laws for implementing complex water monitoring programs and (ii) the lack of an integrated view of ecological conditions of rivers and small streams (Hughes *et al.* 2000, Terra *et al.* 2013).

Small streams are commonly regarded as objects of community ecology and conservationist studies because of their reduced size, which makes them more susceptible to anthropogenic impacts with potential to compromise the stability of fish species populations (Ferreira & Casatti 2006, Frota *et al.* 2016). According to the most recent data from the Brazilian Ministry of Environment, there are currently 310 threatened freshwater bony fish species in the country, mainly members of the Rivulidae family (ICMBio 2018). In the state of Espírito Santo, 18 freshwater fish species are at some level of threat, particularly members of the Characiformes and Siluriformes orders, according to the latest list of endangered species (Hostim-Silva *et al.* 2019).

The Rio Itaúnas valley, near the Espírito Santo-Bahia state border, has experienced widespread deforestation, with the *Eucalyptus* plantations and livestock pastures becoming the main land-use type (Sarmento-Soares & Martins-Pinheiro 2013, Hostim-Silva *et al.* 2019, Sarmento-Soares *et al.* 2019). This is particularly a concern for Córrego Grande Biological Reserve, a small conservation unit mostly surrounded by *Eucalyptus* plantations, where an abrupt transition is observed between the protected forest and the extractive area, the latter corresponding to the buffer zone of the preserved area (Figure S1, Sarmento-Soares & Martins-Pinheiro 2017). Streams that run in agroecosystems can exhibit a gradient of physical habitat conditions, from degraded, less complex sites to slightly altered reaches (Casatti *et al.* 2009a, Ribeiro *et al.* 2016). Therefore, the need for monitoring studies on the ecological quality of water bodies, especially those surrounding protected areas in agroecosystemic regions, is proving essential for the development of biomonitoring programs, assessment of the disturbance gradient, headwaters protection and species conservation programs.

Here we determined the disturbance gradient of stream sites inside and surrounding the Córrego Grande Biological Reserve, northern Espírito Santo, developing an index for this region based on fish metrics responsiveness to disturbance categories, in three steps: (a) assessment of disturbance classes for the sites evaluating impacts on local and landscape scales; (b) selection of indicators derived from the fish assemblages capable of distinguishing among those disturbance classes; and (c) lastly, development, scoring, and validation of a MMI.

MATERIALS AND METHODS

Study area

The Córrego Grande Biological Reserve (hereafter referred to as CGBR, Figure 1) is in the municipality of Conceição da Barra, northern Espírito Santo state, bordering the state of Bahia (Sarmento-Soares & Martins-Pinheiro 2013). The name of protected area derives of the stream that delimits the reserve in its western portion – Córrego Grande stream, an affluent of Rio Itaúnas, the largest drainage in the north of the state (ICMBio 2019). In addition to Córrego Grande stream, the preserved area has in its domain the headwaters of the Córrego Taquaruçu stream, another sub-basin of Rio Itaúnas.

Despite the great importance for the preservation of the protected area, the buffer zone of the CGBR presents legalized extractive activities that include extensive livestock farming, *Eucalyptus* monocultures, and communities of small farmers, along with illegal activities such as wildlife hunting and fires. In this intensely exploited area, there are several headwaters and tributaries both from the Rio Itaúnas basin, such as the Córrego Dourado stream, to the west of the

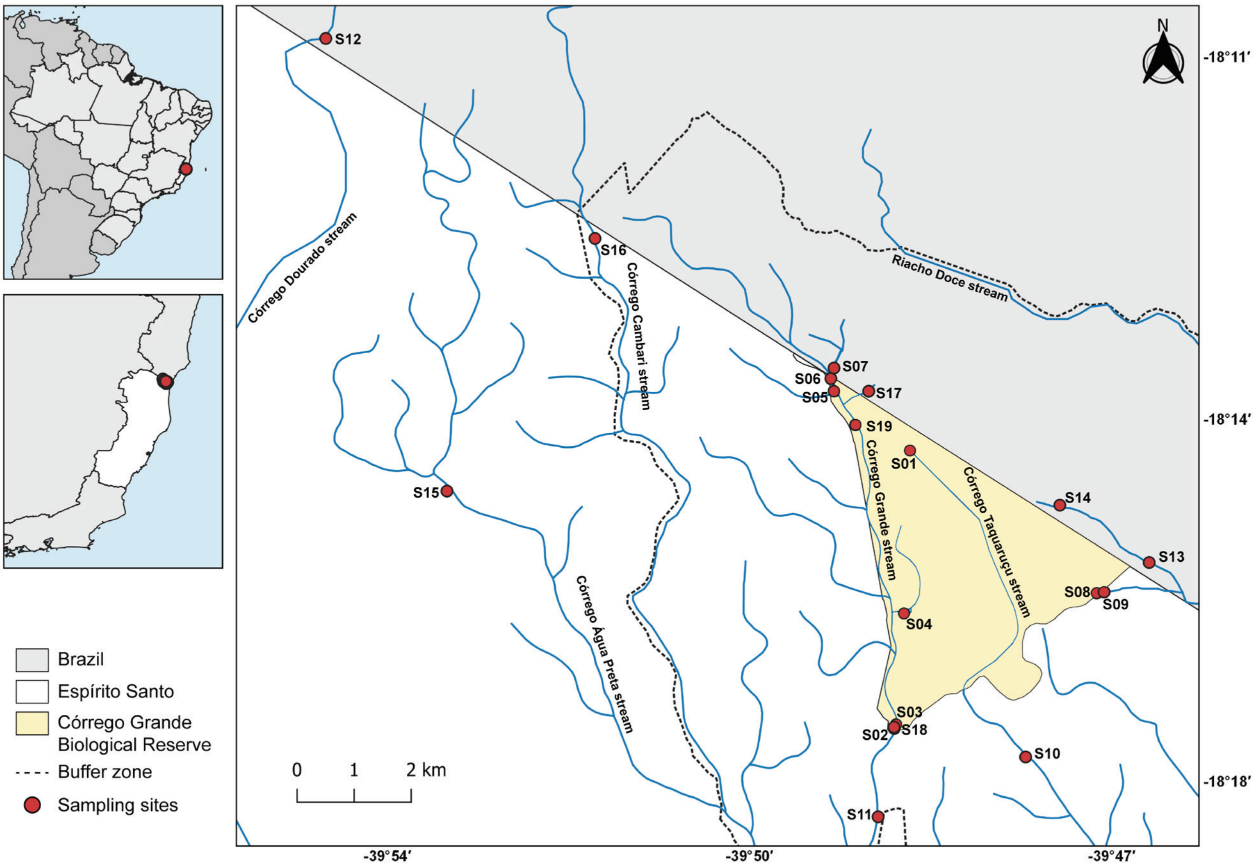

Figure 1. Map showing the location of the study area (red circle) in Brazil (top left map), in the state of Espírito Santo (bottom left map) and the set of sub-basins the drain Córrego Grande Biological Reserve and its buffer zone (right map).

reserve, and the microbasin of the Riacho Doce stream, which runs in Bahia but has tributaries on its right margin in Espírito Santo. The drainage of the Córrego Grande stream has a total area of 35 km², whereas the catchments of Taquaruçu, Doce, and Dourado have, respectively, 61 km², 140 km² and 372 km² (Sarmento-Soares & Martins-Pinheiro 2013). In the classification of Abell *et al.* (2008), the study area is in ecoregion 328, Northeastern Atlantic Rainforest.

The phytophysiognomy of the region is composed of coastal tablelands forest, a formation of the Atlantic Forest that consists of predominantly sandy and fragile soils covered by dense forest (ICMBio 2019) and pioneer vegetation, in mostly flat to wavy terrains (Sarmento-Soares & Martins-Pinheiro 2013, Sarmento-Soares *et al.* 2019). Water accumulates in flooded areas that, because of the concentration of large quantities of leaves and tannin, display a tea-like or amber coloration. The climate in the region is Am (tropical monsoon), according to the climatic classification system of Köppen (Dubreuil *et al.* 2018), with average monthly temperatures between 22-24 °C and annual rainfall ranging from 1200 to 1300 mm in the coastal tablelands, where rainfall seasonality becomes more evident (Alvares *et al.* 2013).

Sampling

The samples used in this study were obtained in three years: 2012 (Sarmento-Soares & Martins-Pinheiro 2013), 2018, and 2019. Nineteen georeferenced sites were sampled in five small sub-basins that drain CGBR and its vicinities: Grande, Taquaruçu, Água Preta, and Dourado sub-basins, and the Doce micro-basin, in an area totaling 688 km². The sites were chosen according to their proximity to the biological reserve and the human settlements in the area. For each site, physical data on climate, altitude, presence and type of marginal and riparian vegetation, substrate type, width, and depth were obtained using measuring tapes and visual or photo assessment. To define the percentage of native vegetation cover for a site, a buffer of 100 m x 100 m was delimited around it. The proportion of native vegetation cover in the area was estimated using shapefiles obtained from the online database GEOBASES and using high resolution images from the Landsat 8 satellite available in the Landviewer online database and Google Earth Pro software, obtained for the years 2020 and 2012, respectively.

We standardized the effort by performing 1h of sampling in 30 to 60 m long reaches, with seines and sieves. As the sampling stretches varied in size, the effort was adjusted according to the length of the stretch, to ensure equivalent sampling effort. To have the least possible impact to the ichthyofauna, the specimens were packed in 5 L drums with water, transported to the margin, identified, photographed, counted, and released. Most of the sampled individuals were released, since this is a conservation unit where most species may be sensitive to population decreases, especially endangered species. We deposited some specimens of each species as voucher material at the Museu Nacional (MNRJ/UFRJ), Museu de Biologia Mello Leitão (MBML/INMA) and Coleção Zoológica Norte Capixaba (CNZC/UFES) (Appendix 1). Those were photographed in a field aquarium, alive, euthanized with a 10% eugenol solution, fixed in 10% formaldehyde solution, transported to the laboratory, and transferred to 70% ethanol. Some specimens intended for genetic analysis were preserved in 99% ethanol. The specimens were identified to the lowest taxonomic level possible with the assistance of inventories published in scientific papers and identification manuals, such as those of Roldi *et al.* (2014), Sarmento-Soares and Martins-Pinheiro (2010, 2012, 2013, 2014a, 2014b), in addition to consulting specialists. The samplings were carried out under environmental permits no. 20096-1, no. 27880-1, and no. 63125-1, provided by the Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio).

Determination of disturbance gradient

First, an adaptation of the environmental degradation index presented by Ligeiro *et al.* (2013) was applied, later replicated by authors such as Terra *et al.* (2013) and Macedo *et al.* (2016). These authors proposed combining a catchment disturbance index (CDI) and a local disturbance index (LDI) into an integrated disturbance index (IDI) (Fierro *et al.* 2018). The land use percentages were estimated for each site by vectorizing satellite images available from the Landviewer online viewer and Google Earth Pro software using Landsat 8 satellite images. To estimate the land use, we created area maps of 0.1 km² around each site, built in the software QGis 3.14 (Qgis Development Team 2020) in scale 1:25,000. We considered four types of land use: urban, agricultural, pasture (Ligeiro *et al.* 2013) and tree plantation (Fierro *et al.* 2018). The types of land use have different weights according to their disturbance potential on the natural environments (Ligeiro *et al.* 2013, Macedo *et al.* 2016). The CDI was calculated as the sum of these land use types:

$$CDI = 4 * \%urban + 2 * \%agricultural + 1 * \%pasture + 0.5 * \%treeplantation$$

LDI followed an adaptation of the W1_hall metric, proposed by Kaufmann *et al.* (1999), which considers different scores in relation to the proximity of anthropogenic impacts to the river, on a 10 m x 10 m plot. This index summarizes the presence and proximity of 11 types of disturbances observed in the riparian zone of stream sites, such as buildings, channel revetment, pavement, roads, trash, agriculture, among others (Ligeiro *et al.* 2013, Carvalho *et al.* 2017). In our approach of this metric, we changed the "mining" disturbance type for "water abstraction", which is a common practice in the streams of the study area. We also reduced the number of transects, adapting the calculation of the metrics accordingly, ranging from four to 10 transects per site. Because the CDI and LDI indices have different numerical scales, we standardized their values by dividing the maximum theoretical value of each by its 75th percentile (*i.e.* CDI values were divided by 300 and LDI values were divided by 5; Fierro *et al.* 2018). The IDI for each site was calculated as follows:

$$IDI = \left[\left(\frac{LDI}{5}\right)^2 + \left(\frac{CDI}{300}\right)^2\right]^{\frac{1}{2}}$$

Adapting the methodology proposed by Carvalho *et al.* (2017), to categorize sites into disturbance categories, we defined the sites as least-disturbed (equal or below the 10th percentile of the maximum IDI value) and most-disturbed (equal or above the 90th percentile). The sites with IDI scores between these thresholds were classified as intermediate.

Assemblage-based metrics and data analyses

We tested 38 metrics (Table S1) related to diversity and taxonomic composition of fish assemblages, tolerance to disturbances, trophic ecology, and habitat use, from information obtained in several studies (*e.g.* Bozetti & Schulz 2004, Pinto & Araújo 2007, Casatti *et al.* 2009b, Teresa & Casatti 2012, Cruz *et al.* 2013, Terra *et al.* 2013, Casatti *et al.* 2015, Teresa *et al.* 2015, Terra *et al.* 2016, Ávila *et al.* 2018). When no information could be obtained for the species, we extrapolated the data to genera and families.

We first eliminated metrics with a range < 5%. Then a pairwise Spearman correlation was performed to remove strongly correlated metrics ($r > |0.75|$, $p < 0.05$). For redundant pairs, we removed the metric with higher mean collinearity with all the others (Terra *et al.* 2013). Metric responsiveness to disturbance was tested with a permutational analysis of variance (PERMANOVA), retaining only metrics with the ability to distinguish between disturbance classes. Box-and-Whisker plots of the final metrics values were produced for visual comparison among the disturbance classes.

To validate the IDI model, we followed Terra *et al.* (2013) and Fierro *et al.* (2018). We performed a Principal Components Analysis (PCA), using the physical variables mean depth, marginal vegetation type (vegetation directly adjacent to the body of water, which can be used as shelter), riparian vegetation type (vegetation in the 10 m buffer from the margins), substrate type (transformed categorical variables), and percentage of native vegetation cover in a 0.1 km^2 buffer with the IDI classes as factors. Finally, to further determine the position of the sites along an environmental gradient, we performed a non-metric multidimensional scaling (NMDS) ordination, using the PCA1 scores against the IDI scores. All analyses were performed in the software PAST v. 4.03 (Hammer *et al.* 2001).

RESULTS

Taxonomic composition

We collected 2,257 specimens belonging to five orders, 11 families, 21 genera and 24 species (Appendix 2). The most representative order and family were Characiformes (N = 1,855, 82.2%) and Characidae (N = 1,722, 76.3%), respectively. The most abundant species was *Mimagoniates microlepis*, about 35% (N = 800) of the collected specimens, followed by *Hyphessobrycon bifasciatus* (N = 411, 18.2%). The richness in the sites ranged from one to 14 species (mean = 5.9 ± 3.9), and the abundance from one to 537 specimens (mean = 118.7 ± 164.8).

Disturbance assessment

From the 19 sites, nine were classified as least-disturbed (IDI = 0.00-0.03), eight as intermediate (IDI 0.14-0.63), and two as most-disturbed (IDI > 0.89) (Table 1). Of the 10 stream sites within the biological reserve, nine presented reference environmental conditions (forested riparian zone, no disturbances at local or catchment scales). The first and second axis of the PCA explained 43.11% and 28.05% of the variation of the data, respectively, showing that least-disturbed sites were strongly influenced by the percentage of native vegetation cover and most-disturbed sites were correlated with types of substrates, marginal and riparian vegetation characteristic of altered environments (sand and silt substrates, grass, and sparse bushes). The NMDS analysis distinctly separated sites according to their disturbance classes (2D stress = 0.01) (Figure 2).

Metric selection and validation

Among the initial 38 metrics (Table S1), only "percentage of common species individuals" was eliminated because it presented a range of less than 5%. Spearman resulted in 19 highly correlated variables, subsequently eliminated. Among the 18 remaining variables, only three met the criteria of distinguishing between disturbance classes through PERMANOVA: percentage of Siluriformes and Characiformes species, percentage of nektonic species, and percentage of omnivorous individuals (Table 2, Figure 3). Both percentage of Siluriformes and Characiformes

Table 1. Sites in Córrego Grande Biological Reserve and surroundings. IDI = Integrated disturbance index, LD = Least-disturbed, IN = Intermediate, MD = Most-disturbed. *Sites located inside the conservation unit.

Code	Site	Coordinates	IDI	Disturbance
S01	Córrego Taquaruçu stream headwater*	18°14’44,5”S 39°48’49,6”W	0.00	LD
S02	Córrego Grande stream, reserve 01*	18°17’30,3”S 39°48’59,0”W	0.00	LD
S03	Córrego Grande stream, reserve 02*	18°17’27,9”S 39°48’57,8”W	0.00	LD
S04	Guaxos lagoon*	18°16’21,7”S 39°48’53,2”W	0.00	LD
S05	Córrego Grande stream, reserve 03*	18°14’09,1”S 39°43’34,9”W	0.00	LD
S06	Córrego Grande stream, reserve 04*	18°13’58,8”S 39°49’36,9”W	0.03	LD
S07	Tributary lagoon of Córrego Grande stream	18°13’55,3”S 39°49’34,9”W	0.28	IN
S08	Pequi lagoon*	18°16’09,5”S 39°46’58,2”W	0.00	LD
S09	Sucuri lagoon	18°16’08,9”S 39°46’53,8”W	0.53	IN
S10	Córrego Taquaruçu stream	18°17’47,3”S 39°47’40,6”W	0.23	IN
S11	Córrego Grande stream, near road	18°17’47,3”S 39°47’40,6”W	0.24	IN
S12	Córrego Dourado stream	18°10’48,7”S 39°54’38,1”W	0.89	MD
S13	Riacho Doce stream tributary 01	18°15’51,3”S 39°46’26,9”W	0.93	MD
S14	Riacho Doce stream tributary 02	18°15’17,1”S 39°47’20,2”W	0.63	IN
S15	Córrego Água Preta stream	18°15’08,7”S 39°53’25,8”W	0.48	IN
S16	Córrego Cambari stream	18°12’38,0”S 39°51’57,5”W	0.35	IN
S17	Tributary of Córrego Grande stream*	18°14’07,4”S 39°49’12,8”W	0.00	LD
S18	Córrego Grande stream, reserve 05*	18°17’30,3”S 39°48’59,0”W	0.14	IN
S19	Córrego Grande stream, reserve 06*	18°17’30,3”S 39°48’59,0”W	0.00	LD

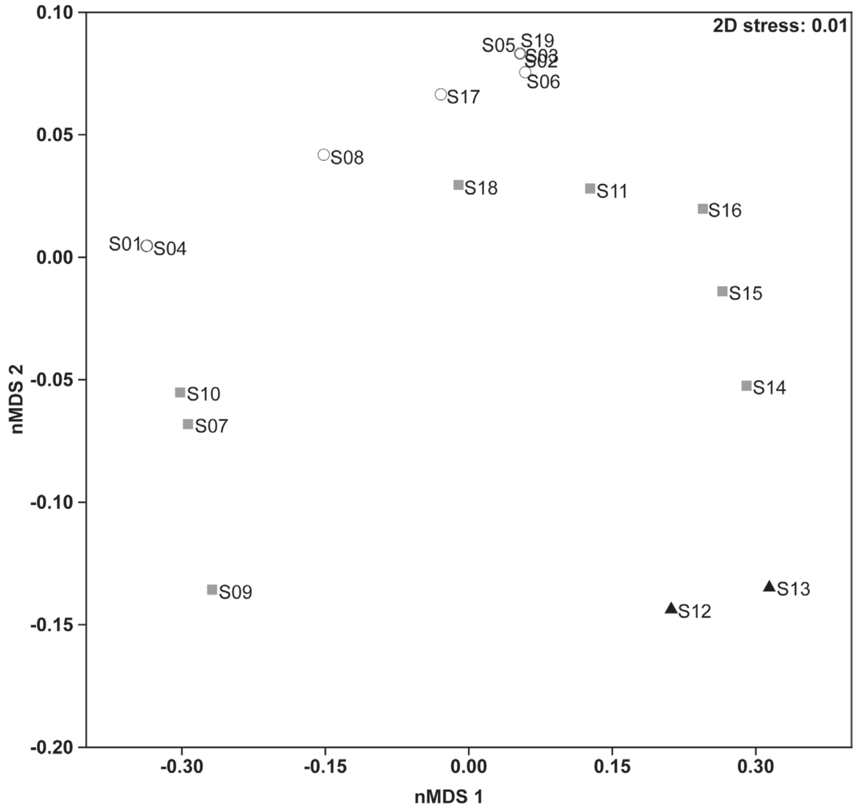

Figure 2. Non-metric Multidimensional Scaling ordination on PCA 1 scores and IDI values. White circles are least-disturbed sites, gray squares are intermediate, and black triangles are most-disturbed.

species and percentage of omnivorous individuals were only able to distinguish least-disturbed from intermediate sites (p = 0.02 and p = 0.05, respectively). Percentage of nektonic species was able to distinguish least-disturbed from intermediate (p = 0.01) and most-disturbed sites (p = 0.02). The outlier in the "percentage of omnivorous individuals" graph represents a discrepant sample point (S01) in which all individuals of the species were omnivorous.

DISCUSSION

In the Neotropical region, especially in the Atlantic Forest biome, there is a recognized predominance of Characiformes and Siluriformes, as attested by Lowe-McConnell (1999) and Menezes *et al.* (2007). Similar occurrence is observed in the sub-basins of CGBR and its surrounding, where these two orders account for about 80% of the sampled species. Regarding families, the Characidae is also known to be the most diverse in the Neotropical region as well as in this study area, mainly because of characteristics that facilitate their occupation of several environments (Lowe-McConnell 1999, Buckup *et al.* 2007, Furtado *et al.* 2018). One factor to consider in the high representativeness of characids in the study is the inherent selectivity of the sampling methodology. Because we performed our samplings during the day, primarily diurnal species were collected, such as *Deuterodon janeiroensis* or *Mimagoniates microlepis* (Mazzoni & Iglesias-Rios 2002), which may have affected the development of the multimetric index.

Species of small-sized Neotropical freshwater fish, including those considered threatened, generally inhabit modestly sized aquatic environments, such as streams, ponds, and marshes and are generally very dependent on the existence of riparian vegetation for their food, shelter, and breeding sites (Castro 1999, Castro *et al.* 2005). Although not presenting high biomasses, these small-sized species represent most of the Neotropical freshwater fish biodiversity (Castro & Polaz 2020). Despite their extraordinary diversity, these fishes are not interesting as targets of commercial, sustenance, or food purposes, making their conservation a challenge (Castro & Polaz 2020).

The conservation unit protects two endangered species: *Acentronichthys leptos* and *Mimagoniates sylvicola*. Both are on the endangered species list of the state (Hostim-Silva *et al.* 2019), the latter appearing as endangered on the last national red list (ICMBio 2018). In fact, *M. sylvicola* is found in clear or matte tea-colored waters with abundant marginal vegetation, exclusively in forested environments and with shading vegetation cover, feeding on allochtonous invertebrates (Sarmento-Soares *et al.* 2019). In Espírito Santo state, *M. sylvicola* was found only in the CGBR, evidencing the role of this protected area in the maintenance of the flora, fauna, and streams. Its congener, *M. microlepis*, was found both inside and in the buffer zone of the conservation unit, as well as elsewhere in the headwaters and lower portions of the Rio Itaúnas basin and in streams draining the Sooretama Biological Reserve (Sarmento-Soares & Martins-Pinheiro 2012, 2013, Plesley 2017). *Acentronichthys leptos* is a monotypic species described from the Rio São Mateus basin (Eigenmann & Eigenmann 1889), the neighboring basin in the south of the Rio Itaúnas basin, and has a wide distribution, from Santa Catarina to Bahia (Sarmento-Soares & Martins-Pinheiro 2009). In the north of Espírito Santo state, *A. leptos* populations are restricted to forested areas and shaded environments of streams with acidic, dark, black tea-colored water, with weak to moderate current (Sarmento-Soares *et al.* 2019) According to

Table 2. Final MMI fish-based metrics. F = test result for metric responsiveness between disturbance classes, LD = Least-disturbed (mean ± SD), IN = Intermediate (mean ± SD), MD = Most disturbed (mean ± SD), ER = Expected response to disturbance. Degrees of freedom: 2.

Metric	F ($p \leq 0.05$)	LD	IN	MD	ER
% Siluriformes + Characiformes species	4.26 (0.03)	87.18 ± 15.24	67.55 ± 9.72	74.11 ± 11.61	Decrease
% nektonic species	6.14 (0.01)	18.44 ± 17.14	44.43 ± 15.64	50±0	Increase
% omnivorous individuals	2.18 (0.05)	19.62 ± 29.56	53.24 ± 31.33	67.85 ± 11.26	Increase

Sarmento-Soares *et al.* (2019), in CGBR these fish are primarily invertivorous, feeding on aquatic invertebrates especially insects including larvae, pupae, arachnids, and crustaceans, besides vegetal fragments and detritus. However, the intense threats to the species, especially habitat loss and fragmentation, have contributed to its disjunct distribution, and probably for these reasons the species has not been found for a long time in the Rio São Mateus basin.

According to the Brazilian Federal Law No. 9985/2000, which establishes the National System of Conservation Units (Sistema Nacional de Unidades de Conservação, SNUC), the buffer zone of a conservation unit aims to filter the negative impacts of human activities on the protected area, as well as mitigate edge effects and protect springs (Vio 2001). There are intense extractive activities in the surroundings of CGBR, especially livestock farming and *Eucalyptus* monocultures. These activities have a great disturbance potential in natural environments, both by the removal of native vegetation, soil degradation, and discharge of pesticides in the streams that cross this area, since the riparian vegetation prevents agrochemicals from being carried into the water bodies (Martinelli & Filoso 2007, Teresa & Casatti 2010). Allied to these factors, the intense traffic on the Picadão da Bahia road, which borders the northern limit of the reserve, throws a large amount of dust on the limits of the protected area, increasing the mortality of the vegetation (ICMBio 2019). Sarmento-Soares *et al.* (2019) list a series of measures that could be applied in the CGBR buffer zone (as well as in other conservation units in a similar condition) in order to mitigate the anthropic impacts of these activities, such as the non-use of herbicides or insecticides in the surrounding *Eucalyptus* plantations and the recomposition of riparian forests around springs and streams by the implementation of Areas of Permanent Protection, with the installation of fences to prevent cattle from entering the margins of streams.

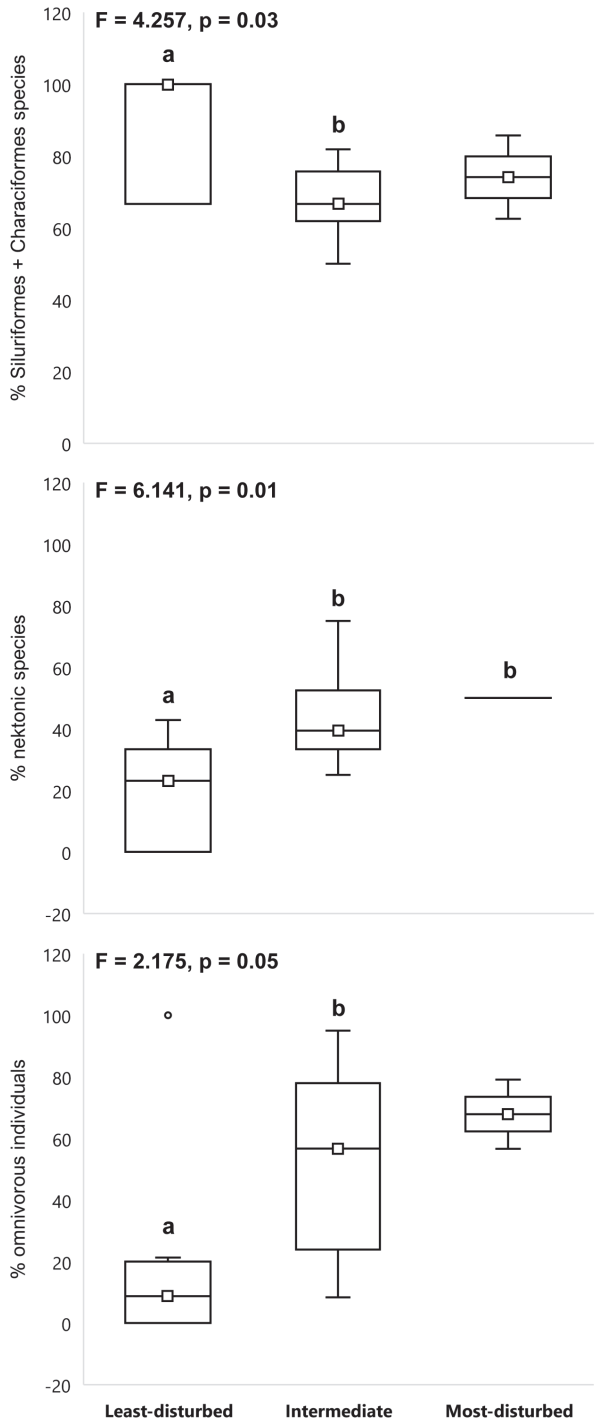

Figure 3. Box-and-Whisker plots of the final metrics derived from the fish assemblages. Rectangles represent the 1st and 3rd quartiles, bars are minima and maxima, and small squares represent the medians. Different letters indicate statistically significant divergences.

Our results indicate that, despite being in a region with high rates of anthropogenic impacts and being a small conservation unit, strongly surrounded by agricultural and livestock influences, almost all sites within the CGBR demonstrated good environmental quality, sustaining a portion of the native aquatic fauna in the region. The exception was a single site (S18), located almost on the edge of the reserve, which despite being inserted in native forest, is close to a pasture area with scarce vegetation.

All sites outside the protected area demonstrated intermediate or poor conditions, which by considering the habitat characteristics considered most relevant for the definition of disturbance classes, such as native vegetation cover, reinforces the potential condition of shelter for species from this conservation unit. *Eucalyptus* plantations reduce riparian forest diversity and potentially yield agricultural chemicals and fine sediments to streams (Sarmento-Soares & Martins-Pinheiro 2018, Carneiro *et al.* 2019). However, the ichthyofauna appears to have remained in good condition within the reserve, with some exceptions, such as *M. sylvicola*, which was not collected in 2018 and 2019.

The use of multimetric indices to evaluate the environmental quality of rivers and streams has become a regular feature in the literature, having methods extensively described in recent studies for streams in the Atlantic Forest (Terra *et al.* 2013) and Cerrado (Ligeiro *et al.* 2013, Macedo *et al.* 2016, Carvalho *et al.* 2017). Considering that there are anthropogenic impacts at different spatial extents (Ligeiro *et al.* 2013, Fierro *et al.* 2018), these indices, coupled with variables from the faunal assemblages, generate scores that make the definition of environmental condition more complete. To adapt an MMI to our study area, some metrics commonly used in the literature were disregarded, such as biomass of native species, percentage of species and individuals of Heptapteridae, percentage of reophilic species and abundance of *Poecilia reticulata* (Araújo 1998, Ferreira & Casatti 2006, Casatti *et al.* 2009b, Terra *et al.* 2013, Ávila *et al.* 2018). Those metrics were not applicable because of the absence or low representativity of fish that meet these parameters or because of methodological limitations.

Although the percentage of Characiformes and Siluriformes species is higher in less disturbed locations (a result generated mainly by the presence of siluriforms unique to sites with low to intermediate disturbance conditions, such as *Acentronichthys leptos* and *Aspidoras virgulatus*), the abundance of Characidae species and individuals increases in disturbed sites. Ávila *et al.* (2018), who found similar results regarding Characidae, suggest that some of these fish, such as *Astyanax* spp. and *Deuterodon* spp., exploit the water column and have great trophic flexibility, independent of structures associated with more intact environments such as leaf litter and large wood (Bozzetti & Schulz 2004, Moraes *et al.* 2013). This result is correlated with two of the sensitive metrics: percentage of nektonic species and omnivorous individuals. Both metrics increased in sites with intermediate and greater disturbance because of the greater abundance of water column dwellers and omnivorous characids and cichlids, which often occur in sites with reduced environmental quality (Casatti *et al.* 2009b).

Assuming that several characid species, such as *Mimagoniates*, are known to be intolerant of environmental degradation (Bozzetti & Schulz 2004, Cruz *et al.* 2013), we hypothesize that the high relevance of these genera in disturbed sites is a result of other factors, such as increased availability of food resources. In disturbed sites, more generalist and opportunistic species (mainly omnivorous) tend to be favored, replacing more specialized species (Karr 1981, Casatti *et al.* 2009b, Ávila *et al.* 2018). The most disturbed sites here are also the streams with the greatest size and availability of food and habitat, which is in line with the River Continuum Concept (Vannote *et al.* 1980).

The metrics derived from the fish assemblages were able to indicate the increase of environmental degradation in the region, despite not being able to differentiate between reference, intermediate, and disturbed sites at the same time. The reduced discriminatory power of the index may be related to the low number of sampling points and the lack of adjustment of the index to the landscape, an important factor in generating reliable results (Macedo *et al.* 2016). In a future perspective, we expect to apply this MMI to the Rio Itaúnas basin, a scenario in which the number of sampling points would increase substantially. The choice of robust tests, the use of metrics directly related to fauna distribution, abundance, and composition, and the understanding of land use make this index potentially replicable and reliable, with appropriate adjustments for the study area. Therefore, as suggested by Ávila *et al.* (2018), these metrics are complementary, because while they are not sufficiently informative when used separately, they provide an efficient bioindicator when used together to assess the conservation status at a local and landscape extent. Because

Córrego Grande Biological Reserve is inserted in a strongly extractive environment—as much of northern Espírito Santo and southern Bahia—and presents in its area two endangered species, in a region of high endemism rates (Sarmento-Soares & Martins-Pinheiro 2012, 2013), we expect that this study will support the implementation of more efficient protective measures around the conservation unit.

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APPENDIX

Appendix 1. Voucher material deposited in zoological collections.

a. Museu Nacional, Universidade Federal do Rio de Janeiro (MNRJ/UFRJ) – 34 vouchers

Acentronichthys leptos MNRJ 51547; *Aspidoras virgulatus* MNRJ 51481, MNRJ 51548; *Deuterodon intermedius* MNRJ 51479, MNRJ 51483, MNRJ 51543; *Astyanax* sp. MNRJ 51457, MNRJ 51737; *Characidium cricarense* MNRJ 51542; *Characidium* sp. MNRJ 51486; *Cyphocharax gilbert* MNRJ 51456; *Geophagus brasiliensis* MNRJ 51451, MNRJ 51482, MNRJ 51540, MNRJ 51541; *Hoplias malabaricus* MNRJ 51454; *Hyphessobrycon bifasciatus* MNRJ 51450, MNRJ 51738, MNRJ 51549; *Hyphessobrycon* sp. MNRJ 51739, MNRJ 51581; *Mimagoniates microlepis* MNRJ 51455, MNRJ 51544, MNRJ 51580; *Mimagoniates* sp. MNRJ 51480; *Moenkhausia vittata* MNRJ 51453; *Otothyris travassosi* MNRJ 51485, MNRJ 51546, MNRJ 51582; *Phalloceros ocellatus* MNRJ 51484, MNRJ 51545; *Pimelodella vittata* MNRJ 51487; *Poecilia vivipara* MNRJ 51542; *Rhamdia quelen* MNRJ 51488.

b. Coleção Zoológica Norte Capixaba, Universidade Federal do Espírito Santo (CZNC/UFES) – 16 vouchers

Astyanax sp. CZNC 3561, CZNC 3565, CZNC 3569; *Corydoras nattereri* CZNC 3560; *Geophagus brasiliensis* CZNC 3556; *Hoplias malabaricus* CZNC 3555, CZNC 3567; *Hyphessobrycon bifasciatus* CZNC 3563, CZNC 3570; *Hyphessobrycon reticulatus* CZNC 3564, CZNC 3568; *Mimagoniates microlepis* CZNC 3562, CZNC 3566; *Otothyris travassosi*; CNZC 3558; *Phalloceros ocellatus* CZNC 3559; *Poecilia vivipara* CZNC 3557.

c. Museu de Biologia Mello Leitão, Instituto Nacional da Mata Atlântica (MBML/INMA) – 155 vouchers

Astyanax sp. aff. *A. lacustris* MBML 4730; *Deuterodon janeiroensis* MBML 4680, MBML 5613, MBML 4686, MBML 4721, MBML 4744, MBML 4749, MBML 4751, MBML 4820, MBML 4821, MBML 6360, MBML 4832, MBML 4773; *Deuterodon* sp. aff. *D. intermedius* MBML 4690, MBML 4698, MBML 5509, MBML 4734, MBML 4809, MBML 4759, MBML 4827, MBML 4766; *Hyphessobrycon bifasciatus* MBML 4707, MBML 4795, MBML 4710, MBML 4797, MBML 4720, MBML 4806, MBML 4726, MBML 4812, MBML 4742, MBML 4747, MBML 4816, MBML 4822, MBML 3656, MBML 4758; *Hyphessobrycon* sp. aff. *H. reticulatus* MBML 4743, MBML 4814, MBML 4825, MBML 4757, MBML 4828; *Mimagoniates microlepis* MBML 4684, MBML 4691, MBML 4700, MBML 4722, MBML 4802, MBML 4745, MBML 4815, MBML 5518, MBML 4756, MBML 4826, MBML 4772, MBML 4833, MBML 4778, MBML 4837; *Mimagoniates sylvicola* MBML 4705, MBML 4753; *Moenkhausia vittata* MBML 4728, MBML 4731; *Characidium* sp. aff. *C. cricarense* MBML 4679, MBML 4689, MBML 4717, MBML 4807, MBML 4733, MBML 4808, MBML 7369, MBML 4769, MBML 4830; *Hoplias malabaricus* MBML 4677, MBML 4685, MBML 4694, MBML 4703, MBML 4708, MBML 4796, MBML 4709, MBML 4724, MBML 4736, MBML 4737, MBML 4746, MBML 4754, MBML 4761, MBML 4763, MBML 4764, MBML 4779; *Aspidoras virgulatus* MBML 4682, MBML 4687, MBML 4697, MBML 4704, MBML 4714, MBML 4774; *Callichthys callichthys* MBML 4675, MBML 4692; *Corydoras nattereri* MBML 4727; *Acentronichthys leptos* MBML 4676, MBML 4718, MBML 4804, MBML 4768; *Pimelodella* sp. MBML 4732, MBML 4770; *Otothyris travassosi* MBML 4683, MBML 4688, MBML 4695, MBML 4701, MBML 4793, MBML 4800, MBML 4715, MBML 4801, MBML 4771, MBML 4831, MBML 4776, MBML 4834; *Trichomycterus pradensis* MBML 4729; *Australoheros capixaba* MBML 4739, MBML 4767; *Geophagus brasiliensis* MBML 4681, MBML 4699, MBML 4706, MBML 4792, MBML 4711, MBML 4798, MBML 4713, MBML 4799, MBML 4716, MBML 4725, MBML 4810, MBML 4735, MBML 4738, MBML 4740, MBML 4813, MBML 4750, MBML 4819, MBML 4762, MBML 4765, MBML 4775, MBML 4835; *Phalloceros ocellatus* MBML 4678, MBML 4693, MBML 4702, MBML 4794, MBML 4712, MBML 4719, MBML 4805, MBML 4741, MBML 4817, MBML 4752, MBML 4818, MBML 4824, MBML 4760, MBML 4829, MBML 4777, MBML 4836; *Poecilia vivipara* MBML 4723, MBML 4811; *Synbranchus marmoratus* MBML 4748, MBML 4755, MBML 4823.

Appendix 2. Abundance of the sampled species per disturbance classes. LD = Least-disturbed, IN = Intermediate, MD = Most-disturbed.

Order	Family	Species	LD	IN	MD	Total
Characiformes	Characidae	*Astyanax* sp. 2 aff. *A. lacustris*	0	0	1	1
		Deuterodon janeiroensis (Eigenmann, 1908)	12	95	103	210
		Deuterodon sp. aff. *D. intermedius*	33	91	34	158
		Hyphessobrycon bifasciatus Ellis, 1911	0	190	221	411
		Hyphessobrycon reticulatus Ellis, 1911	0	41	80	121
		Mimagoniates microlepis Steindachner, 1877	373	381	46	800
		Mimagoniates sylvicola Menezes & Weitzman, 1990	14	2	0	16
		Moenkhausia vittata Castelnau, 1855	0	0	5	5
	Crenuchidae	*Characidium cricarense* Malanski, Sarmento-Soares, Silva-Malanski, Lopes, Ingenito & Buckup, 2019	12	40	32	84
	Curimatidae	*Cyphocharax gilbert* Quoy & Gaimard, 1824	0	0	2	2
	Erythrinidae	*Hoplias malabaricus* Bloch, 1794	32	10	5	47
Siluriformes	Callichthyidae	*Aspidoras virgulatus* Nijssen and Isbrücker, 1980	16	5	0	21
		Callichthys callichthys (Linnaeus, 1758)	4	0	0	4
		Corydoras nattereri Steindachner, 1876	2	0	2	4
	Heptapteridae	*Acentronichthys leptos* Eigenmann and Eigenmann, 1889	1	8	0	9
		Pimelodella sp. n. sp. 2. sensu Sibien, 2019	1	1	16	18
		Rhamdia quelen (Quoy & Gaimard, 1824)	1	0	0	1
	Loricariidae	*Othothyris travassosi* Garavello, Britski & Schaefer, 1998	50	41	0	91
	Trichomycteridae	*Otothyris pradensis* Sarmento-Soares, Martins-Pinheiro, Aranda & Chamon, 2005	0	0	5	5
Cichliformes	Cichlidae	*Australoheros capixaba* Ottoni, 2010	0	1	3	4
		Geophagus brasiliensis Quoy & Gaimard, 1824	12	66	25	103
Cyprinodontiformes	Poeciliidae	*Phalloceros ocellatus* Lucinda, 2008	25	43	48	116
		Poecilia vivipara Bloch & Schneider, 1801	0	0	23	23
Synbranchiformes	Synbranchidae	*Synbranchus marmoratus* Bloch, 1795	0	3	0	3
Total			**588**	**1,018**	**651**	**2,257**