Structure of fish assemblages with the bioinvasion of *Clarias gariepinus* in a river in the Guapimirim Environmental Protection Area, southeastern Brazil.

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Abstract

The African sharptooth catfish (*Clarias gariepinus*) is the most important catfish species for aquaculture. It has great capacity to endure several stressing factors, such as adverse environmental conditions, as well as a broad feeding flexibility. Notwithstanding, the presence of this alien fish in the Guapimirim Environmental Preservation Area, in Southeast of Brazil, may generate negative impacts on the community of native fish. In 2018, during dry and rainy seasons, samples from the fish community were collected in thirty-two sites of the Guapi-Macacu River, and the abiotic variables (salinity, pH, temperature, turbidity, dissolved oxygen, and transparency) were assessed to diagnose which factors influence in the distribution of the alien fish along the river. Moreover, species within the ichthyofauna were evaluated as bioindicators to identify possible alterations in the community. Multivariate analyzes indicated that the African sharptooth catfish dominates the buffer zone of the environmental protection area, benefiting from higher levels of dissolved oxygen and temperature. However, *C. gariepinus* still do not dominate the most protected area of Guapimirim, where a higher percentage of native fish species dwell. The alterations in abiotic factors, related to the increase in the temperature, may significantly contribute to the domination of this invading alien fish in this protected area, being necessary the constant monitoring of some key species, as well as the population of the invading species in this environmental preservation area.

Introduction

Invasive alien species (IAS) are considered one of the main threats to the biodiversity and an important component of global environmental change (Clavero & García-Berthou, 2005). Once present and acclimated, the control of the IAS is challenging and results in marked changes in the native fauna, compromising the balance and stability of the ecosystem by reducing their stocks, causing extinctions, competition, pathogen transmission, and hybridization (Agostinho & Júlio Jr, 1996; Hill & Lodge, 1999; Miller, 1989; Omeji et al., 2013; Thrush & Peeler, 2006).

The African sharptooth catfish, *Clarias gariepinus* (Burchell, 1822), is a native species in great part of the African continent and parts of Southeast Asia, such as Israel, Syria, and South Turkey (Graaf & Janssen, 1996). It is considered as one of the most important catfish species for aquaculture (Osibona et al., 2009) and the third most important commercial species in countries such as Egypt (Khallaf & Gaber, 1991). Additionally, it has exceptional capabilities: it can move to the terrestrial environment; can breathe the atmospheric air through pseudolungs, which make them tolerate adverse conditions of temperature and low oxygen concentrations (Graaf & Janssen, 1996). *Clarias gariepinus* was introduced in Brazil in the 1980’s for aquaculture and pay-to-fish businesses (Agostinho et al., 2007; Alves et al., 1999). At present, this species is produced commercially mainly in the state of Santa Catarina (58.9% of the total fishing in 2007), followed by Espírito Santo (39.7%) and Rio de Janeiro (1.3%) states (IBAMA, 2009). The escapes of African catfish from aquaculture systems to the natural environment has been reported and is threatening several native fish species (Umbria, 2008), becoming a potent IAS for these ecosystems.

In this regard, it is imperative the detection of changes in the biotic community and the environmental parameters that may be influencing on the establishment of alien species. In Brazil, some studies report the existence of *C. gariepinus* in natural environments (Alves et al., 1999; Mili & Teixeira, 2006; Vitule et al., 2006), among others) in environmental protection areas (Rocha & Schiavetti, 2007; Rocha, 2008). According to Weyl et al. (2016), that extensively reviewed studies on the establishment of alien populations of African sharptooth catfish, few biological evaluations are available, especially studies directed to their persistency and establishment for long term periods using empirical ecological data. For these authors, the establishment is the most important phase in the invading process, for the negative impacts are most likely to occur when alien species are settled.

To determine the integrity of the ecosystems, the characterization of physical and chemical parameters is not sufficient; and the paucity of methods to objectively incorporate the degradation of the biological component, also implicates in a lack of responses to the increasing negative effects of IAS (Hermoso & Clavero, 2013). An alternative to obtain a response on the biotic integrity is to look for “bioindicators”, whose presence and abundance reflect changes that occur to the whole community or to the ecosystem level (Hermoso & Clavero, 2013; Parker et al., 1999).

In Guapi-Macacu River, located at the Guapimirim Environmental Protection Area, in the southeast of Brazil, the riverside human population acknowledge the presence of the African catfish, although their distribution and possible impacts on the native ichthyofauna of this area is unacknowledged. Therefore, in order to assess the integrity, and establishment, of this IAS in this preservation area, it is necessary to assess the local fish community and the main abiotic components for the structure. In this sense, the current study characterizes which are the favorable environmental conditions for the alien species, by contemplating the physical and chemical parameters of the water, as well as suggesting possible bioindicator species of bioecological impacts on the fish community. Moreover, we characterize the distribution of the African sharptooth catfish and the native fish community along the Guapi-Macacu River.

Methods

Area of study

The Guapimirim Environmental Protection Area (APA Guapimirim) is located to the northeast of the Guanabara Bay, one of the most critical seaside areas of Brazilian coastline, in Rio de Janeiro, Brazil. The APA Guapimirim is a Federal Conservation unity, created in 1984 with the primary objective to protect the mangrove remnants and to ensure the permanence and survival of human populations that maintain a close relationship with the environment (Ecologus-Agrar, 2005). Within the APA, there is the Guanabara Ecological Station (Guanabara ESEC), a fully protected Conservation Unity, with the no admittance of people within its borders except for scientific or educational purposes.

The APA Guapimirim has several rivers and canals, standing out as the Guapi-Macacu River, known for water output (Fries et al., 2019), supplying potable water to most municipalities in its east part. The Guapi-Macacu River has its physiography divided into three segments or areas. The first segment flows down the slope of the Serra do Mar in rapids-shapes and waterfalls, with its margins covered with the Atlantic Forest. A second area runs through a transition between the cliffs and the plains, with less rugged formation. In this stretch, the rivers bordered by riparian forest, surround hills with rounded and massive shapes with altitudes lower than 1000 m. And a third area, which is longer and runs through lowland areas, with flat terrain and minimal unevenness, that is easily flooded and subjected to tidal influences, due to its proximity to the Guanabara Bay. The vegetation in this segment is composed of small shrubs, pastures and mangroves (Costa, 1999) and is closer to the most expressive urban centers (Ferreira, 2012).

Sampling

Community sampling was conducted in February (rainy season) and August 2018 (dry season) in the Guapi-Macacu River, using nets with different meshes, fishing traps, fyke nets, hooks, and lines. The fishing gear was installed and used at night, as the alien species is of nocturnal habits. At the same time, we used a multiparameter probe Hanna (model HI 9828), measuring *in situ* the physical and chemical variables of the water: temperature (°C), pH, dissolved oxygen (mg/L); transparency (cm), turbidity (FNU); and salinity (PSU). Sampling was distributed along thirty-two points: ten points between the mouth of the river and the inner limit of the Guanabara ESEC (downstream area); 12 points between the external boundary of ESEC Guanabara and the internal perimeter of APA Guapimirim (intermediate zone); and ten points distributed between the outer limit of APA Guapirim to the dam (upstream area), within the Buffer Zone of the Guanabara Ecological Station (Guanabara BZ), totaling 64 sampling points in both seasons (Figure 1). Gillnets were removed 24 hours after installation. Fish captured were grouped at each sampling point and packed in plastic bags, labeled, and refrigerated on ice, and then transferred to the Laboratory of Applied Ecology at UFF.

Laboratory activities, data processing and statistical analysis

The collected fish were identified, weighed and measured. In order to characterize the fish communities, the following indices were assessed: species richness, abundance, biomass, diversity, dominance, and equitability. Richness was calculated using the total number of species collected at each sampled point, as well as abundance. For the analysis of diversity, the Shannon-Wiener index, the Simpson dominance index, and the Pielou's uniformity index were used (Magurran, 1988).

The Multivariate Permutation Analysis of Variance (PERMANOVA) was used in the R Program (R Core Team, 2020), available in the VEGAN package (Oksanen et al., 2020), to test for spatial and seasonal differences in the physical and chemical variables of water and ecological descriptors of the ichthyofauna. PERMANOVA was also applied to test the spatial and temporal variation of species richness, abundance and fish biomass, in addition to the indices of diversity, equitability and dominance. PERMANOVA is similar to traditional ANOVA, though does not require the assumptions of normality and homoscedasticity (Anderson, 2001; McArdle & Anderson, 2001). Bray-Curtis Distance was used in all PERMANOVA tests, permuted 4999 times per analysis.

The Multinomial Species Classification Method (CLAM) was used, through the function "*CLAMTEST*" available in the VEGAN package (Oksanen et al., 2020), to classify the species into generalists or specialists during rainy and dry seasons without excluding rare species (Chazdon et al., 2011). This method uses a multinomial model to estimate the relative abundance of species in two groups (A, B), minimizing adverse effects due to differences or insufficiency of sampling within each habitat (Solymos, 2020). A threshold of 50% specialization in each period was set, with 95% of significance level for individual tests.

Cluster Analysis was used in order to assess the spatial proximity of species and the formation of groups, according to the sampling points in both periods (taking into account the river as a whole), by using the "*HCLUST*" function of the package DENDEXTEND, using "ward. D2" (Galili, 2015), with the Bray-Curtis distance in the dissimilarity matrix of the VEGAN package (Oksanen et al., 2020). The sum of squares criterion was used for this agglomerative method, producing groups that minimize the scattering within the group in each binary merger (Murtagh & Legendre, 2014). This analysis was used to infer about the similarity in the distribution of species in the river stretches, according to their abundance.

As a way of assessing potential bioindicator species of the fish community, the specificity and fidelity analysis (*IndVal*) was used, in which a permutation test is performed (with a significance of 0.05). To do so, the "*MULTIPATT*" function was used, available in the INDICSPECIES package (De Caceres et al., 2016), both to determine the indicator species between seasons (rainy and dry) and river areas, and between river segments, regardless the season. The *IndVal* method has some advantages when compared to other bioindication methods, being calculated for each species independently, where the categorization of habitats occurs without restrictions, and may be grouped subjectively or quantitatively (McGeoch & Chown, 1998).

The Canonical Correspondence Analysis (CCA), which is considered one of the best methods for the analysis of direct gradient in community ecology (Rodríguez & Lewis Jr, 1997; Ter Braak, 1986), was used in the R program version 4.0.2 (R Core Team, 2020), available in the VEGAN package (Oksanen et al., 2020), and applied to the environmental and biological data matrix (excluding the rare species previously selected in the CLAM model). It was used to identify which physical and chemical variables of the water contributed the most to the characterization of the river points in the rainy and dry seasons, as well as to verify the distribution of the abundances of the fish species found, correlating them with the environmental variables (complete model). The multicollinearity of the model was diagnosed through the "*ORDISTEP*" function, which performs an automatic selection, based on the permutation test using the P-value. The variable selection procedure based on the P-value aims to find the ideal model, in which only the most significant environmental variables explain the model (Blanchet et al., 2008). Subsequently, the significance of both models (full model and reduced model) was tested, as well as which axes and terms were significant, by analysis of variance (ANOVA), using the "*ANOVA.CCA*" function. Therefore, an ideal and reduced model for the Guapi-Macacu River was obtained, which displayed the environmental variables able to predict changes in the composition and abundance of the ichthyofauna and indicate which species will be affected in this relationship.

Results

PERMANOVA identified significant differences for all environmental variables between the areas and seasons studied, except for temperature, which did not exhibit evident differences between the river segments (Table 1). In general, the water temperature was higher in the rainy season. Salinity was higher downstream in the dry season, but much lower in the rest of the groups (location and season combined). Transparency was higher in the dry season upstream. Dissolved oxygen (DO) was higher in the dry season than in the rainy season. Higher OD values occurred in the upstream segment during the dry season, followed by the intermediate and downstream areas. In the rainy season, DO was higher in the upstream region, followed by the intermediate and downstream regions. The pH was also higher in the dry season than in the rainy season. Higher values were recorded in the upstream segment during the dry season, followed by the downstream and intermediate zones. During the rainy season, the pH had similar values in three stretches of the river. The highest turbidity values occurred in the rainy season downstream, followed by intermediate and low segments. During the rainy season, turbidity did not vary significantly between the three areas of the river (Figure 2).

Thirty-one species were collected, with a total abundance of 428 specimens, in terms of ichthyofauna, distributed in nine orders and 21 families (Table 2). The distribution of the relative abundance of the fish community along the Guapi-Macacu River between the areas and seasons sampled is shown in Figure 3. PERMANOVA detected significant differences for fish abundance in relation to the area ($F= 2.6214$; $p = 0.0454$) and periods ($F=6.9902$; $p=0.0020$). The highest abundance of medians occurred in the dry season downstream.

PERMANOVA for species richness had significant differences only between the dry and rainy seasons ($F= 4.8069$; $p = 0.0226$). This index indicated the highest medians in the dry season downstream and the lowest median in the rainy season downstream. Biomass had significant differences in PERMANOVA only between the dry and rainy seasons ($F=5.8107$; $p=0.0032$). The dominance had differences among the river segments, detected in the PERMANOVA analysis ($F=3.6422$; $p=0.0326$).

PERMANOVA did not have significant differences between the other zones and seasons, as well as for diversity and equitability (p>0.05, in all) (Figure 4).

The CLAM classification indicated that 22.6% of the species collected were generalists in the dry and rainy seasons, with frequent occurrence in the two seasons studied, while 9.7% were classified as specialists in the rainy season and 9.7% in the dry season. Rare species in the samples corresponded to 58.1% (Figure 5).

As for the cluster analysis, they coincided with the previous locations shown in Figure 1, portraying the physiography of the Guapimirim APA in the spatial separation of the ichthyofauna (Figure 6). The main species that make up the Guapimirim APA were located mainly to the left of the dendrogram. The species on the right are distributed along the upstream area and cover the entire river. The last two groups were formed by rare species. In this classification, the target species *C. gariepinus* is close to groups with broad distribution in the river, such as *L. castaneus*, *T. striatulus* and *H. auroguttatus*. Furthermore, *C. gariepinus* occurs similarly to *R. quelen* and *T. striatulus*, sharing the same habitats.

The specificity and fidelity measures (*IndVal*) had as indicators the most important species that make up the two main groups formed in the cluster analysis (red and brown lines). As for the specificity and fidelity between areas and seasons, the analysis selected six species. In the dry season downstream, the selected species were *B. pectinata* (67.9%; p = 0.001), *M. furnieri* (58.0%; p = 0.008), and *E. saurus* (48.0%; p = 0.021). *Genidens genidens* had high fidelity and specificity in the downstream segment in the two seasons sampled (76.9%, p=0.001). *Acestrorhynchus lacustres* was selected as an indicator species in the upstream segment in the rainy season (52.2%; p = 0.008), while *T. striatulus* in the same segment in the two seasons sampled (53.4%, p=0.035). When we performed the *IndVal* analysis only for the river segments, seven species were selected, and they were grouped in the two seasons analyzed. In the group formed only upstream, the species *C. gariepinus* (54.8%, p=0.013), *T. striatulus* (52.6%, p=0.007), and *A. lacustres* (39.7%; p=0.025) had fidelity and specificity. In the downstream segment, an exclusive area of Guanabara ESEC, the function selected *G. genidens* (76.9%, p=0.001), *B. pectinata* (52.2%, p=0.003), and *M. furnieri* (45.2%, p=0.043). *C. undecimalis* was the only species selected for both regions of the Guapimirim APA (downstream and intermediate area) (p=0.022), demonstrating 51.2% of specificity and fidelity.

The CCA ordination diagram defined 40% (ANOVA, F=1.956; p=0.001) of the total distribution of the species abundance (generalists and specialists selected in the CLAM model) with abiotic variables on the river segments and in the rainy and dry seasons (Figure 7). The complete model (Figure 7A) displayed the distribution of species with all abiotic variables, with the first axis defining 39.60% of the samples (ANOVA, F= 4.9758; p = 0.003) and the second axis defining 22.58 % of the samples (ANOVA, F = 2.8375; p = 0.111), with respect to the differences in the distribution of fish species. Among the selected environmental variables, ANOVA exhibited significant differences for dissolved oxygen (F=2.2514; p=0.033), transparency (F=2.0854; p=0.028), temperature (F=1.6246; p=0.057), and pH (F = 3.7788; p=0.017), which was related to the distribution and abundance of fish species, as well as to the river segments. ANOVA had no significant differences in CCA for turbidity ($F = 1.9987$; $p = 0.061$) and salinity ($F = 1.0788$; $p = 0.372$).

On the other hand, the significance of the axes is altered in the reduced model, obtained through the "*ORDSTEP*" function (Figure 7B). In the second model, the three axes are significant ($p<0.02$, to all), which explained 73.19% of the samples together. The most significant variables for each analyzed area of the river were distributed as follows: pH had a significant correlation with the species distributed further downstream of the river ($F=3.3683$; $p=0.010$); transparency was mainly correlated with species in the intermediate area ($F=3.1519$; $p=0.001$); and OD was the most important Physical and chemical parameter for the species upstream of the river ($F=2.5752$; $p=0.004$), which was highly statistically significant.

Discussion

Abiotic variables in the Guapi-Macacu River

In the Guapi-Macacu River, salinity was higher downstream in both seasons, while DO and pH values were higher in the dry season upstream. Partially similar results were reported by Macêdo et al. (2000) in the Rio Formoso estuary (Pernambuco, Brazil), that detected the highest pH values in the lower estuary region, in addition to higher DO and salinity. According to Macêdo et al. (2000), salinity and oxygenation levels are influenced by tidal cycles and photosynthesis and respiration rates. The neutralization capacity existing in the aquatic ecosystem due to the buffering effect prevents great variations in the pH. Therefore, the maximum values were obtained in areas with more significant saline influence. In the Guapi-Macacu River, the highest salinity is expected at the mouth of the Guanabara Bay because it is a coastal segment, and its intensity may vary within the rainy season, which favors its dilution. The higher rates of DO and pH reflect a rainless season, with greater water transparency, favoring photosynthesis, which removes CO_2 and, consequently, raising the pH of the water due to H^+ ion consumption.

When analyzed individually, it was observed that temperature and pH were the environmental attributes that were not related to the different segments of the river, but to the periods of the year. The highest temperature in the rainy season corresponds to the hottest season of the year in South America. However, there is collinearity of environmental attributes. When presented in the reduced model and correlated with species abundance, pH is displayed as a high significant variable for the downstream area, probably due to its higher value in the dry season. The association between the other variables analyzed, such as the predominance of increased transparency in the intermediate region of the river, contributed to a better distinction between the river segments in the dry and rainy seasons, corroborating the environmental characteristics of the Guapi-Macacu river in the Guapimirim APA, and serving as predictors for related fish species in this habitat. According to Blaber (2002), fish from tropical estuaries are subject to a series of interactions of Physical and chemical and biological factors that determine their patterns of occurrence, distribution, and movement. According to this author, in the Rio Formoso estuary (Pernambuco, Brazil), temperature, salinity, pH, and dissolved oxygen were higher in the lower estuarine zone and in the dry season. However, inefficient levels of DO can be observed in the area downstream of the river, mainly in the rainy season, coinciding with the lowest richness and abundance registered. For Edokpayi et al. (2017), concentration levels of DO below 5.0 mg/L impairs the aquatic life. Furthermore, during the rainy season, the currents in the Guanabara Bay carry organic materials to and concentrates contaminants during the dry season in the area downstream of the Guapi-Macacu River, in addition to the sediment resuspension, which explains the lower oxygenation during the rainy season in this area.

The fish community of the Guapi-Macacu River

The fish community of the Guapi-Macacu River, within the Guapimirim APA and its Buffer Zone, has freshwater species with the presence of marine species, many of which are euryhaline. Marine species were concentrated in the lower part of the river (downstream), at the mouth of Guanabara Bay, with some species migrating inland, such as sea basses, gray mullets, mojarras, and whitemouth croakers. This segment consisted of resident species, marine and freshwater migrants, that use estuaries for feeding, larvae and juveniles rearing, or for reproduction (Blaber, 2002). These habitats favor the presence of several fish populations on their shores (Vidy, 2000), consisting mainly of juveniles of marine species (Rozas & Zimmerman, 2000). Thus, the greater abundance of fish downstream of the Guapi-Macacu River is probably due to the availability of food from primary production, the structural complexity of the mangrove vegetation, which provides refuge, especially for young fish, and the high turbidity of the water, as well as for being a nursery for coastal species.

In estuarine environments, mangroves provide a natural refuge for young individuals due to the protection supplied by the root structure of their trees. Most fish caught in tropical coastal areas appreciate this protection during the juvenile phase and at the time of spawning and, therefore, are intimately dependent on the integrity of this ecosystem (Lacerda, 1984). Thus, the area with the highest occurrence of native species is also located downstream of the river, within the Guanabara ESEC, the most preserved area within the Guapimirim APA and with the best ecological indices, such as abundance and richness of the species found, mainly in the dry season. According to Teixeira et al. (2005), the determination of biodiversity, especially of the fish community and the patterns of spatial and temporal variation, is of great relevance for the assessment of environmental quality.

The Siluriformes was the most abundant fish Order in the Guapi-Macacu River. The dominance of Siluriformes over others is a pattern of the eastern region of Brazil, being particularly accentuated in areas of high river courses, where the high hydrodynamic condition favors the occupation of demersal species (Bizerril & da Silveira Primo, 2001). *Genidens genidens*, a representative of the second most abundant family, Ariidae, occurs in coastal areas and is generally more significant in shallow coastal waters on muddy or sandy bottoms (Andreata et al., 1989; Araújo, 1988). The presence of *G. genidens* downstream from the river may be related to the spawning season. The species seek the mouth of rivers with the males performing oral incubation, and rarely females, carrying eggs and initial forms of the offspring until they complete embryonic development (Reis, 1986; Yáñez-Arancibia & Sánchez-Gil, 1988), therefore explaining the presence of specimens downstream of the Guapi-Macacu River. Furthermore, *G. genidens* exhibited high levels of fidelity (59.09%) and specificity (100%), considered a perfect indicator species in the analysis of *IndVal*. Thus, the *IndVal* analysis determined that for this specific area, regardless of the season analyzed, three species were considered as indicators of this habitat: *G. genidens*, *B. pectinate*, and *M. furnieri* (Figure 8-A). In addition to these species, *E. saurus* was selected only in the dry season in the downstream area. All species selected for the downstream area were considered as asymmetric indicators, as they contribute more to habitat specificity than to fidelity (Dufrêne & Legendre, 1997). This segment was also particularly evident in the cluster analysis, which showed that the main species selected by *IndVal* share this river segment.

The area within the Guapimirim APA, disregarding the ESEC Guanabara (ie, the intermediate segment of the river), presented ecological indices similar to the other stretches of the river. However, greater abundance and richness can be observed (Figure 8A). Thus, the downstream and intermediate segments (i.e. comprising the Guanabara ESEC and APA de Guapimirim) showed only *C. undecimalis* as an indicator of the APA regardless of the time of year assessed (Figure 8A; 8B). *Centropomus undecimalis* belongs to the order Perciformes, the second most abundant in the Guapi-Macacu River. According to Peterson & Gilmore (1991), seabasses do not undergo major migratory cycles, being a relatively fast-growing fish that spawn large numbers of eggs in brackish waters during late spring and early summer. Juvenile seabasses have a great affinity for fresh water and have higher survival rates than adults in waters with lower oxygen levels, being found upstream of rivers at all times of the year (Ager et al., 1976). Its primary or nursery habitat has been described as shallow warm streams or drainage canals, with low current and bare bottoms or bordering mangroves (McMichael Jr et al., 1989). As they develop, they move from shallow water habitats to estuaries, mangroves, and deeper waters (Tucker Jr & Campbell, 1988). According to cluster analysis, the river segment shared with *C. undecimalis* and other species provides this species with a habitat with abundant food resources and protection for its development.

The intermediate region of the river was characterized by a very sinuous zone with greater diversity in physiography, constantly flooded with deeper portions, and a salinity gradient that decreases from the river mouth to the interior. This segment presents a vegetation composed of mangrove forests, of riverine type. This area is directly related to samples with greater transparency, showing *E. brasilianus* and *O. niloticus* with intermediate values of this attribute (Figure 8B). *Eugerres brasilianus* is a species of marine origin that tolerates significant variations in salinity (Ramos et al., 2016). It is anadromous, migrating from the sea to rivers, living in coastal waters of warm seas, penetrating coastal lagoons and estuaries to complete its life cycle (Yáñez-Arancibia, 1986). Furthermore, *E. brasilianus* is a nocturnal, generalist, and opportunistic species; it is also an epibenthic and demersal species. That is, it presents patterns strictly linked to the substrate, being considered an excellent biological resource, mainly because it is considered an abundant fishery resource (Araújo & Santos, 1999; Barletta & Blaber, 2007; Barletta & Costa, 2009; Cyrus & Blaber, 1983; Tapia-García & Ayala-Pérez, 1996).

On the other hand, the cluster analysis did not show the intermediate region with a specific community for this segment of the river, showing itself as a transition area and occupied mainly by species that travel along the river, such as *H. littorale*, *Hypostomus auroguttatus* and *Loricariichthys castaneus* (Figure 8C). *Hypostomus auroguttatus* and *L. castaneus* belong to the most abundant family, Loricaridae, common in areas with muddy river bottoms and may occur even in lentic environments. These two species recorded in the three segments of the Guapi-Macacu River during the dry and rainy seasons, were considered as generalists in the present study.

The species *A. lacustris* (Figure 5), which were considered rare in the analysis presented in CLAM TEST, was selected by *IndVal* for the stretch upstream of the river, as well as in the rainy season, with high specificity and low fidelity, according to Dufrêne & Legendre (1997). Rare species can receive the same *IndVal* value as indicator species and are called asymmetric indicators. On the other hand, in the same river stretch, disregarding the attributes of the seasons (dry or rainy), *IndVal* selected *T. striatulus* and *C. gariepinus* (Figure 8D), both with high specificity. Which means that these fish can also be considered asymmetric indicators, which contribute to habitat specificity, but do not serve to predict groups (Dufrêne & Legendre, 1997).

From the CCA, it is noticed that the stretch upstream of the river presents higher values of DO and abundance of *C. gariepinus*. The upstream part are the headwaters of the river, which have a humid tropical climate, high and variable slope, determining the dynamic character of the fluvial system, with the presence of rapids, characteristic of mountainous and plateau regions. Barrella et al. (2000) ponders on the vital role of riparian forests in providing resources for feeding aquatic fauna and attracting dispersers, making the riparian environment a fundamental element in the sustainability of rivers and lakes and connecting the different systems that make up the rural landscape. The CCA also highlights the most protected area of the Guapimirim APA, downstream of the river, with greater diversity and richness, with greater attributes of salinity, pH, and turbidity. In general, the most abundant species in river flow are intermediate values of the abiotic variables analyzed, except for *C. gariepinus*, which correlates with higher dissolved oxygen samples, and *M. furnieri*, which presents more significant correlations with salinity values. *C. parallelus*, *H. auroguttatus*, *H. littorale*, and *L. castaneus* express abundances related to intermediate values of transparency, revealing correlations in all river segments. As well as the CCA, the cluster analysis also evidenced the group formed by *G. genidens*, *B. pectinata* and *M. furnieri*, disclosing a sharing proximity in the same region, mainly in the dry season, with dominance of *G. genidens*, as it presents greater abundance in this group. The CCA also highlights a similar spatial distribution of *T. striatulus* with *C. gariepinus* (with greater representation in abundance), corroborating the cluster analysis.

African sharptooth catfish in the Guapi-Macacu river

The African sharptooth catfish, *C. gariepinus*, is among the most abundant species in the river, but it still does not show a significant abundance in the most preserved area of the Guapimirim APA. It is also noted that the species that present ecological equivalence to the African sharptooth catfish, *R. quelen* and *T. striatulus*, despite of having a similar distribution, presented lower abundance, which denotes the overlapping of IAS habitats over native ones (Figure 9A). In addition, the population of *C. gariepinus* presented higher abundance contributions in the dry season, mainly upstream, compared to the other species, demonstrating a high potential to colonize the entire river (Figure 9B).

Although the CCA analysis does not show similar correlations of the abiotic factors *R. quelen* with *Clarias gariepinus*, group exposed in the cluster analysis, it is clear that these species share the same river segments. *Rhamdia quelen* prefers lakes and river bottoms, selecting calmer water environments with sandy and muddy bottoms, along the river banks and the vegetation (Gomes et al., 2000). They are omnivores species with a clear preference for fish, crustaceans, insects, plant remains, and organic debris (Guedes, 1980; Meurer & Zaniboni Filho, 1997). Therefore, they are considered generalists in terms of food choice (Guedes, 1980). Among the many biological similarities that *R. quelen* has with *C. gariepinus*, stands out the food preference and habitat use; however, *R. quelen* has a disadvantage in terms of its development, as females can reach up to 66.5 cm and males up to 52.0 cm (Gomes et al., 2000).

According to our analysis, dissolved oxygen was the attribute directly correlated with the distribution of *C. gariepinus* (Figure 7B). Thus, dissolved oxygen (DO) plays a key role in regulating the metabolic functions of the organism, including the aquatic community, in addition to being an environmental indicator of water quality (Anyachor & Sikoki, 2021). On the other hand, African sharptooth catfish can tolerate low concentrations of dissolved oxygen (Adewolu et al., 2008) due to an accessory air-breathing organ, which can absorb oxygen from atmospheric air (Moussa, 1956), allowing the fish to survive out of water for long hours or even weeks or in muddy swamps (Idahor et al., 2014).

Another significant environmental attribute in our analysis was the temperature, and this seems to be an essential factor in the distribution of the species in the Guapi-Macacu River. The highest temperature values in the upstream area corroborate the results found in the CCA, where the highest abundance of the species was correlated with this stretch of the river. According to Hecht (2013), the African sharptooth catfish larvae have an optimal development around 28ºC, a value that was recorded in the rainy season upstream. Indeed, it could be observed during the dry season, in which there was a large increase in the population of the IAS, favored by the increase in temperature recorded previously. On the other hand, the current study indicated that, although the average temperature values measured in the Guapi-Macacu River are not considered ideal for the development of catfish, the adaptability of the species allowed it to develop well in this ecosystem and to colonize other segments of the river. However, climate change resulting from global warming may contribute to a gradual increase in temperature, significantly altering this scenario, which may favor the rapid development of this alien species and, possibly, the decline of native populations.

Salinity is also an attribute that limits the occurrence of the alien species at the mouth of Guanabara Bay. De Melo et al. (2014) had detected an intrusion of salinity into the river in the dry season of the year, corroborating our findings, where salinity varied considerably in this season, reaching 31. This factor may have contributed to the lower abundance of IAS in this stretch of the river. This occurs because African sharptooth catfish are stenohaline, with limited ability to tolerate increased salinity in the environment at more advanced stages (Hogendoorn, 1981). Borode et al. (2002) carried out a study on the effect of salinity on the early development of the African catfish. They concluded that increasing salinity delays the hatching and development of African sharptooth catfish eggs and larvae, but they tolerate variations of up to 6 ppt for their growth. Even so, we can observe that despite the significant abundance of this species, the specificity and fidelity analysis does not list *C. gariepinus* as an indicator species, taking into account both the seasons studied and the collecting areas. However, this pattern diverges when considering only the upstream zone, when the species specificity index rises up to 81% and the fidelity to 36% for this area, demonstrating *C. gariepinus* with a probability of 53% to be an asymmetric indicator species of this area. According to Dufrêne & Legendre (1997), a species can be an asymmetric indicator without high fidelity.

Conclusion

The analyzes presented herein contributed to the knowledge of the ichthyofauna of the Guapi-Macacu River and how they may help future studies of the impacts related to the presence of the alien species. Even so, we reveal that this species still does not colonize the Guanabara Bay, nor the downstream part of the river. These factors may be related to a lower temperature, higher salinity, and potential predators and competitors that occupy this stretch of the river. In addition, we show that this conservation area still fulfills its role of protecting the resident ichthyofauna, harboring a larger contingent of typically estuarine juvenile fish, portraying this area as a natural breeding ground and shelter for several species of fish of ecological and economic importance. However, the gradual increase in temperature in aquatic ecosystems is one of the factors that may favor the development of African sharptooth catfish. Southeastern part of Brazil has been experiencing more intense summers and prolonged periods of droughts, consequently, an increase in the temperature in stretches of the river may favor the successful development of African sharptooth catfish. Moreover, the IAS has been competing for habitats with native species, which have ecological equivalence, such as *R. quelen*, and *T. striatulus*, thus impacting the local ichthyofauna, with a visible decline in the abundance of native species where African catfish settles. Therefore, it is necessary to constantly monitor key species, such as *G. genidens* and *C. undecimalis*, as well as the population of the invasive species in this area of environmental preservation.

Declarations

Financing

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AUTHOR CONTRIBUTION

MTD analyzed the data and wrote the manuscript. AFGNS performed field activities, assessed the data and wrote the manuscript.

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Tables

Table 1. PERMANOVA analyses of environmental variables in the Guapi-Macacu River with segments and seasons (dry/rainy) as factors.

Parameter	Area		Season	
	F	*p*	*F*	*p*
Salinity	19.93	< 0.001	11.799	< 0.001
Temperature	0.4311	0.660	299.6786	0.0002
pH	4.2159	0.020	92.8138	0.0002
DO	5.1768	0.008	27.1637	0.0002
Transparency	15.038	< 0.001	189.762	< 0.001
Turbidity	17.625	< 0.001	102.902	< 0.001

Table 2. List of species (authors), analysis codes, abundance, biomass, size classes, and CLAM classification of the icthyofauna collected in 2018 along the Guapi-Macacu River-RJ.

Species	Code	Abundance	Biomass (g)	Size class (mm)	Classification (CLAM)
Acestrorhynchus lacustres (Lütken, 1875)	ALA	4	261.068	130-257	Rare
Astyanax altiparanae Garutti&Britski, 2000	AAL	7	80.16	90-100	Rare
Astyanax fasciatus (Cuvier, 1819)	AFA	1	15.867	115	Rare
Brevoortia pectinata (Jenyns, 1842)	BPE	15	1000.301	143-300	Specialist/Dry
Centropomus parallelus Poey, 1860	CPA	28	5563.947	120-415	Specialist/Rainy
Centropomus undecimalis (Bloch, 1792)	CUN	14	3365.919	125-455	Generalist
Clarias gariepinus (Burchell, 1822)	CGA	30	43970	345-830	Generalist
Cynoscion acoupa (Lacepède, 1801)	CAC	1	372	350	Rare
Cyphocharax gilbert (Quoy&Gaimard, 1824)	CGI	7	986.376	178-208	Rare
Diapterus rhombeus (Cuvier, 1829)	DRH	7	243.309	105-159	Rare
Elops saurus Linnaeus, 1766	ESA	8	758.023	201-293	Rare
Eucinostomus argenteus Baird&Girard, 1855	EAR	4	274.482	153-195	Rare
Eugerres brasilianus (Cuvier, 1830)	EBR	30	2794.393	114-370	Specialist/Dry
Genidens genidens (Cuvier, 1829)	GGE	57	5163.797	105-320	Generalist
Geophagus brasiliensis (Quoy&Gaimard, 1824)	GBR	8	1336.527	78-250	Rare
Gymnotus carapo Linnaeus, 1758	GCA	1	272	390	Rare
Hoplias malabaricus (Bloch, 1794)	HMA	10	5576	280-395	Rare
Hoplosternum littorale (Hancock, 1828)	HLI	22	1528.875	86-190	Specialist/Rainy
Hypostomus auroguttatus Kner, 1854	HAU	51	7647.396	180-334	Generalist
Leporinus friderici (Bloch, 1794)	LFR	1	242	264	Rare
Loricariichthys castaneus (Castelnau, 1855)	LCA	30	4379.137	259-354	Generalist
Micropogonias furnieri (Desmarest, 1823)	MFU	33	4003.924	110-335	Specialist/Dry
Mugil curema Valenciennes, 1836	MCU	1	148	250	Rare
Mugil liza Valenciennes, 1836	MLI	3	2043.7	230-425	Rare
Oligosarcus hepsetus (Cuvier, 1829)	OHE	6	414.461	128-223	Rare
Oreochromis niloticus (Linnaeus, 1758)	ONI	16	3108.394	126-362	Specialist/Rainy
Plagioscion squamosissimus (Heckel, 1840)	PSQ	1	630	330	Rare
Prochilodus lineatus (Valenciennes, 1837)	PLI	1	13.461	110	Rare
Rhamdia quelen (Quoy&Gaimard, 1824)	RQU	11	2889.96	119-375	Generalist
Trachelyopterus striatulus (Steindachner, 1877)	TST	18	2496.304	154-225	Generalist
Trinectes microphthalmus (Chabanaud, 1928)	TMI	2	14.468	50-55	Rare

Figures

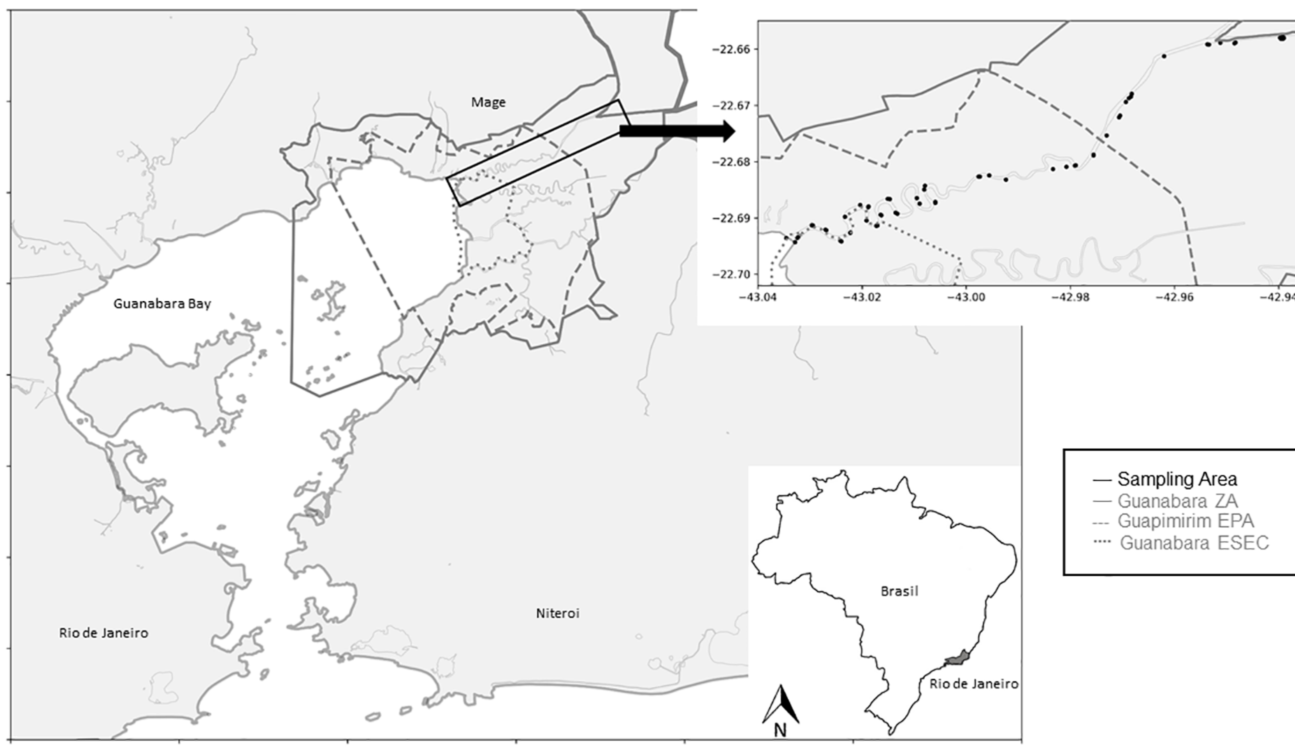

Figure 1

Map of the Guanabara Bay with the representation of the limits of the Guapimirim Environmental Preservation Area (APA Guapimirim), Guanabara Ecological Station (Guanabara ESEC), and Buffer Zone of the Guanabara Ecological Station (Guanabara BZ). The sampling area is highlighted, with sampling spots in the dry and rainy seasons distributed along the Guapi-Macacu River.

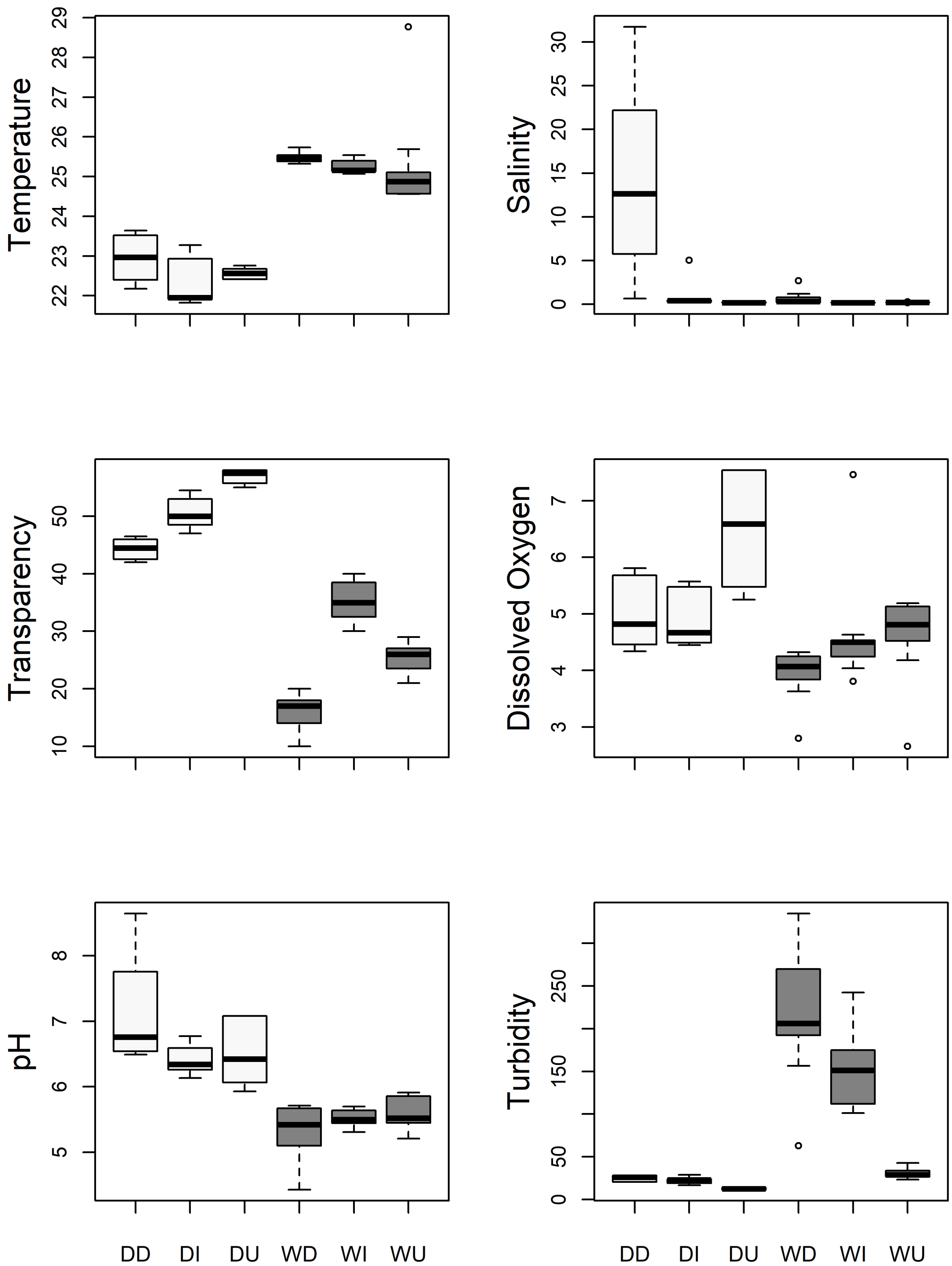

Figure 2

Boxplot of environmental variables by segments and seasons of the year in the Guapi-Macacu River. DD: dry downstream; DI: dry intermediate; DU: dry upstream; WD: wet downstream; WI: wet intermediate; and WU: wet upstream. White: dry season; grey: rainy season.

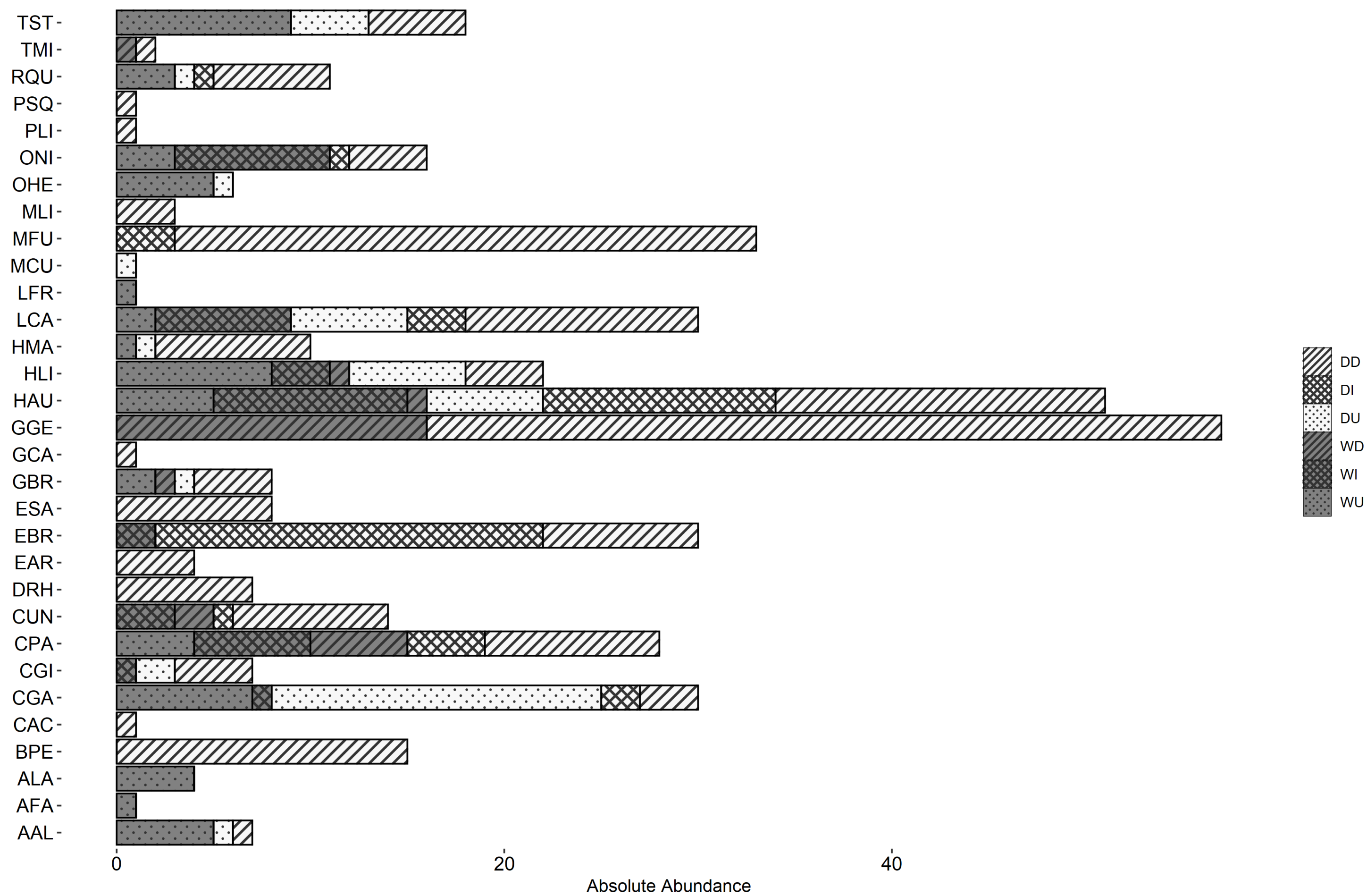

Figure 3

Distribution of absolute abundance of fish community along the Guapi-Macacu River between areas and seasons of the year. DD: dry downstream; DI: dry intermediate; DU: dry upstream; WD: rainy downstream; WI: rainy intermediate; and WU: rainy upstream. The codes corresponding to each species are on Table 2.

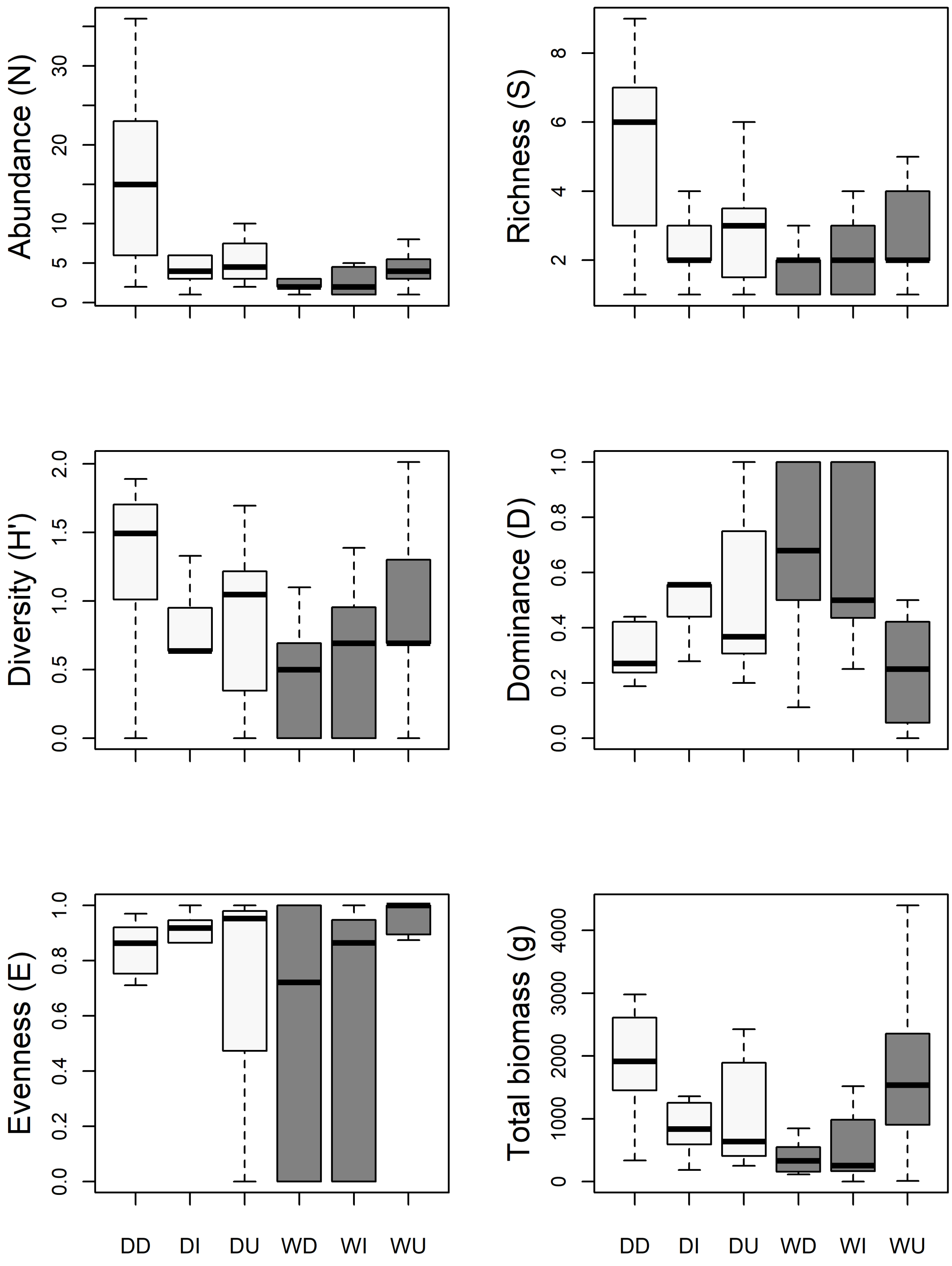

Figure 4

Boxplot with the distribution of abundance, richness, Shannon-Wienner diversity, Pielou's equitability, dominance, and total biomass (g) between the stretches and seasons in the Guapi-Macacu River. DD: dry downstream; DI: dry intermediate; DU: dry upstream; WD: wet downstream; WI: wet intermediate; and WU: wet upstream. White: dry season; grey: rainy season.

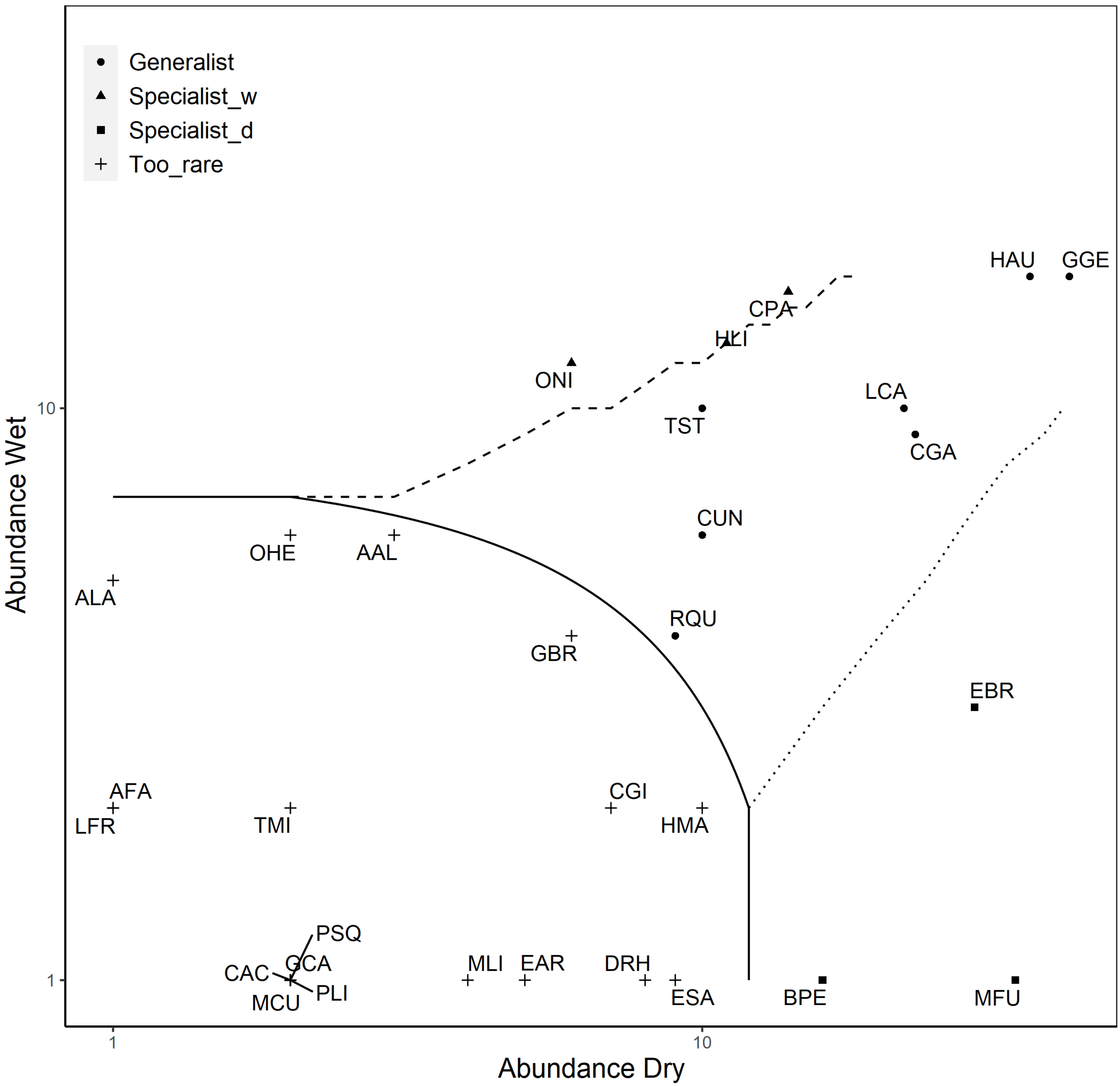

Figure 5

CLAM classification of the ichthyofauna in the dry and rainy seasons (p = 0.05). lines represent the specialization limit. Traced line: rainy season; dotted line: dry season; circle: generalist; triangle: rainy season specialist; square: dry season specialist; cross: rare species. The codes corresponding to each species are on Table 2.

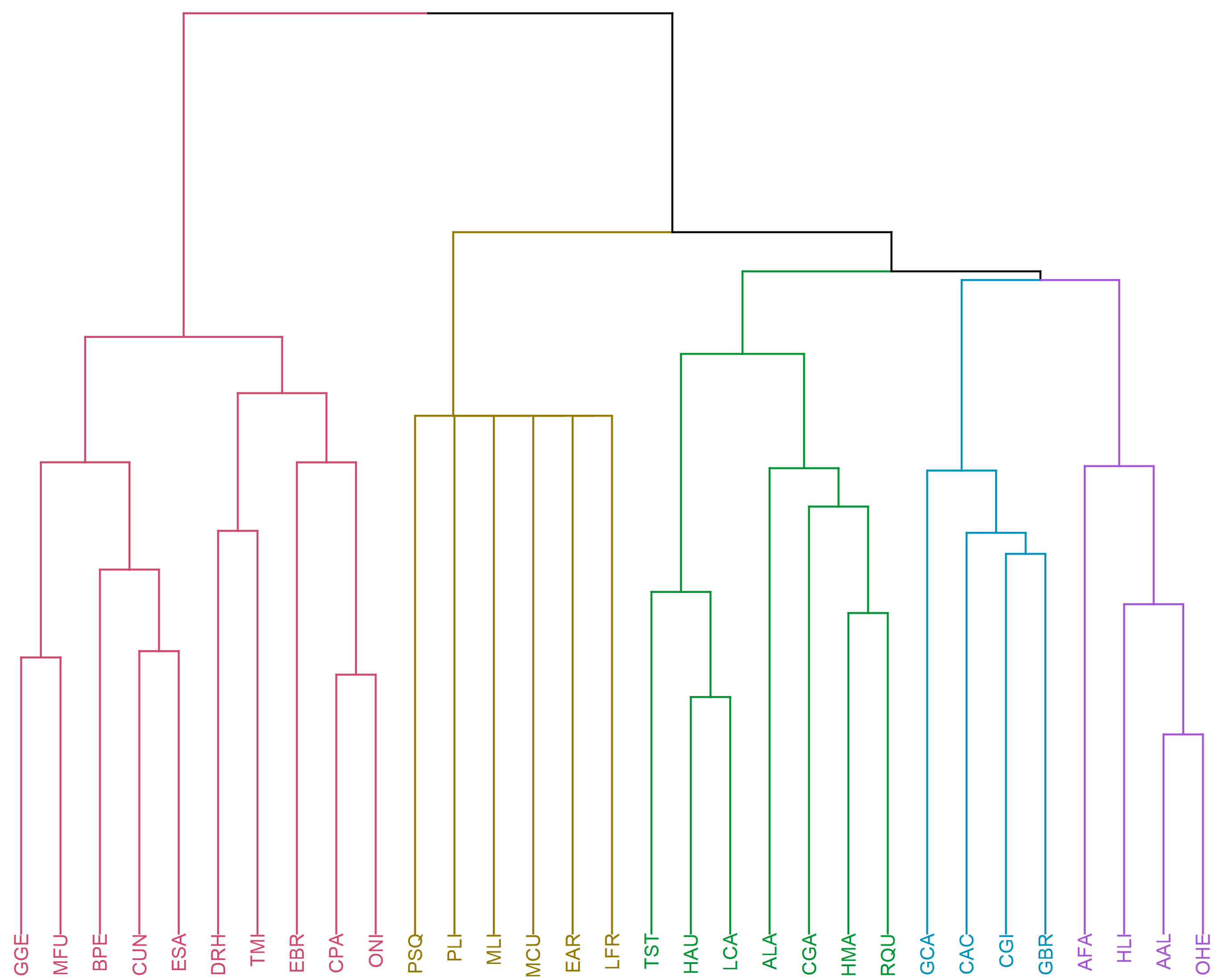

Figure 6

Cluster analysis of fish abundance between the areas of the Guapi-Macacu River. Different types of line correspond to the formed groups. The codes corresponding to each species are on Table 2.

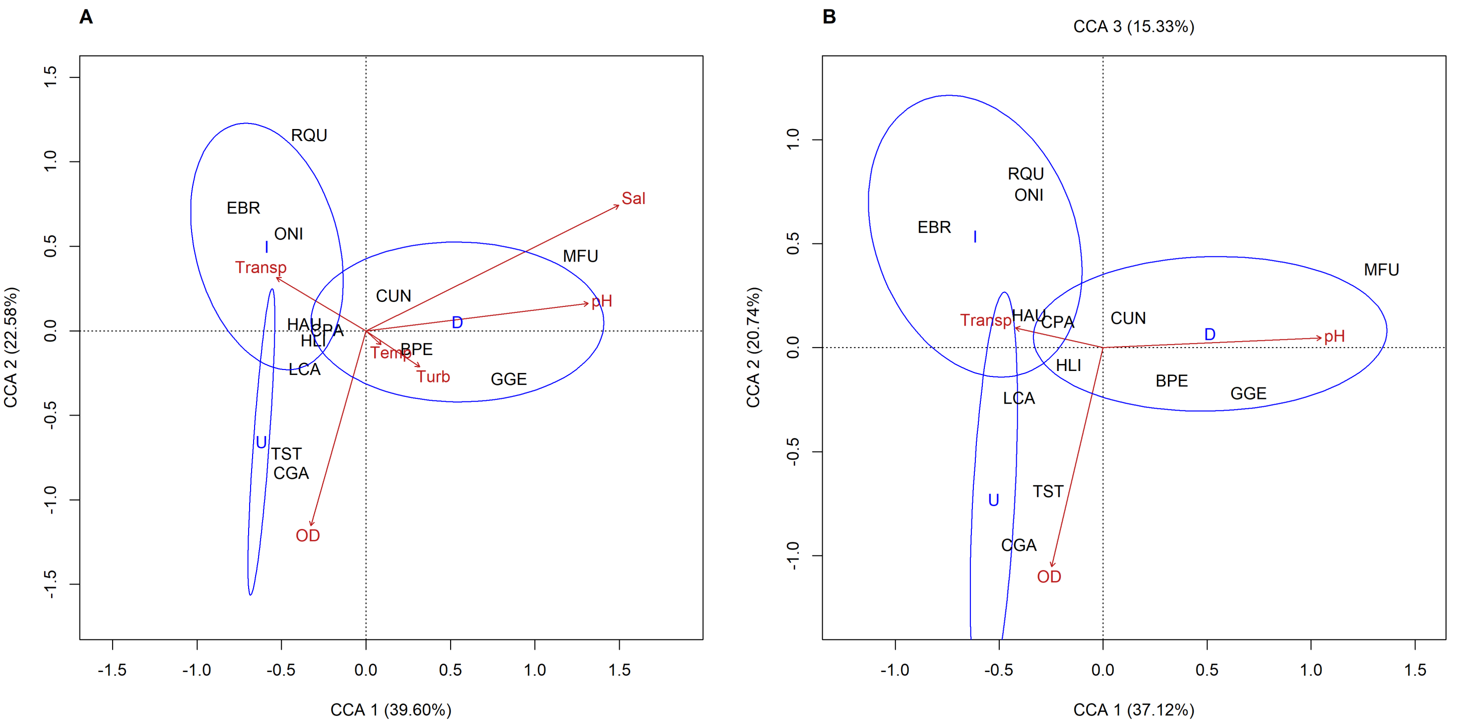

Figure 7

Canonical correspondence analysis (CCA) applied to the matrix of abundance of generalist and specialists species selected in the CLAM model and the environmental variables. In blue, the downstream (D), intermediate (I) and upstream (U) segments of the Guapi-Macacu River. In red, arrows indicate a contribution of each environmental variable. The codes corresponding to each species are on Table 2. (A) Analysis of CCA with all environmental variables measured; (B) reduced model, obtained through the *ORDSTEP* function, removing the multicollinearity of the environmental variables.

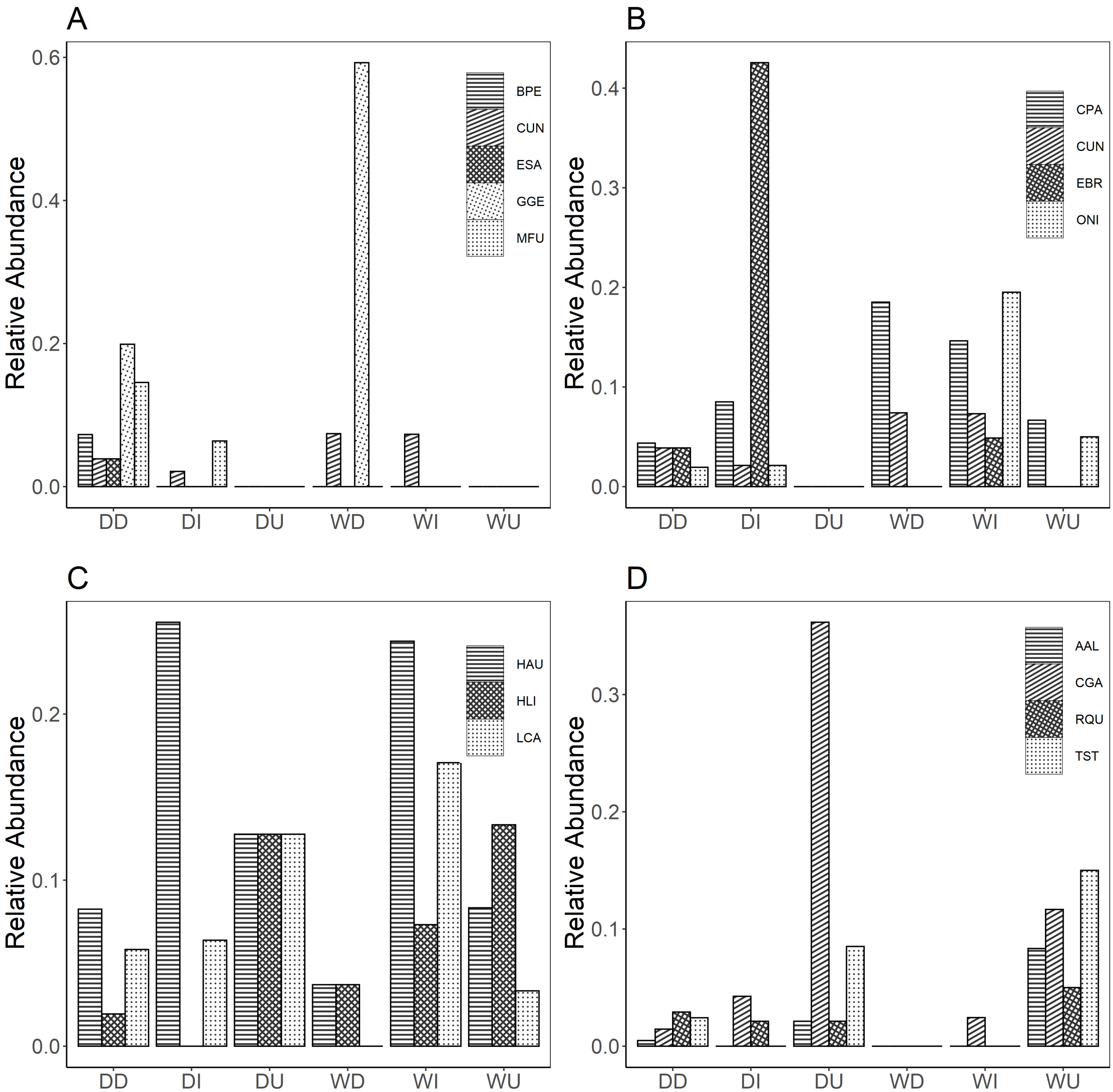

Figure 8

Relative abundance of main fish species of the Guapi-Macacu River that stretches: (A) downstream area; (B) intermediate area; (C) the whole river; and (D) upstream area. DD: dry downstream; DI: dry intermediate; DU: dry upstream; WD: wet downstream; WI: wet intermediate; and WU: wet upstream. White: dry season; grey: rainy season. The codes corresponding to each species are on Table 2.

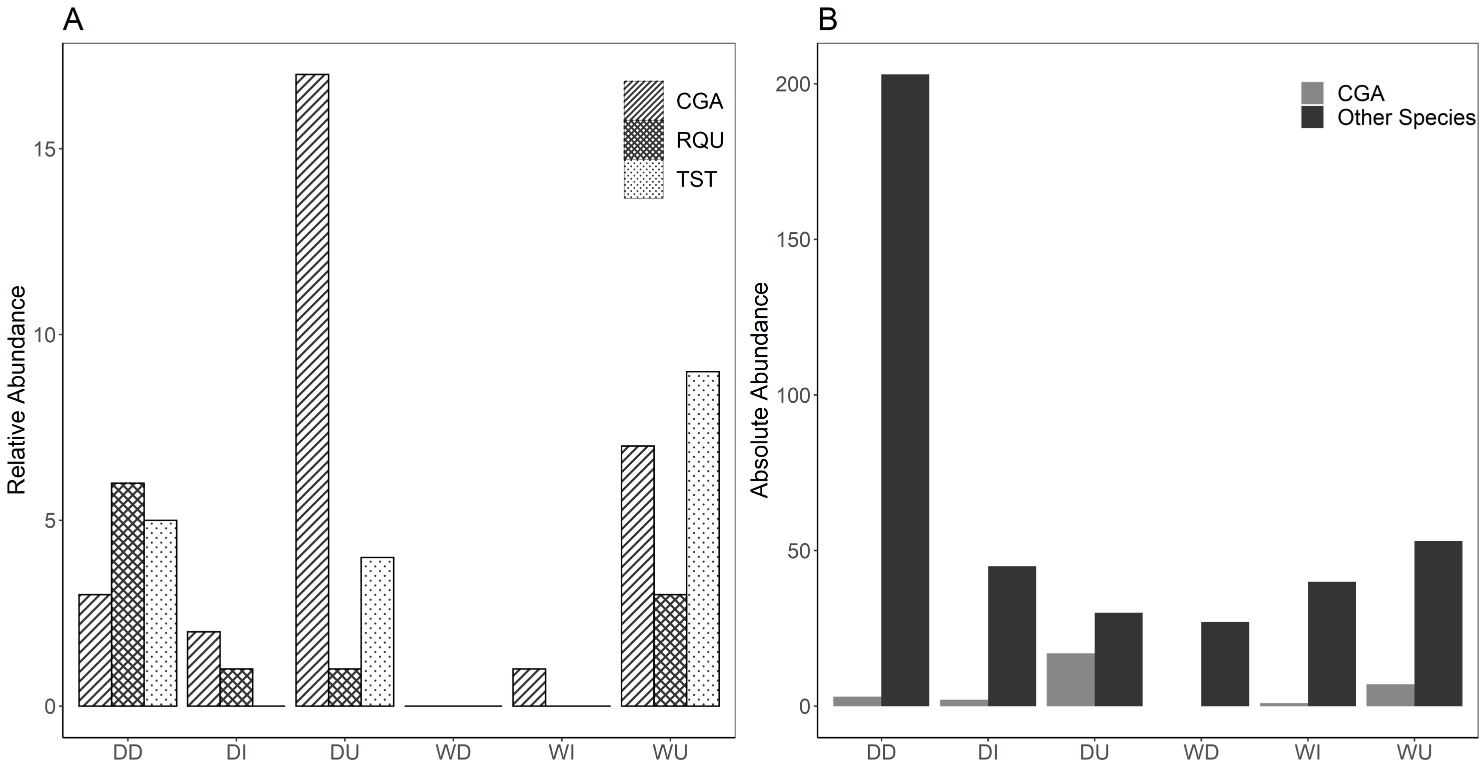

Figure 9

(A) Captures of the population of *Clarias gariepinus* and of the two species with the ecological equivalents, between the different river segments and seasons sampled. (B) Captures of *Clarias gariepinus* and other species of the community, between the different parts of the Guapi-Macacu River and sampled seasons. DD: dry downstream; DI: dry intermediate; DU: dry upstream; WD: wet downstream; WI: wet intermediate; and WU: wet upstream. White: dry season; grey: rainy season. The codes corresponding to each species are on Table 2.