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DEPARTAMENTO DE GEOLOGIA E GEOFÍSICA/LAGEMAR  
PÓS-GRADUAÇÃO EM DINÂMICA DOS OCEANOS E DA TERRA**



**Michelle Torres Dumith**

**Bioinvasão do bagre africano *Clarias gariepinus* (Burchel, 1822) numa área de preservação ambiental de uma baía do Sudeste do Brasil.**

**Niterói, 2024**

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Tese apresentada ao Programa de Pós- Graduação em Dinâmica dos Oceanos e da Terra da Universidade Federal Fluminense, como requisito parcial para obtenção de Título de Doutor em Dinâmica dos Oceanos e da Terra - Área de Concentração: Ecologia Marinha

Orientadora: Prof<sup>a</sup> Dr<sup>a</sup> Alejandra Filippo Gonzalez Neves dos Santos

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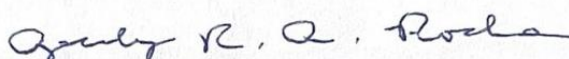
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## RESUMO

DUMITH, Michelle Torres. **Bioinvasão do bagre africano *Clarias gariepinus* (Burchel, 1822) numa área de preservação ambiental de uma baía do Sudeste do Brasil.** 2024. 189 f. Tese de Doutorado, Pós-graduação Dinâmica dos Oceanos e da Terra, Universidade Federal Fluminense, Niterói, RJ, 2024.

A conservação da biodiversidade é dependente de vários mecanismos, sendo de fundamental importância a criação de áreas para a preservação ambiental. A Baía de Guanabara, é um ambiente impactado, por diversos fatores, como poluição, destruição de áreas naturais e perda de qualidade dos ecossistemas. A Área de Proteção Ambiental de Guapimirim (APA de Guapimirim) foi criada, para proteção dos resquícios da biodiversidade que a baía apresenta, promovendo a preservação ambiental. A APA também abriga a espécie exótica *Clarias gariepinus* (bagre africano), considerada uma importante espécie invasora, por causar diversos impactos ambientais. Este peixe possui alta versatilidade alimentar e resistência a condições ambientais adversas, como baixas concentrações de oxigênio e variações de temperatura. Sua presença na APA foi registrada desde 2001, e sua população tem sido pouco estudada. A presente tese teve como objetivo, identificar os fatores biológicos e ambientais relacionados ao estabelecimento do bagre africano ao longo do Rio Guapi-Macacu, até seu deságue na Baía de Guanabara. A pesquisa foi dividida em quatro capítulos: (1) Avaliação da ictiofauna e da invasão do bagre africano; (2) Caracterização da dieta e ecologia trófica do bagre africano invasor, comparado com outras espécies de bagres nativos; (3) Confirmação da origem genética da população do bagre africano; e (4) Análise da biologia reprodutiva do bagre africano. Foram realizadas coletas de peixes em 32 pontos amostrais (10 a jusante, 12 no trecho intermediário e 10 a montante) no rio Guapi-Macacu, através de rede de espera com malhas variadas, tarrafas, covos, e anzol e linha, durante o período de cheia (fevereiro) e de seca (setembro) em 2018. O esforço de pesca foi de 24 h de pesca em cada trecho, com despescas noturnas. Os resultados indicaram que a APA, abriga uma diversidade espécies menor, quando comparada a estudos pretéritos antes da chegada do bagre africano, representando um sinal de alerta para sua integridade biótica. A população do bagre africano denota ser consistente neste ecossistema, com espécimes robustos e numerosos. O bagre africano, habita preferencialmente áreas a montante do rio: com maior oxigênio dissolvido, baixa salinidade e maior temperatura da água. No entanto, também foram coletados espécimes a jusante do rio - trecho de maior salinidade. O bagre africano pode ser considerado uma espécie bioindicadora ambiental, em função de sua distribuição e abundância. A dieta do bagre africano, foi comparada a duas espécies equivalentes nativas e foi constatado, que não há sobreposição alimentar entre as espécies. O bagre africano é onívoro, se alimentando oportunamente de qualquer item disponível, afetado indiretamente por variações nos componentes físico-químicos da água. A confirmação da origem genética do Rio Congo (Congo, África), de *Clarias gariepinus* no rio Guapi-Macacu foi confirmada e suas características morfométricos (diâmetro do olho, comprimento da cabeça, pedúnculo caudal e tamanho da boca) são compatíveis com a espécie proveniente de pisciculturas, confirmando sua origem na região. Por fim, constatamos, que águas com temperatura mais elevada e menor salinidade contribuem para o sucesso reprodutivo do bagre africano, que se mantém ativo neste ecossistema, há mais de 40 anos. Sugere-se com essa tese, nortear a necessidade de implementação de políticas públicas no estado do Rio de Janeiro no controle do bagre africano, além de programas de monitoramento contínuo, visando reduzir seus impactos sobre a ictiofauna nativa, à produtividade pesqueira, e a integridade biológica deste ecossistema.

Palavras-chaves: *Espécie exótica, Ecologia trófica, Reprodução, Conservação Ambiental.*

## ABSTRACT

DUMITH, Michelle Torres. **Bioinvasion of the African catfish *Clarias gariepinus* (Burchel, 1822) in an environmental protection area of a bay in Southeast Brazil.** 2024. 189 pages. Doctoral Thesis, Postgraduate Program in Ocean and Earth Dynamics, Fluminense Federal University, Niteroi, RJ, 2024

Biodiversity conservation depends on several mechanisms, and creating areas for environmental preservation is of fundamental importance. Guanabara Bay is an environment impacted by several factors, such as pollution, destruction of natural areas and loss of ecosystem quality. The Environmental Protection Area of Guapimirim (Guapimirim APA) was created to protect the remnants of biodiversity that the bay presents, promoting environmental preservation. The APA is also sheltered by the exotic species *Clarias gariepinus* (African catfish), which is considered an important invasive species as it causes several environmental impacts. This fish has high food versatility and resistance to adverse environmental conditions, such as low oxygen concentrations and temperature variations. Its presence in the APA has been recorded since 2001, and its population has been little studied. This thesis aimed to identify the biological and environmental factors related to the establishment of African catfish along the Guapi-Macacu River, until its confluence in Guanabara Bay. The research was divided into four chapters: (1) Assessment of the ichthyofauna and African catfish invasion; (2) Characterization of the diet and trophic ecology of the invasive African catfish, compared with other native catfish species; (3) Confirmation of the genetic origin of the African catfish population; and (4) Analysis of the reproductive biology of the African catfish. Fish were collected at 32 sampling points (10 downstream, 12 in the intermediate section and 10 upstream) on the Guapi-Macacu river, using a gill net with different meshes, cast nets, pits, and hook and line, during the period flood (February) and drought (September) in 2018. The fishing effort consisted of 24 hours of fishing in each section, with nighttime fishing. The results indicated that the APA harbours a lower species diversity when compared to previous studies before the arrival of the African catfish, representing a warning sign for its biotic integrity. The African catfish population appears to be consistent in this ecosystem, with robust and numerous specimens. The African catfish preferentially inhabits areas upstream of the river: with higher dissolved oxygen, low salinity and higher water temperature. However, specimens were also collected downstream of the river - a stretch of higher salinity. The African catfish can be considered an environmental bioindicator species, due to its distribution and abundance. The diet of the African catfish was compared to two equivalent native species and it was found that there is no dietary overlap between the species. The African catfish is omnivorous, feeding opportunely on any available item, indirectly affected by variations in the physical-chemical components of the water. The genetic origin of *Clarias gariepinus* in the Guapi-Macacu River from the Congo River (Congo, Africa) was confirmed, and its morphometric characteristics (eye diameter, head length, caudal peduncle, and mouth size) are compatible with the species originating from fish farms, confirming its origin in the region. Finally, we found that waters with higher temperatures and lower salinity contribute to the reproductive success of the African catfish, which has remained active in this ecosystem for over 40 years. This thesis suggests the need to implement public policies in the state of Rio de Janeiro to control the African catfish, in addition to continuous monitoring programs, aiming to reduce its impacts on native ichthyofauna, fishing productivity, and the biological integrity of this ecosystem.

Keywords: *Exotic species, Trophic ecology, Reproduction, Environmental conservation.*

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## INTRODUÇÃO GERAL

A Baía de Guanabara é um dos trechos costeiros mais importantes da costa brasileira, localizada no Estado do Rio de Janeiro. Essa relevância vem, não só do ponto de vista ambiental, mas também pela influência de inúmeras atividades econômicas nas suas margens, e pelas características peculiares da sua biota (Monte et al. 2018). Sua biodiversidade é considerada relevante, abrigando diversos ecossistemas em sua composição, como manguezais, ilhas, praias, costões rochosos, entre outros (Teixeira-Leite e Vianna 2023). A noroeste da Baía de Guanabara, está localizada a Área de Proteção Ambiental (APA) de Guapimirim, que é uma Unidade de Conservação federal criada em 1984.

A APA de Guapimirim, também considerada como o “Pantanal Fluminense” ainda apresenta um papel fundamental para a proteção das espécies de peixes que a utilizam como berçário e reprodução, fornecendo abrigo e manutenção dos estoques pesqueiros para áreas adjacentes, e para a Baía de Guanabara (de Melo et al. 2014). Além da ampla diversidade biológica, que uma área de proteção ambiental deve conter, a APA de Guapimirim ainda acolhe em seu interior os remanescentes de manguezal situados no seu recôncavo, que favorece a proteção de diversas espécies de peixes nativos ali presentes (Sales et al. 2018). Com diversos rios e canais em seu interior, que oscilam em volume e extensão durante os períodos de seca e cheia do ano, sendo o Rio Guapi-Macacu o mais caudaloso entre eles (Ferreira 2012). Este Rio em especial, serve como fonte do abastecimento de água para parte da região metropolitana do Rio de Janeiro, e sua bacia de drenagem foi considerada como um “bolsão de biodiversidade” da Baía de Guanabara (Bizerril e Primo 2001). No entanto, esta importante área, também apresenta inúmeros fatores relacionados à qualidade ambiental (Fistarol et al. 2015; Vicente et al. 2016; Costa et al. 2018), devido à proximidade com a Baía de Guanabara. Outros fatores também contribuem para a piora na qualidade ambiental em suas águas, como polos industriais, falta de tratamento de esgotos sanitários, desmatamento das matas ciliares, e assoreamento dos rios. Não obstante, e além de todos estes fatores ambientais, a APA de Guapimirim também hospeda em seu interior peixes exóticos, como a tilápia e o bagre africano.

O bagre africano, *Clarias gariepinus* (Burchel, 1822) (Siluriformes, Clariidae) foi introduzido para a Aquicultura na região. Contudo, a não apreciação do sabor da sua carne no mercado interno (Gutierrez et al. 2014), acarretou o abandono da piscicultura e o consequente escape da espécie exótica para a APA. Uma das primeiras menções do bagre africano na APA de Guapimirim foi informada por Bizerril e Primo (2001), que fizeram um levantamento das espécies das águas interiores no Estado do Rio de Janeiro. Embora o abandono da piscicultura tenha ocorrido na década de 80, ainda pouco se conhece sobre sua população nesta área.

Conhecidamente como uma das espécies mais cultivadas mundialmente, *C. gariepinus* é a principal espécie de bagre produzido e consumido na África e na Ásia, e em algumas partes da Europa (Dauda et al. 2018). Possui carne saborosa, sem espinhos e com baixo teor de cinzas (Oladipo e Bankole 2013; Adebayo et al. 2016; Karim et al. 2017), sendo estas características nutricionais importantes para consumo no mercado aquícola (Papuc et al. 2019). Embora, do ponto de vista comercial, o bagre africano apresente um rápido e fácil crescimento, além suportarem baixas concentrações de oxigênio dissolvido na água (Moussa 1956; Belão et al. 2011), e grande densidade de estocagem na aquicultura (Kucharczyk et al. 2019), no Brasil o mercado consumidor não foi receptivo a sua carne.

Natural do continente africano, e de partes da Ásia como Israel, Síria e sul da Turquia (De Graaf & Janssen, 1996), o bagre africano habita ecossistemas aquáticos de água doce. Sua ampla versatilidade alimentar e a capacidade de resistir a grandes oscilações de fatores ambientais como temperatura e oxigênio (Appelbaum e Kamler 2000), favorece sua capacidade de aclimação em ambientes diversos, conseguindo em situações de estresse, se deslocar em ambiente terrestre, e respirar ar atmosférico através de pseudopulmões (De Graaf & Janssen, 1996). No Brasil, *C. gariepinus* foi introduzida na década de 80 para cultivo e recreação (Agostinho et al., 2007). E na história recente do país, foi produzido comercialmente em Santa Catarina (58,9% da produção total de peixes em 2007), Espírito Santo (39,7%) e Rio de Janeiro (1,3%) (IBAMA, 2009). No entanto, diante de sua rusticidade e hábitos alimentares, *C. gariepinus*, tem sido uma ameaça a diversas espécies de peixes nativos (Umbria, 2008), devido a eventuais escapes de sistemas de produção para o ambiente natural, em muitas regiões do mundo. Mesmo decorrido mais de 40 anos desde a desativação do sistema de produção na APA de Guapimirim, e por consequência, seu estabelecimento neste ambiente, ainda carece de estudos.

De modo geral, espécies exóticas invasoras são consideradas uma das principais ameaças à biodiversidade e um componente importante das mudanças ambientais globais (Clavero e García-Berthou 2005). Estas espécies, uma vez introduzidas e aclimatadas, são de difícil controle e resultam em alterações marcantes à fauna nativa, comprometendo o equilíbrio e a estabilidade do ecossistema através de reduções de seus estoques, extinções, competição, nanismo, disseminações de patógenos e até mesmo hibridações (Agostinho, 1996). O planejamento do controle e da gestão dessas espécies invasoras são cruciais para preservar a integridade dos ecossistemas aquáticos e minimizar os efeitos adversos sobre a fauna e flora nativas (Carey et al. 2012; Woodford et al. 2016).

As medidas e alternativas de controle de espécies exóticas invasoras, foram ratificadas pelo Brasil, ao assinar e promulgar na forma de Lei a Convenção da Biodiversidade, emanada da Rio-92 e ratificada na Rio+20. No entanto, é imprescindível para isso que as informações sejam detalhadas, sobre os fatores que podem limitar a expansão da espécie exótica invasora e seus impactos, baseadas em informações cientificamente obtidas.

Nesse sentido, e diante de tantos aspectos e condicionantes, a presente tese de doutorado teve como objetivo principal, identificar as condicionantes biológicas e ambientais relacionadas com o estabelecimento de populações do bagre africano, ao longo do rio Guapi-Macacu até seu deságue na Baía de Guanabara. Também consideramos, que a presente tese, possa nortear a elaboração de programas de conservação e manejo pesqueiro, que resultem no seu controle sustentável, com menores riscos à ictiofauna nativa, à produtividade pesqueira, ou para a integridade biológica destes ecossistemas. Para tal, conduzimos um inventário sobre as populações do bagre africano ao longo do rio Guapi-Macacu (cerca de 14 Km), até seu deságue na Baía de Guanabara. As amostragens ocorreram no ano de 2018, cobrindo os períodos de cheia e seca, por meio de capturas com esforço padronizado, e através de petrechos de pesca variados, além da mensuração de amostras de variáveis ambientais (i.e. variáveis físicas e químicas) da água.

A presente tese foi dividida em quatro capítulos, apresentados em formato de artigos publicados, de acordo com o periódico selecionado:

### **Capítulo 1: Ichthyofauna structure at risk due to the bioinvasion of *Clarias gariepinus* in a river at Guapimirim Environmental Protection Area, southeastern Brazil.**

O Capítulo 1 objetivou mapear as populações do bagre africano ao longo do rio Guapi-Macacu até a Baía de Guanabara – RJ, Brasil; este estudo teve como objetivo caracterizar as condições ambientais favoráveis às espécies exóticas, contemplando os parâmetros físico-químicos da água, bem como inferir possíveis espécies bioindicadoras de impactos bioecológicos sobre a comunidade de peixes. Este capítulo foi publicado na *Fisheries and Aquaculture Journal*.

DUMITH, M. T.; SANTOS, A. F. G. N. Ichthyofauna structure at risk due to the bio invasion of *Clarias gariepinus* in a river at Guapimirim environmental protection area, Southeastern Brazil. *Fisheries and Aquaculture Journal*, 13:305, 2022.

### **Capítulo 2: Use of trophic ecology of omnivorous fish and abiotic factors as supporting tools for assessing environmental impacts in a neotropical river.**

O Capítulo 2, objetivou caracterizar a dieta e ecologia trófica de *C. gariepinus*, comparando-as com outras duas espécies de bagres nativos, com distribuição semelhante ao longo dos trechos do rio Guapi-Macacu, e por apresentarem hábitos e preferências alimentares análogas. Este capítulo foi publicado na *Journal of Fish Biology*.

DUMITH, M. T.; SANTOS, A. F. G. N. Use of trophic ecology of omnivorous fish and abiotic factors as supporting tools for assessing environmental impacts in a neotropical river. *Journal of Fish Biology*, 1:1-17, 2023.

### **Capítulo 3: Genetic origin and morphometric traits of invasive African catfish (*Clarias gariepinus*) in a protected area in southeastern Brazil**

O Capítulo 3 teve como objetivo confirmar, a origem genética da população coletada nas amostras de *Clarias gariepinus* no rio Guapi-Macacu e assim confirmar a bioinvasão. Analisamos dois loci mitocondriais, permitindo a correta identificação desta espécie invasora e ajudar na reconstrução de sua invasão no Brasil. Além disso, as características morfométricas foram analisadas, para auxiliar na identificação das espécies e examinar hipóteses ecomorfológicas que puderam confirmar também sua origem na APA de Guapimirim. Este capítulo foi publicado na *Environmental Biology of Fishes*.

NÓBREGA, FELIPE; DUMITH, MICHELLE TORRES; SALGUEIRO, FABIANO; SANTOS, ALEJANDRA F. G. N. S. Genetic origin and morphometric traits of invasive African catfish (*Clarias gariepinus*) in a protected area in southeastern Brazil. *Environmental Biology of Fishes*, 107:369–380, 2024.

### **Capítulo 4: The Rusticity of the African Catfish (*Clarias gariepinus*): Reproductive Aspects of the Invader in the Environmental Context.**

O Capítulo 4 analisou a biologia reprodutiva da espécie exótica invasora, evidenciando seus aspectos reprodutivos. Neste capítulo a composição dos estágios reprodutivos foram avaliados, evidenciando o potencial risco da população da espécie invasora manter-se reprodutivamente ativa, aumentando sua população, e assim potencializando a ameaça a comunidade de peixes nativos, que também utilizam este ambiente como criadouro e berçário. Para isso, selecionamos três índices relacionados a: condição ecológica (fator de condição), índice hepatossomático e de reprodutibilidade da espécie exótica invasora. A qualidade ambiental da água foi avaliada com relação as variáveis físico-químicas da água, sendo relacionadas a atividade reprodutiva do bagre africano no Rio Guapi-Macacu. Este capítulo foi

enviado e está sob revisão na revista *Biological Invasions*, sob o número: BINV-D-23-00501-R1.

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## 1. CAPÍTULO 1

### **Ichthyofauna structure at risk due to the bioinvasion of *Clarias gariepinus* in a river at Guapimirim Environmental Protection Area, southeastern Brazil**

#### **1.1. ABSTRACT**

The African sharptooth catfish (*Clarias gariepinus*) was the most important catfish species for aquaculture. It has a great capacity to endure several stressing factors, such as adverse environmental conditions, besides having broad feeding flexibility. Notwithstanding, the presence of this alien fish species in the Guapimirim Environmental Protection Area, in southeast Brazil, may have negative impacts on the community of native fish. In 2018, during dry and rainy seasons, samples from the fish community were collected in 32 sites of the Guapi-Macacu river, in addition to abiotic variables (salinity, pH, temperature, turbidity, dissolved oxygen, and transparency) to diagnose which factors influence the distribution of the alien fish along the river. Moreover, species were evaluated as bioindicators in the ichthyofauna to identify potential alterations in the community. Multivariate analyses 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 does not dominate the most protected area of Guapimirim, where a higher percentage of native fish species dwell. Alterations in abiotic factors, related to the increase in the temperature, can significantly contribute to the dominance of this invasive alien fish in this protected area, requiring constant monitoring of some key species, as well as the population of the invasive species in this environmental preservation area.

**Keywords:** invasive alien fish, overlapping habitats, abiotic variables, bioindicators, climate change

#### **1.2. INTRODUCTION**

Invasive alien species (IAS) are considered one of the main threats to biodiversity and an important component of global environmental change (Clavero & García-Berthou, 2005). Once present and acclimated, the IAS becomes difficult to control and results in marked changes in the native fauna, compromising the balance and stability of the ecosystem by reducing their stocks and causing extinctions, competition, pathogen transmission, and hybridization (Agostinho et al., 2007; Hill & Lodge, 1999; McMichael & Peters, 1989; Miller et al., 1989; Omeji et al., 2013; Peeler et al., 2006).

The African sharptooth catfish – *Clarias gariepinus* (Burchell, 1822) – is a native species to much of the African continent and parts of southwest Asia, such as Israel, Syria, and southern Turkey (De Graaf & Janssen, 1996). It has the exceptional ability to migrate to the terrestrial environment and breathe atmospheric air through *pseudolungs*, which allows it to endure adverse conditions of temperatures and low oxygen concentrations (De Graaf & Janssen, 1996). *Clarias gariepinus* was introduced in Brazil in the 1980s for aquaculture and fish-and-pay lakes (Agostinho et al., 2007; Alves et al., 1999). The escape of the African catfish from aquaculture systems to the natural environment has threatened several species of native fish (Umbria, 2008), making it a potent IAS for these ecosystems. The potential invasions of *C. gariepinus* have shown that the species can progressively establish itself depending on the source of the invader's dispersion (Singh, 2021), causing a reduction in food availability (Ellender et al., 2015) and pressure on the ecosystem's trophic chain (Khan et al., 2021). Added to this factor, the invasiveness of the African catfish can happen in a short period, resulting in malnutrition, low growth, or even elimination of some native species (Khan et al., 2021). In other cases, unplanned hybridization, with an escape into the natural environment, can cause the irreversible loss of native biodiversity (Parvez et al., 2022).

The Guapimirim Environmental Protection Area (Guapimirim APA) is located northeast of the Guanabara Bay, one of the most environmentally critical coastal areas of the Brazilian coastline, in Rio de Janeiro, Brazil. The Guapimirim APA is a Federal Conservation unit, created in 1984 to protect the remaining mangrove and to ensure the survival of human populations that still depend on this environment. The Guanabara Ecological Station (Guanabara ESEC) is located within the APA. The ESEC is a fully protected Conservation Unit, with no admittance of people within its borders, except for scientific or educational purposes. The Guapimirim APA has several rivers and canals; among them, the Guapi-Macacu river stands out, due to its water output (Fries et al., 2019), supplying drinking water to most municipalities in its eastern portion. In this river, in particular, the riverine population is aware of the presence of the African catfish; however, we know little about its distribution and its possible impacts on the native ichthyofauna of this area.

In this regard, the riverine population must receive detailed information on the distribution of invasive species, so that they can detect potential changes in the biotic community of aquatic ecosystems, considering the fish community as a whole, and which abiotic or environmental factors may be involved in the establishment of exotic species. In Brazil, some studies report the occurrence of *C. gariepinus* in natural environments (Alves et al., 1999; Vitule et al., 2006) and environmental protection areas (Rocha, 2008; Rocha &

Schiavetti, 2007). Weyl et al. (2016), who conducted an extensive review of studies on the establishment of alien populations of African sharptooth catfish, mention that few biological assessments are available, especially studies focused on their persistence and long-term establishment using empirical ecological data. For these authors, establishment is the most important phase of the invasion process, as negative impacts are more likely to occur when alien species become established.

To determine the integrity of ecosystems, the characterization of physicochemical parameters is not sufficient; and the scarcity of methods to objectively incorporate the degradation of the biological component also implies a lack of responses to the increasingly negative effects of IAS (Hermoso & Clavero, 2013). An alternative to obtaining a response regarding the biotic integrity is to look for “bioindicators”, whose presence and abundance reflect changes that occur throughout the community or at the ecosystem level (Hermoso & Clavero, 2013; Parker et al., 1999).

Therefore, to assess the integrity of the fish community and the establishment of this IAS in this protection area it is necessary to first assess the fish community itself and the main abiotic components for its structure. In this sense, this study aims to characterize the environmental conditions favourable to the alien species, by contemplating the physicochemical parameters of the water, as well as inferring possible bioindicator species of bioecological impacts on the fish community. To accomplish this, it is essential to characterize the distribution of the African catfish population and the native fish community along the Guapi-Macacu river.

### **1.3. METHODS**

#### ***1.3.1. Area of Study***

The physiography of the Guapi-Macacu river is divided into three segments or areas. The first flows down the slope of the Serra do Mar in the form of rapids and waterfalls, with its banks covered with Atlantic Forest. A second area runs through a transition between the cliffs and the plains, with a less rugged formation. In this stretch, the river is surrounded by riparian forest and hills with rounded, massive shapes with altitudes below 1000m. And a third area, which is longer and runs through a floodplain, with flat terrain and minimal unevenness, is easily flooded and subject to tidal influences due to its proximity to the Guanabara Bay. The vegetation of this segment is composed of small shrubs, pastures, and mangroves (Dantas et al., 2007) and is closer to the most significant urban centres (Ferreira, 2012).

### 1.3.2. 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 invasive species, the target of the study, is nocturnal. At the same time, a Hanna multiparameter probe (model HI 9828) was used to measure *in situ* the physicochemical variables of the water: temperature (°C), pH, dissolved oxygen (mg/L); transparency (cm), turbidity (FNU); and salinity (PSU). Sampling was distributed along 32 points: 10 points between the mouth of the river and the inner limit of the Guanabara ESEC (downstream area); 12 points between the external boundary of the Guanabara ESEC and the internal perimeter of the Guapimirim APA (intermediate zone); and 10 points distributed between the outer limit of the Guapimirim APA and the dam (upstream area), within the Buffer Zone of the Guanabara Ecological Station (Guanabara BZ), totalling 64 sampling points in both seasons (Figure 1). Gillnets were removed 24 hours after installation. The fish captured were grouped at each sampling point and packed in plastic bags, labelled, and refrigerated on ice, and then transferred to the Laboratory of Applied Ecology at UFF.



Figure 1. Map of the Guanabara Bay with the representation of the limits of the Guapimirim Environmental Protection Area (Guapimirim APA), 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.

### ***1.3.3. Laboratory Activities, Data Processing, and Statistical Analysis***

The collected fish were identified, weighed, and measured. 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 uniformity index were used (Magurran, 1988).

The Permutational Multivariate Analysis of Variance (PERMANOVA) was used in the R Program (R Core Team, 2020), available in the VEGAN package (Oksanen et al., 2022), to test for spatial and seasonal differences in the physicochemical 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 the traditional ANOVA, although it does not require the assumptions of normality and homoscedasticity (Anderson, 2001; McArdle & Anderson, 2001). The Bray-Curtis distance was used in all PERMANOVA tests, permuted 4999 times per analysis, as recommended by Manly (2018).

The *Multinomial Species Classification Method* (CLAM) was used, through the "*CLAMTEST*" function available in the VEGAN package (Oksanen et al., 2022), 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 (Oksanen et al., 2022). A threshold of 50% specialization in each period was set, with a 95% significance level for individual tests.

To analyse the spatial proximity of the species and the formation of groups, according to the sampling points in both periods (considering the river as a whole), Cluster Analysis was used, through the "*HCLUST*" function of the package DENDXEXTEND, using the "ward. D2" (Galili, 2015) with the Bray-Curtis distance in the dissimilarity matrix of the VEGAN package (Oksanen et al., 2022). The sum of squares criterion was used for this agglomerative method, producing groups that minimize the dispersion within the group in each binary merger (Murtagh & Legendre, 2014). This analysis was used to infer the similarity in the distribution of species in the river stretches, according to their abundance.

As a way of assessing potential species as bioindicators of the fish community, the specificity and fidelity analysis (*IndVal*) was used, which performs a permutation test (with a significance of 0.05). For this, the "*MULTIPATT*" function, available in the INDICESPECIES

package (Caceres et al., 2016), was used both to determine the indicator species between seasons (rainy and dry) and river areas and between river segments, regardless of the season. The *IndVal* method has some advantages when compared to other bioindication methods; it is 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, 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., 2022), 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 physicochemical 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, displaying the environmental variables that can predict changes in the composition and abundance of the ichthyofauna and indicate which species will be affected in this relationship.

#### **1.4. 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, 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 DO 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. 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).

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

Parameter	Area		Season	
	<i>F</i>	<i>p</i>	<i>F</i>	<i>p</i>
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

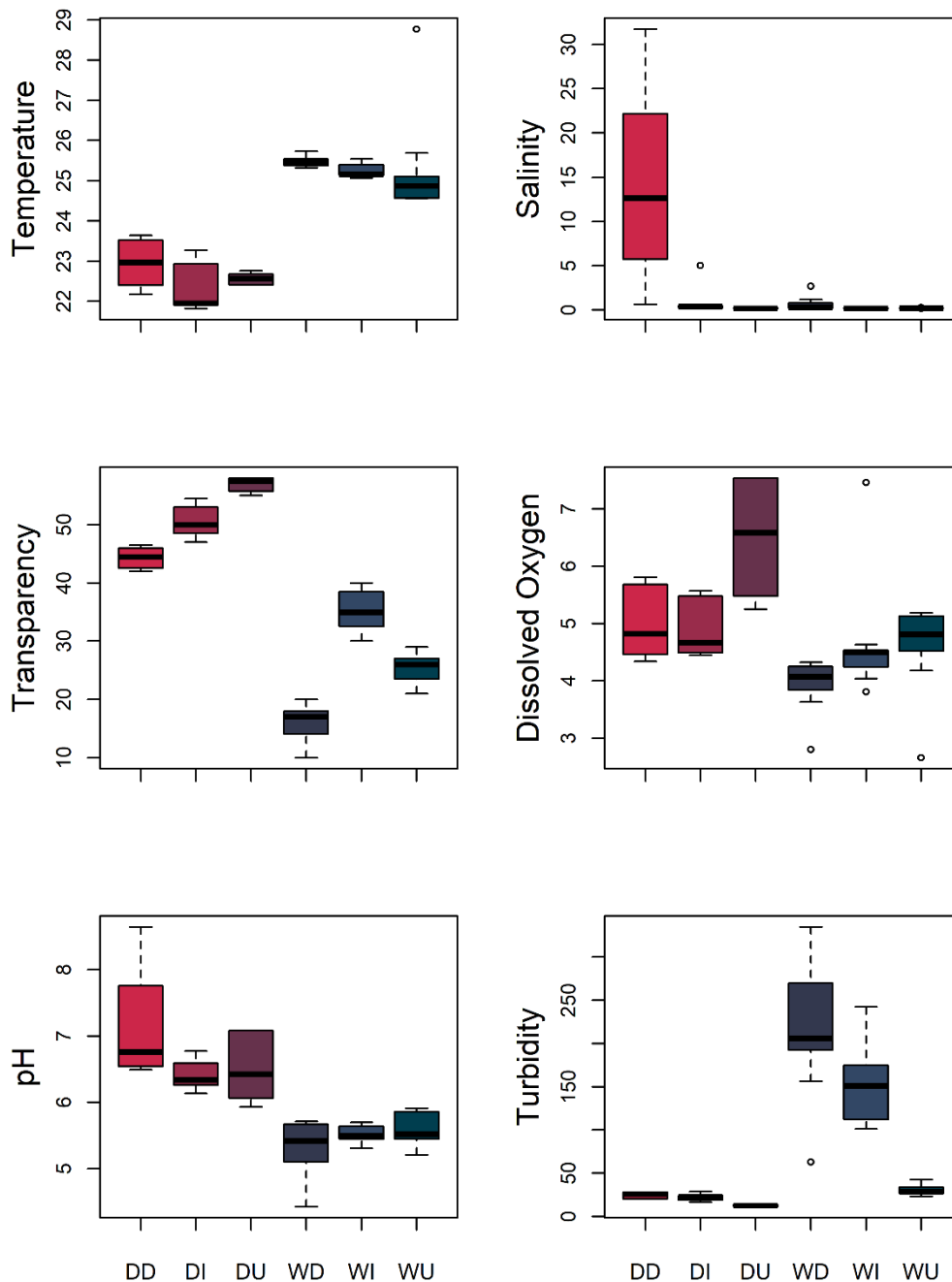


Figure 2. Boxplot of environmental variables by segments and seasons of the year in the Guapi-Macacu river. DD: dry downstream; DI: dry intermediate; DU: dry upstream; WD: wet downstream; WI: wet intermediate; and WU: wet upstream.

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.

Table 2. List of species (authors), code in the analyses, abundance, biomass, size classes, and CLAM classification of the ichthyofauna collected in 2018 along the Guapi-Macacu river – RJ.

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

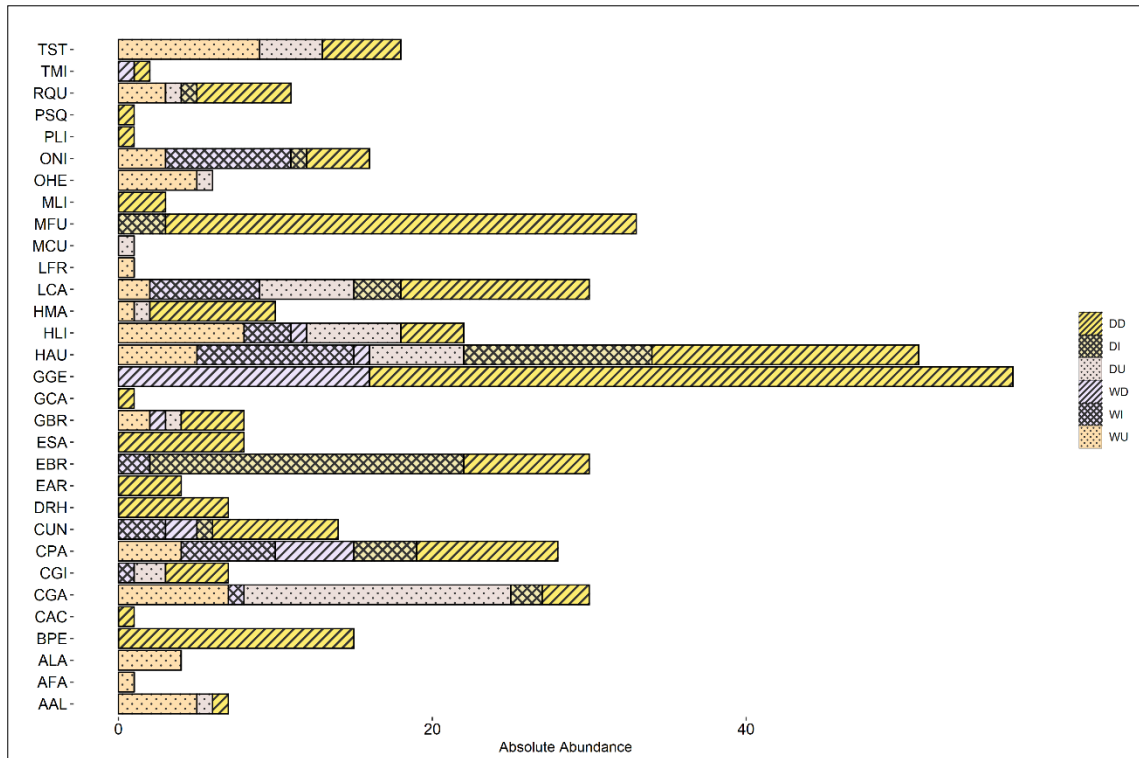


Figure 3. Distribution of absolute abundance of fish community along the Guapi-Macacu river between areas and seasons of the year. DD: dry downstream; DI: dry intermediate; DU: dry upstream; WD: rainy downstream; WI: rainy intermediate; and WU: rainy upstream. The codes corresponding to each species are shown in Table 2.

For species richness, PERMANOVA showed 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 show significant differences between the other zones and seasons, as well as for diversity and equitability ( $p>0.05$ , for all) (Figure 4).

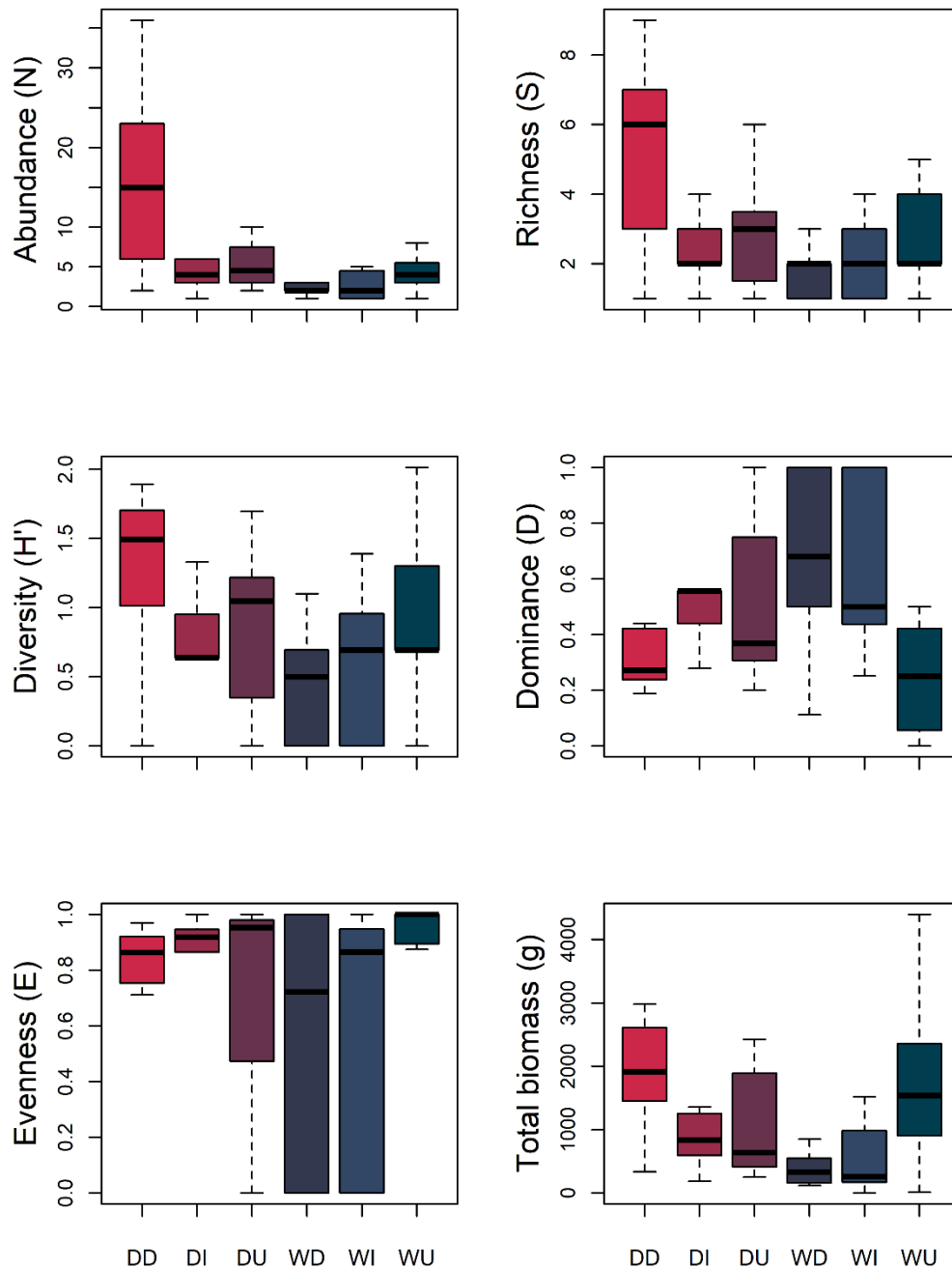


Figure 4. Boxplot with the distribution of abundance, richness, Shannon-Wiener diversity, Pielou equitability, dominance, and total biomass (g) between the stretches and seasons in the Guapi-Macacu river. DD: dry downstream; DI: dry intermediate; DU: dry upstream; WD: wet downstream; WI: wet intermediate; and WU: wet upstream.

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).

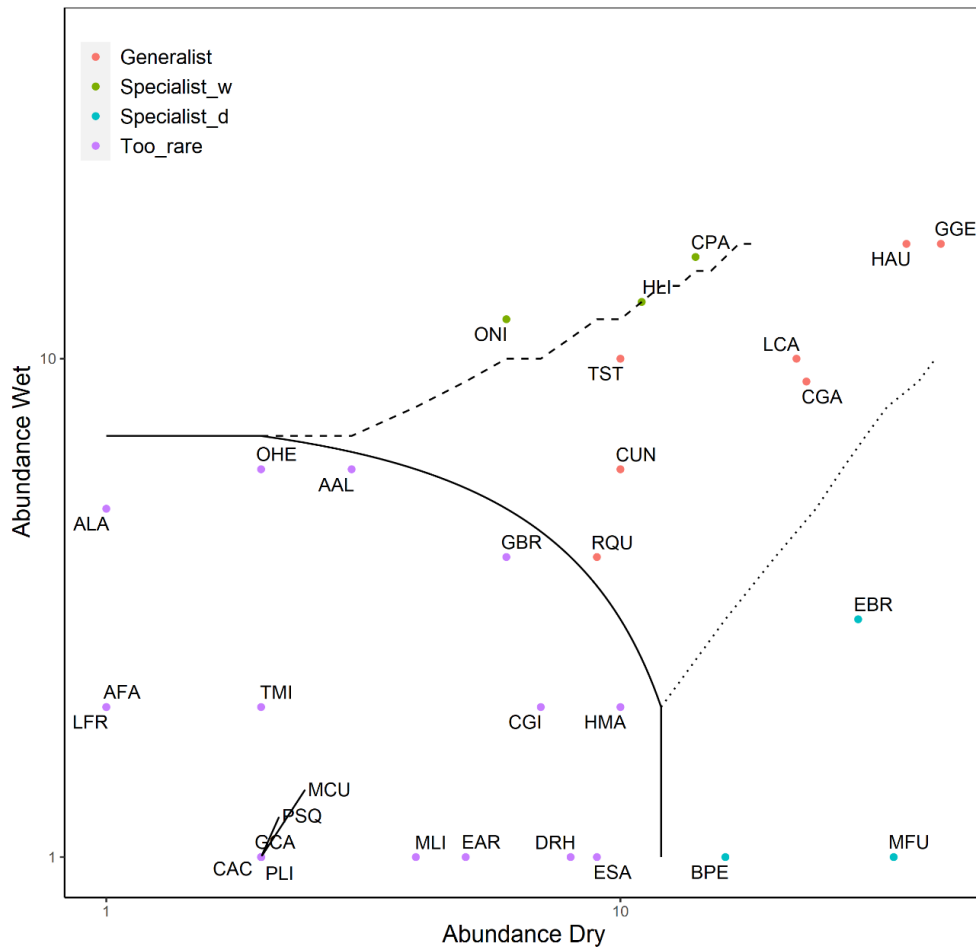


Figure 5. CLAM classification of the ichthyofauna in the dry and rainy seasons ( $p = 0.05$ ). Lines represent the specialization limit. Dashed line = rainy season; dotted line = dry season; solid line = rare and abundant species. The codes corresponding to each species are shown in Table 2.

The cluster analysis results coincided with the previous locations shown in Figure 1, representing 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. gariépinus* is close to groups with broad distribution in the river, such as *L. castaneus*, *T. striatulus*, and *H. auroguttatus*. Furthermore, *C. gariépinus* occurs similarly to *R. quelen* and *T. striatulus*, sharing the same habitats.

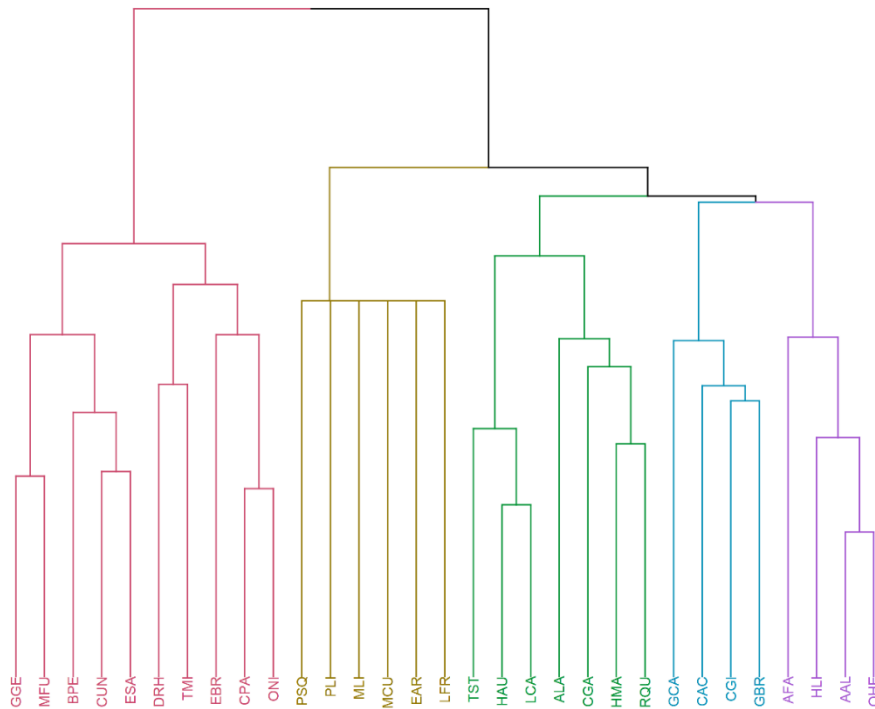


Figure 6: Cluster analysis of fish abundance between the areas of the Guapi-Macacu river. The codes corresponding to each species are shown in Table 2.

The specificity and fidelity analysis (*IndVal*) showed as indicators the most important species that make up the two main groups formed in the cluster analysis (red line and brown line). 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* was selected in the same segment in the two seasons sampled (53.4%,  $p=0.035$ ). When the *IndVal* analysis was performed only for the river segments, seven species were selected and grouped in the two analysed seasons. In the group formed only upstream, the *C. gariepinus* (54.8%,  $p=0.013$ ), *T. striatulus* (52.6%,  $p=0.007$ ), and *A. lacustres* (39.7%;  $p=0.025$ ) showed fidelity and specificity. In the downstream segment, an exclusive area of the 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* ( $p=0.022$ ) was the only species selected for both regions of the Guapimirim APA (downstream and intermediate area), 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 a relation between 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 were related to the distribution and abundance of fish species, as well as to the river segments. ANOVA showed 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, which was obtained through the "*ORDSTEP*" function (Figure 7B). In the second model, the three axes are significant ( $p<0.02$ , to all), together explaining 73.19% of the samples. The most significant variables for each analysed 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 DO was the most important physicochemical parameter for the species upstream of the river ( $F=2.5752$ ;  $p=0.004$ ), all showing high statistical significance.

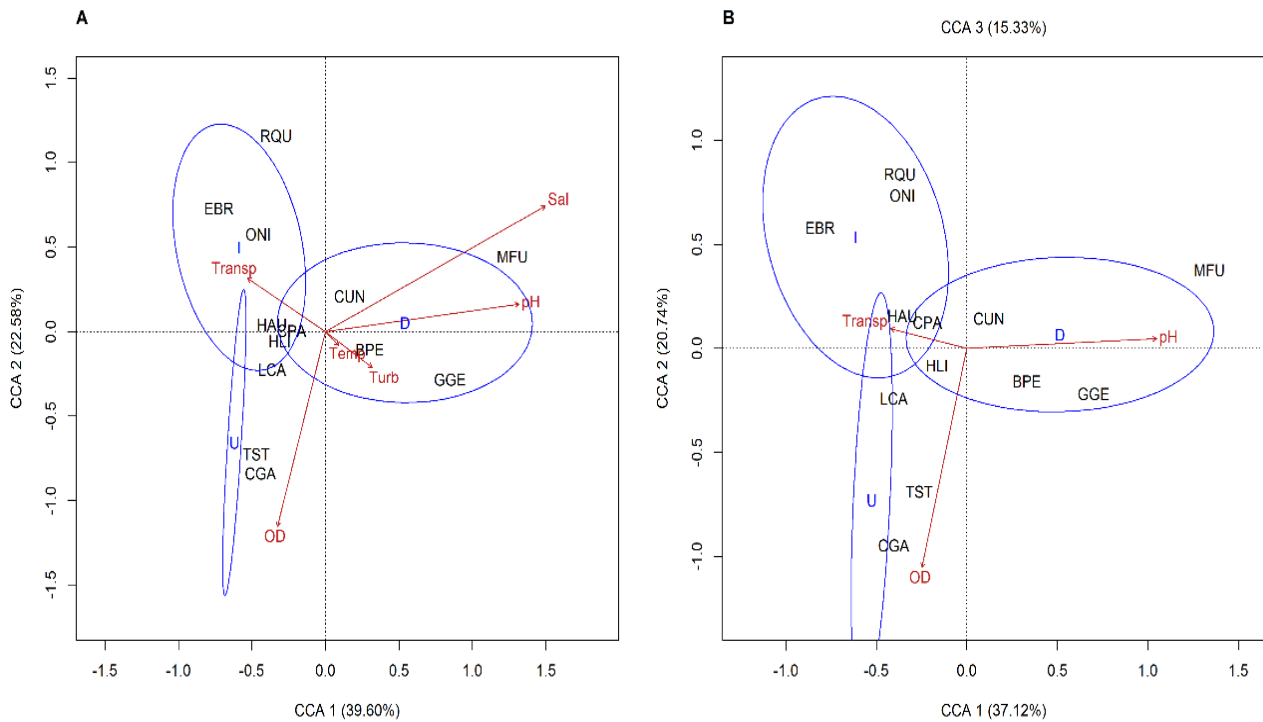


Figure 7: Canonical correspondence analysis (CCA) applied to the matrix of abundance of generalist and specialist species selected in the CLAM model and the environmental variables. In blue, are the downstream (D), intermediate (I), and upstream (U) segments of the Guapi-Macacu river. In red, arrows indicate the contribution of each environmental variable. The codes corresponding to each species are shown in 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.

## 1.5. DISCUSSION

### 1.5.1. 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 da Silva et al. (2009) in the Rio Formoso estuary (Pernambuco, Brazil): they detected the highest pH values in the lower estuary region, in addition to higher DO and salinity. According to da Silva et al. (2009), 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 as it is a coastal segment, and its intensity may vary within the rainy season, which favours its dilution. The higher rates of DO and pH reflect a rainless season, with greater water transparency,

favouring photosynthesis (which removes CO<sub>2</sub>) and, consequently, raising the pH of the water due to the consumption of H<sup>+</sup> ions.

When analysed individually, temperature and pH were the environmental attributes that were not related to the different segments of the river, but 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 variable with high significance for the downstream area, probably due to its higher value in the dry season. The association between the other analysed variables, 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 et al. (2000), fish from tropical estuaries are subject to a series of interactions of physicochemical and biological factors that determine their patterns of occurrence, distribution, and movement. For these authors, in the Rio Formoso estuary (Pernambuco, Brazil), temperature, salinity, pH, and dissolved oxygen were higher in the lower estuarine zone and during 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 impair aquatic life. Furthermore, during the rainy season, the currents in the Guanabara Bay carry organic materials to and concentrate 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.

### ***1.5.2. 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 the Guanabara Bay, with some species migrating inland, such as common snooks, grey mullets, jenny mojarras, and whitemouth croakers. This segment consisted of resident species, marine and freshwater migrants, which use estuaries as feeding areas, for rearing larvae and juveniles, or for reproduction (Blaber et al., 2000). These habitats favour 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 enjoy this protection during the juvenile phase and at the time of spawning and, therefore, are closely dependent on the integrity of this ecosystem (Paiva et al., 2008). 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. For Teixeira et al. (2005), the determination of biodiversity, especially of the fish community and its patterns of spatial and temporal variation, is of great relevance for the assessment of environmental quality.

Siluriformes was the most abundant fish order in the Guapi-Macacu river. The dominance of Siluriformes over others is a characteristic pattern of Brazil's eastern region, being particularly accentuated in areas of high river courses, where the high hydrodynamic condition favours the occupation by demersal species (Passos et al., 2016). *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 (Andreatta et al., 1989; F. G. 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 – and rarely females – performing oral incubation and carrying eggs and initial forms of the offspring until they complete embryonic development (Barbieri et al., 1992; Yáñez-Arancibia & Sánchez-Gil, 1988); this explains 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 *IndVal* analysis. Thus, the *IndVal* analysis determined that for this specific area, regardless of the season analysed, three species were considered indicators of this habitat: *G. genidens*, *B. pectinate*, and *M. furnieri* (Figure 8A). In addition to these species, *E. saurus* was selected only for the dry season in the downstream area. All species selected for the downstream area were considered 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 in *IndVal* share this river segment.

The area within the Guapimirim APA, disregarding the Guanabara ESEC (i.e., the intermediate segment of the river), presented ecological indices similar to the other stretches of the river. However, there is greater abundance and richness (Figure 8A). Thus, the downstream and intermediate segments (i.e., comprising the Guanabara ESEC and Guapimirim APA) 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), common snooks 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 common snooks 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 (Gracia-López et al., 2006). Its nursery or primary habitat has been described as shallow warm streams or drainage canals, with low current speeds and non-vegetated bottoms or at the border of mangroves (McMichael & Peters, 1989). As they develop, they move from habitats with shallow water to estuaries, mangroves, and deeper waters (Martin et al., 1988). According to cluster analysis, the river segment shared with the *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 has vegetation composed of mangrove forests, of the riverine type. This area is directly related to samples with greater transparency, showing *E. brasiliensis* and *O. niloticus* with intermediate values of this attribute (Figure 8B). *Eugerres brasiliensis* is a species of marine origin that tolerates significant variations in salinity (dos Santos et al., 2016). It is anadromous, migrates from the sea to rivers, lives in coastal waters of warm seas, and penetrates coastal lagoons and estuaries to complete its life cycle (Araújo et al., 2016). Furthermore, *E. brasiliensis* is a nocturnal, generalist, and opportunistic species; it is also epibenthic and demersal. That is, it presents patterns strictly linked to the substrate, being considered an excellent biological resource, mainly because it is regarded as an abundant fishery resource (Alvarez-Lajonchère et al., 1996; Nunes et al., 2021; Ramos et al., 2016; Soares et al., 2016).

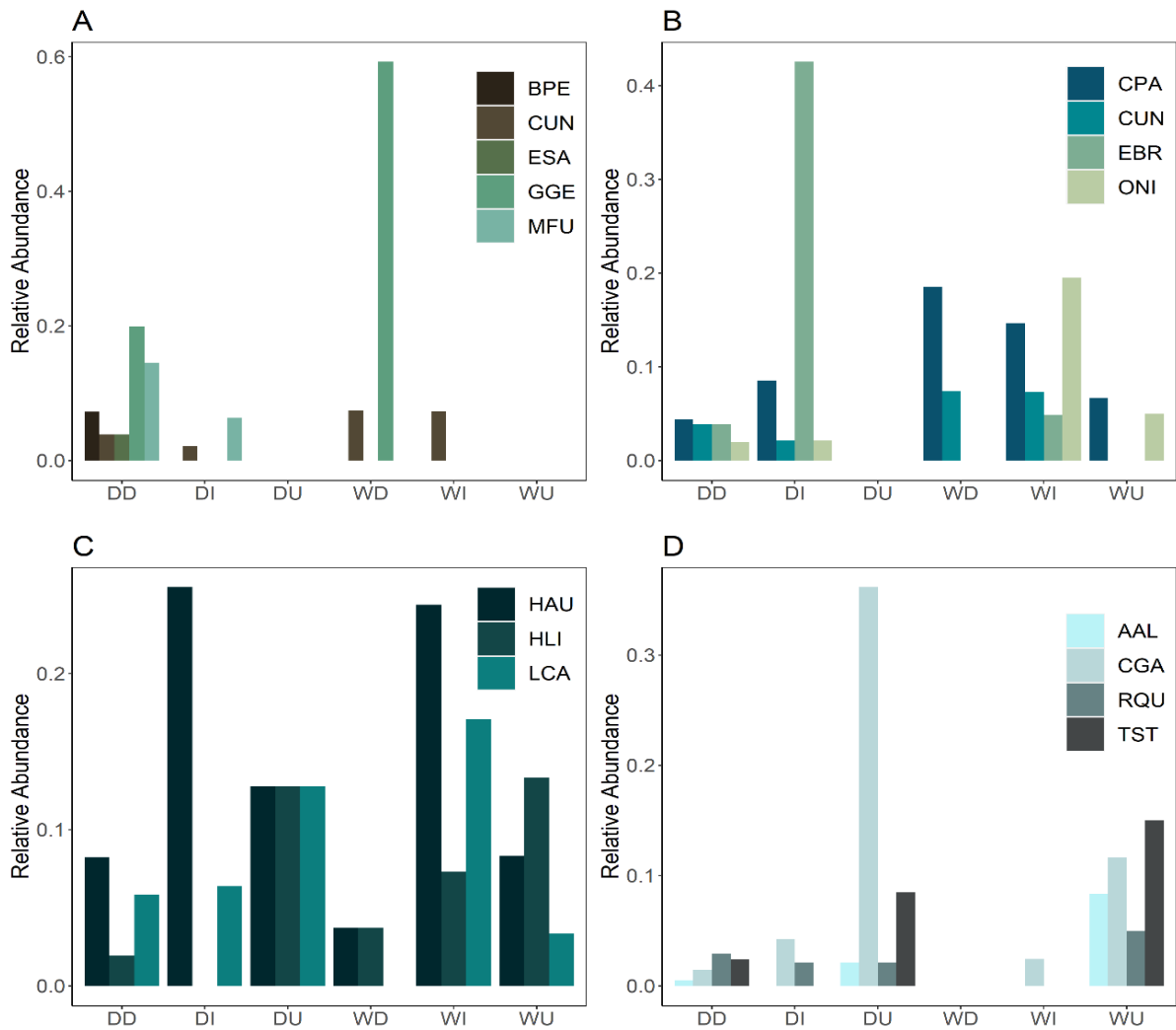


Figure 8. Relative abundance of the main fish species of the Guapi-Macacu river which cover: (A) downstream area; (B) intermediate area; (C) the whole river; and (D) upstream area. DD: dry downstream; DI: dry intermediate; DU: dry upstream; WD: wet downstream; WI: wet intermediate; and WU: wet upstream. White: dry season; grey: rainy season. The codes corresponding to each species are shown in Table 2.

On the other hand, the cluster analysis did not show the intermediate region with a specific community for this segment of the river, presenting itself as a transition area, 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 – the Loricariidae – 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 generalists in this study.

The species *A. lacustris* (Figure 5), considered rare in the analysis presented in the 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. For Dufrêne & Legendre (1997), rare species can get 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, contributing to habitat specificity, but are not useful 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 an abundance of *C. gariepinus*. The upstream part is the headwaters of the river, which have a humid tropical climate, and high and variable slope, determining the dynamic character of the fluvial system, with the presence of rapids, characteristic of mountainous and plateau regions. Riparian forests are a key factor in providing resources for feeding aquatic fauna and attracting dispersers (Yirigui et al. 2019), making the riparian environment a fundamental element in the sustainability of rivers and lakes and in the connection between the different systems that compose them (Casatti et al. 2012; Vieira e Tejerina-Garro 2020). The CCA also highlights the most protected area of the Guapimirim APA, downstream of the river, with greater diversity and richness, presenting greater attributes of salinity, pH, and turbidity. In general, the most abundant species in river flow are intermediate values of the abiotic variables analysed, 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, showing 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*, revealing proximity of sharing in the same region, mainly in the dry season, with a dominance of *G. genidens*, as it presents greater abundance in this group. The CCA also highlights the similar spatial distribution of *T. striatulus* with *C. gariepinus* (with greater representation in abundance), corroborating the cluster analysis.

### **1.5.3. 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. The species that present ecological equivalence to the African sharptooth catfish, namely the *R. quelen* and the *T. striatulus*, despite having a similar distribution, presented lower abundance, which denotes the overlapping of IAS' habitat over the native's habitat (Figure 9A). In addition, the population of *C. gariepinus* presented higher contributions

of abundance in the dry season, mainly upstream, compared to the other species, demonstrating a high potential to colonize the entire river (Figure 9B).

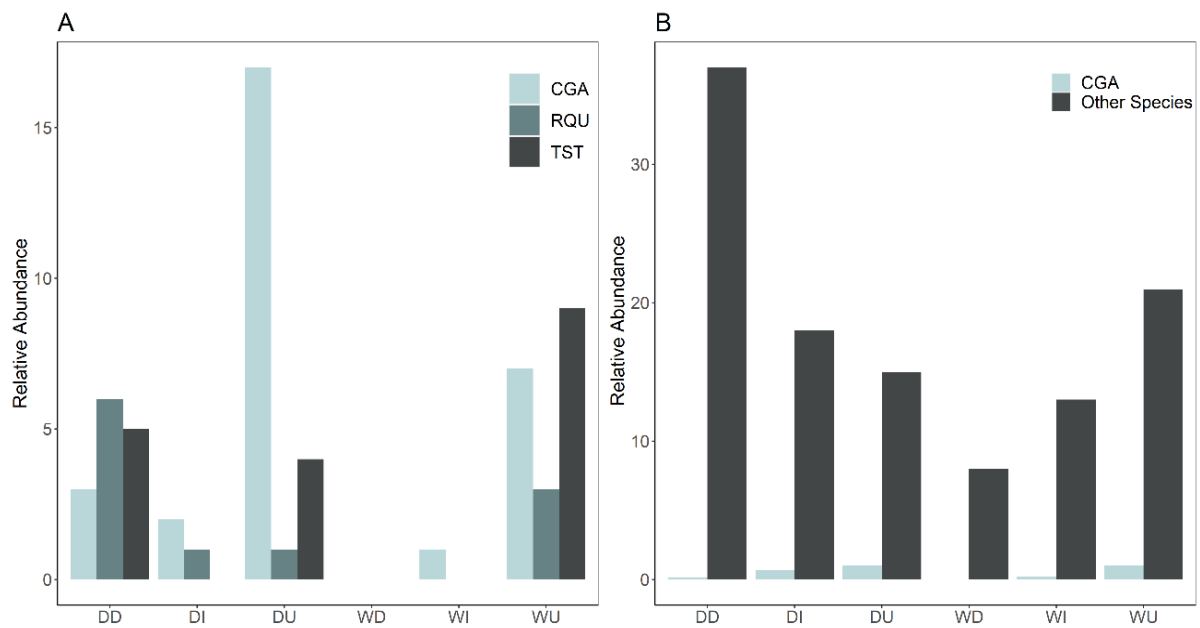


Figure 9. (A) Captures of the population of *Clarias gariepinus* and the two species with the ecological equivalents, between the different river segments and seasons sampled. (B) Captures of *Clarias gariepinus* and other species of the community, between the different parts of the Guapi-Macacu river 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 shown in Table 2.

Although the CCA analysis does not show similar correlations of the abiotic factors *R. quelen* with *Clarias gariepinus*, the 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 environments with calmer water and sandy, muddy bottoms, along the riverbanks and vegetation (Gomes et al., 2000). They are omnivores species with a clear preference for fish, crustaceans, insects, plant remains, and organic debris (Gomes et al., 2000; Guerini et al., 2014); therefore, they are considered generalists in terms of food choice (Gomes et al., 2000). The *R. quelen* and the *C. gariepinus* share many biological similarities, especially the food preference and habitat use; however, the *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, 2022). On

the other hand, the African sharptooth catfish can tolerate low concentrations of dissolved oxygen (Adewolu et al., 2008) due to an accessory air-breathing organ that can absorb oxygen from atmospheric air (Moussa, 1956), allowing it to survive out of water for long hours or even weeks or even in muddy swamps (Idahor et al., 2014).

Another significant environmental attribute in our analysis was the temperature, which 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. For Hecht (2013), the African sharptooth catfish larvae have an optimal development around 28°C, a value that was recorded in the rainy season upstream. Indeed, it could be observed during the dry season, when there was a large increase in the population of the IAS, favoured by the increase in temperature recorded previously. On the other hand, our study indicates that, although the average temperature values measured in the Guapi-Macacu river are not considered ideal for the development of the catfish, the species' adaptability allowed it to develop well in this ecosystem and 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 favour 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 the Guanabara Bay. De Melo et al. (2014) 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 the 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 later stages (Hogendoorn, 1981). Borode et al. (2002) conducted a study on the effect of salinity on the early development of the African catfish. They concluded that increased salinity delays the hatching and development of African sharptooth catfish eggs and larvae, but the African catfish can tolerate variations of up to 6 ppt for its growth. Even so, despite the significant abundance of this species, the specificity and fidelity analysis does not list the *C. gariepinus* as an indicator species, considering both the seasons studied and the collecting areas. However, this pattern diverges when considering only the upstream zone, when the species specificity index rises to 81% and fidelity to 36% for this area, demonstrating that the *C. gariepinus* has a probability of 53% to be an asymmetric indicator species of this area. For Dufrêne & Legendre (1997), a species can be an asymmetric indicator without high fidelity.

## 1.6. CONCLUSION

The analyses 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, our study reveals that this species still does not colonize the Guanabara Bay, nor the downstream portion 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 fulfils its role of protecting the resident ichthyofauna, harbouring a larger contingent of typically estuarine juvenile fish, which portrays 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 favour the development of African sharptooth catfish. Southeast Brazil has been experiencing more intense summers and prolonged periods of droughts, and consequently, an increase in the temperature in stretches of the river that may contribute to the successful development of African sharptooth catfish. Moreover, the IAS has been competing for habitats with native species that 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 the 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.

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## 2. CAPÍTULO 2

### Use of trophic ecology of omnivorous fish and abiotic factors as supporting tools for assessing environmental impacts in a neotropical river.

#### 2.1. ABSTRACT

The study of diet is one of the mechanisms by which competition for resources between species that cohabit in the same ecosystem can be inferred. Therefore, the relationships of the indices that measure specialization in the diet of fish species are necessary to characterize the nutritional quality of these populations and the ecosystem's environmental health. Three species of catfish were selected: one invasive (*Clarias gariepinus*) and two natives (*Trachelyopterus striatulus* and *Rhamdia quelen*), with similar distribution along the Guapi-Macacu River, in the Guapimirim Protection Area (Rio de Janeiro). Fifty-nine catfish of the three species were collected in total, along 32 collection points in the Guapi-Macacu River in two periods (dry and rainy) in 2018. Nonparametric statistics showed the partition of resources between species and the influence of abiotic factors (temperature, pH, transparency, dissolved oxygen) contributing to the selection of available resources in the environment. Diet-related indices – repletion index (RI), condition factor (K), niche width, and trophic position of the specimens collected – contributed to measuring the nutritional status of each of these catfish species, showing that the *R. quelen* species has a relationship between RI and K, tending to absorb and metabolize nutrients faster than other species. In addition, the invasive species occupies a wide range of trophic positions compared to native species, confirming its feeding plasticity. On the other hand, *T. striatulus* needs large amounts of terrestrial insects to maintain its poor condition factor. Also, the RI showed direct influences of abiotic variables, with the temperature being the most prominent. Our results suggest that the invasive species can benefit from this environment that shows signs of environmental degradation.

**Key Words:** Siluriformes; invasive alien fish; trophic position; null model; environmental impacts; repletion index

#### 2.2. INTRODUCTION

Fish is one of the main bioindicators in aquatic ecosystems (Chovanec et al. 2003; Duarte et al. 2020; Kılıç e Yücel 2022), being effective for assessing various types of

environmental contamination (Freitas e Siqueira-Souza 2009; Authman 2015; Araújo et al. 2018). Thus, the assessment of trophic ecology is a powerful tool for describing the dynamic interactions between organisms and their environments (da Silveira et al. 2020). And, studying the diet of fish species in preserved areas also serves as a tool to assess these areas' environmental and especially aquatic health (da Silva et al. 2008). Such information provides subsidies to understanding the mechanisms that allow the coexistence and exploitation of food resources by several species in the same system (Sato e Godinho 2003).

In this sense, ecological indices that measure the specialization of fish species regarding their diet are necessary to characterize the nutritional quality of this fish population (Bolnick et al. 2002) and thus contribute to inferring about food specialization and well-being of these species. In addition, studies on trophic relationships are important to obtain information on interactions between species (Goodale et al. 2010) and their habitat. Therefore, knowing which factors and how they influence the choice of a particular resource allows us to understand the different ecological roles of individuals in a population (Singer e Bernays 2003), as well as which environmental factors may be related to these fishes' nutritional status. Furthermore, slight alterations in individual abiotic characteristics, such as transparency and turbidity, can contribute to preserving native species in bioinvaded areas (Tsunoda e Mitsuo 2012; Yamanaka 2013; Alexander et al. 2015). Thus, abiotic elements can modify predator-prey relationships, mitigating predatory impact on a diversity of prey (Tsunoda e Mitsuo 2018).

The Guanabara Bay (Rio de Janeiro - Brazil) is an ecosystem regularly impacted by sewage emissions, petroleum residues, and industrial effluents; nevertheless, it still presents valuable biodiversity and significant fishing activity (Fries et al. 2019). Several studies in Guanabara Bay are related to its pollutants (Monteiro et al. 2012; Silveira et al. 2017; Fries et al. 2019; Olivatto et al. 2019). Its northeastern portion houses an environmental preservation area with mangroves and several rivers and channels, whose fish community lacks research. This area also exhibits signs of degradation associated with the Bay (Linde-Arias et al. 2008; Ghizelini et al. 2019) and harbours invasive fish species such as *Clarias gariepinus* (Burchell, 1822) and *Oreochromis niloticus* (Linnaeus, 1758) (Dumith e Santos 2022). Both species are African and commonly used in aquaculture, but *C. gariepinus* stands out for its rusticity and impressive dietary plasticity (Umbria 2008). As a result, it can exert an additional impact on the fish community.

We selected three catfish species, *C. gariepinus*, *Trachelyopterus striatulus* (Steindachner, 1877) and *Rhamdia quelen* (Quoy & Gaimard, 1824) are catfish of the

Siluriformes order, considered omnivorous and nocturnal, with similar food preferences (Bruton 1978; Gomes et al. 2000; Silveira et al. 2013). The three catfish species cohabit the same stretches of the Guapi-Macacu River (Dumith e Santos 2022), located within the Guapimirim Environmental Protection Area. Among the criteria for selecting these species as bioindicators are well-known ecological characteristics, such as long life cycle, easy identification, and medium/large size (Freitas e Siqueira-Souza 2009). Furthermore, *R. quelen* has been used as an evaluation tool in various environmental assessments, providing insights into water contaminants (Becker et al. 2009; Ghisi et al. 2011; Marins et al. 2021; Severo et al. 2023). *Trachelyopterus striatulus* has served as a biodiversity bioindicator for insects and is also associated with the types of exogenous food in the water (dos Santos et al. 2009). On the other hand, *C. gariépinus* is an exotic fish, dominant in many rivers in Africa, and can occupy various trophic levels (Khan et al. 2021; Low et al. 2022). The interactions for food resources within this environment among catfish species may have implications for environmental health, as they could potentially compete for supplies in this ecosystem. In addition, studies on the diet of invasive species and their native ecological equivalents are of vital importance in estimating interspecific interactions in the local community. Thus, investigating such interactions can lead us to understand the impacts of biological invasion and habitat changes on ecosystem structure and function, providing a solid foundation for effective ecosystem management and biodiversity conservation (Lodge et al. 2012). Despite the paramount importance of these studies for the knowledge and bioecology and management of exotic species, they are still scarce in the Guapimirim Protection Area.

In this context, the present study aims to: 1) characterize the diet and trophic ecology of three catfish species, 2) analyse the main ecological attributes correlated to the fishes' diet; 3) determine a potential niche overlap between catfish species, and 4) infer about the influence of abiotic variables related to diet. The comparison of the abundances of the three species was also discussed, allowing inferences about the river's environmental health and the potential impacts of the exotic species in the environmental protection area. The present study is expected to enhance the knowledge about the trophic ecology of *C. gariépinus* and its ecological counterparts in the preservation area.

## 2.3. MATERIALS AND METHODS

### 2.3.1. Area of Study

This study focused on the main river of the Guapimirim Protection Area (Guapimirim APA). The APA is a 14,000-hectare conservation unit created in 1984 (Monteiro et al. 2012), important for the preservation and recovery of Guanabara Bay (Fries et al. 2019). With the largest watershed in the region (Cabral e Fiszon 2004), the Guapi-Macacu River has the highest flow within the Guapimirim APA (Dias et al. 2019; Ferreira et al. 2019), in addition to a diverse landscape composed of mangroves, forests and (Ferreira et al. 2019). The Atlantic Forest dominates the headwaters of the river, which descends the slope of Serra do Mar with great volume and force; its energy is dissipated after a sinuous flat course (Ferreira et al. 2019), and with lower speed, it increases the riverbed, forming swamps and mangroves (Fries et al. 2019).

### 2.3.2. Ethical Statement

The care and use of experimental animals complied with the laws, guidelines and animal welfare policies of the Ministry of the Environment (MMA) and the field collections as approved by the Authorization and Information System on Biodiversity and the Chico Mendes Institute for Conservation of Biodiversity (SISBIO/ICMBio) under number 59329-1 (available at ([www.icmbio.gov.br/sisbio](http://www.icmbio.gov.br/sisbio), authentication code: 92527898).

### 2.3.3. Sampling

Three species of catfish from the Guapi-Macacu River were selected, all of them presenting a similar distribution along three areas of the river (downstream, intermediate, and upstream). Two of them are native species – *R. quelen* and *T. striatulus* – and one is an invasive species – *C. gariepinus*. The invasive species is native to the African continent with a generalist feeding habit, being able to occupy any food niche; its closest ecological equivalent in Brazil is *R. quelen*, with which it has several ecological similarities (Dumith e Santos 2022). *Trachelyopterus striatulus* is also an omnivorous species (Chapman e Lowe-McConnell 1976) with high food adaptability in different environments.

The three fish species were collected in the Guapi-Macacu River at 32 sampling points: 10 points downstream, 12 points in an intermediate area, and 10 points in an upstream area, covering the entire river (Figure 10). Gillnets with different meshes (60m x 1.5m, with mesh sizes of 15, 30, and 45mm of adjacent nodes), cast nets (mesh size of 0.5cm), fyke nets (nylon traps with a height of 40cm and a diameter of 35cm), hooks, and lines were used. The fishing gear was set up and used in three replicates (one hour per replicate) at each sampling point,

installed and used throughout the day in two campaigns (February and August) of 2018, totalling 64 sampling points. At the same time, a YSI 6600 multiparameter probe was used to measure *in situ* the physicochemical variables of the water: temperature (°C); dissolved oxygen (mg/L), pH, turbidity (FNU), and pressure (mmHg). Transparency (cm) was measured using the Secchi disk. The variations in these abiotic factors are commonly related to water quality (Bordalo et al. 2001; Ndaruga et al. 2004; Mena-Rivera et al. 2017) and also can interfere with feeding choice, especially in exotic species used for commercial purposes (Santos et al. 2018). After collection, the fish were labelled, refrigerated on ice (still in the field), and transferred to the Laboratory of Applied Ecology, UFF.

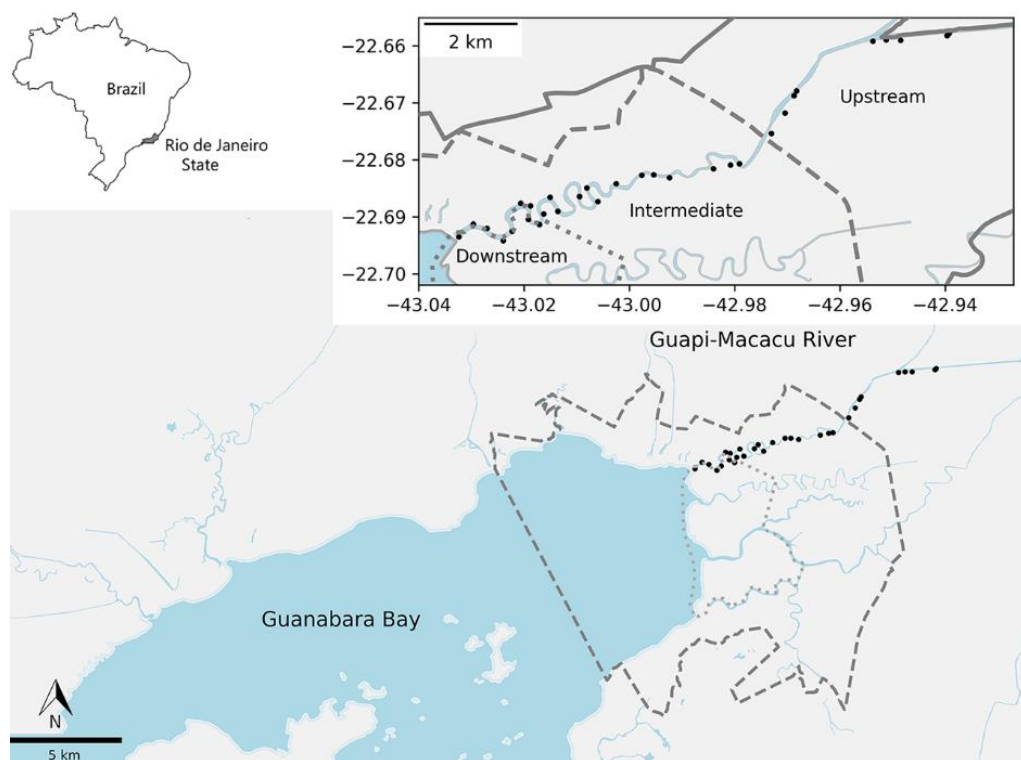


Figure 10. Map of Guanabara Bay with the representation of the boundaries of the Guapimirim Environmental Protection Area (dashed line) and the Guanabara Ecological Station (dotted line). The collection area is highlighted, with the sampling points, in the dry and rainy seasons, distributed along the Guapi-Macacu River.

#### 2.3.4. Laboratory Activities

All the collected fish were identified (Reis 2003; Figueiredo e Menezes 2015a; Froese e Pauly 2021), weighed, measured, and dissected at the Laboratory of Applied Ecology – UFF. Their stomachs were stored in jars with 10% formaldehyde, and their contents were later analysed through magnifying glasses and stereoscopic microscopes. The analysed items were grouped into categories to facilitate the classification of species into trophic guilds, identified

at the lowest possible taxonomic level (by specialists), and subsequently quantified in number (item count) and biomass (total weight of each food category), measured using a precision scale.

### **2.3.5. Data Processing and Statistical Analysis**

The Index of Relative Importance (IRI%) (Pinkas et al. 1971) was used to determine the food importance of the three species, for each food category. IRI is calculated based on the frequency of occurrence (FO%), numerical frequency (FN%), and weight (or biomass) (FW%), according to the following formula:  $IRI\% = (FN\% + FW\%) * FO\%$ . IRI% was calculated considering the IRI value for each food category divided by the sum of the IRI values of all categories multiplied by one hundred. The classification of the trophic guilds of the three species was determined by IRI values  $\geq 50\%$ , as proposed by Cruz *et al.* (2018), including the species in a guild. In this step, we made calculations with items classified into larger groups, with seven food categories, including Fish, Molluscs, Zooplankton, Zoobenthos, Decapoda, Detritus (terrestrial and aquatic plants, sand, mud, wood residues), and Digested Organic Matter (DOM).

As proposed by Zavala-Camim (1996), the food repletion index (RI), condition factor (K), niche width (Levins 1968), and trophic position (Vander Zanden e Rasmussen 1996; Vander Zanden et al. 1997; Fernando e Suárez 2021) were calculated for each individual, and these attributes are directly linked to the diet. The repletion index (RI) (Hyslop, 1980), or stomach fullness, was calculated about stomach biomass ( $S_w$ ) as a function of total fish biomass ( $W$ ), using the following formula:  $RI = (S_w / W) * 100$ . For the condition factor (K), Fulton's factor was used, referred to as the "K factor", which assumes that the isometric growth of the fish can be calculated as  $K = W/L^3$ , where K is Fulton's condition factor; 3 is the exponent indicating isometric growth; L is the total length and W is the total weight (Cren 1951). As Cren (1951) proposed, the total mass of the fish was used, which reflects the weight of gonads and other organs, indicating the condition of the fish. Fulton's K assumes that heavier fish of a given length are in better condition; therefore, this index can be used as an indicator of energy storage (Dibble e Meyerson 2012). Each of these indices estimated the nutritional status of individuals in different stretches of the river; the repletion index estimates the amount of food ingested on a short time scale and the condition factor represents an individual's nutritional status on a larger scale (Pereira et al. 2016). To assess the degree of specialization in resource exploitation, we calculated the niche width of the individual using the Standardized Levins' Index (Levins 1968; Hurlbert 1978), using the "niche.width" function from the "spaa" package (Zhang 2015). To

calculate the trophic position (TP) of the individual, the following formula was used:  $TP = \sum (V_i * T_i) + 1$ , where TP is the individual's trophic position;  $V_i$  is the relative volume (weight) of item  $i$ , and  $T_i$  is the trophic position of item  $i$  (Vander Zanden et al. 1997). In this way, the trophic position was quantified using a continuous variable that represents how many times the biomass consumed by an organism has been metabolically "processed" within the food chain (Vander Zanden et al. 1997). In addition, Permutational Multivariate Analysis of Variance (PERMANOVA) was applied univariately, available in the "vegan" package (Oksanen et al. 2022), to test whether the differences in the four calculated indices ( $\log_{10} x + 1$ ) were significant ( $p < 0,01$ ), among the three analysed species, with the Euclidian distance used in all PERMANOVA tests, the data being permuted 4,999 times in the analysis, as recommended by Kemp & Manly (1997). PERMANOVA has a similar robustness to traditional ANOVA. However, unlike traditional ANOVA, it does not require the assumptions of normality and homoscedasticity to be met (Anderson 2001; McArdle e Anderson 2001). To assess whether changes in the amount of food ingested are related to the condition factor, niche width, and trophic position or if they are related to other environmental changes, a Spearman's correlation was applied (indicated for variables without normal distribution) between the RI and other calculated indices. Correlation is a test to measure the degree to which one dataset varies with another without implying a cause-and-effect relationship (Dytham 2011). Although the fullness index of species provides an immediate picture of food intake before fish collection (Hyslop 1980), it is related to the amount of food ingested and the categories included in their diet, which serves as the basis for classifying the feeding specialization of various fish species (Amundsen e Sánchez-Hernández 2019). Furthermore, the other calculated indices also relate to the feeding and overall condition of the fish and, consequently, their population. Spearman's correlation was also used to infer (when necessary) relationships between the size of the fish and the calculated indices.

To evaluate the niche overlap between catfish species, a null model analysis with 1000 interactions was used, between the values of the biomass of the categories consumed by each species in the three stretches of the Guapi-Macacu River and in the two sampled periods. The null model tests co-occurrence patterns in food items to reveal whether species share (i.e., upper tail significant P-value) or partition (i.e., lower tail, significant P-value) resources (Gotelli et al. 2013). RA3 was chosen as the randomization algorithm, which retains the real niche breadth observed for the species evaluated in the simulated matrices. RA3 also reorganizes the zeros found in the original data matrix, simulating the use of resources different from those on which the evaluated species feed (Gotelli et al. 2015). The metric chosen for this test was the

Czechanowski Index, carried out in the “EcoSim” package (Gotelli et al. 2015) for (R Core Team 2022).

To analyse the distribution of food resources along the environmental gradient, we performed a non-metric multidimensional scaling (NMDS) for each individual with the biomass of the seven food categories ( $\log_{10} x + 1$ ). The Bray-Curtis coefficient was used, with the ordering of the abiotic variables measured in the different stretches of the river on the temporal scale, in the sampling points where the catfish specimens, the object of this study, were obtained. The “*envfit*” function was used, from the “vegan” package (Oksanen et al. 2022), which adjusts vectors or environmental factors in a vector order, where the projections of points in vectors have a maximum significant correlation ( $p < 0.05$ ) with the corresponding environmental variables, where each variable is analysed separately (Oksanen et al. 2022).

Finally, we applied Boosted Regression Trees (BRT) to the abiotic factors (independent variables) and the fullness index (dependent variable) of the sampled fish to detect which environmental changes are related to variations in the amount of food ingested by the three analysed catfish species. In the BRT analysis, we exclusively employed variables obtained through the multiparameter probe measurements. The transparency was excluded from consideration to mitigate potential issues related to multicollinearity within the model. Cross-validation was applied to address the independent and dependent data structure (Fabricius e De’ath 2008; Buston e Elith 2011). Following the procedure by Elith *et al.* (2008), the BRT model was fitted with a tree complexity of 10, a learning rate of 0.001, and a bag fraction of 0.75, using a Gaussian error distribution (for more details on these parameters, see Elith *et al.*, 2008). Although BRT models are complex and used for machine learning, they can also be summarized to provide a good ecological understanding, and their predictive performance is superior to more traditional modelling methods (Elith et al. 2008). Thus, the BRT models result in clear graphics and are suitable for studying ecological questions (Cappo et al. 2005; Fabricius e De’ath 2008). All analyses were performed using the R program version 4.0.2 (R Core Team 2022).

## 2.4. RESULTS

### 2.4.1. Analysis Of Diet and Nutritional Indices

All the sampling efforts resulted in the collection of 30 specimens of *C. gariepinus*, 11 specimens of *R. quelen*, and 18 of *T. striatulus* were obtained (Table 3). The greatest abundance

of the species occurred upstream of the river in August (dry period). The invasive species had the highest biomass and total length among the analysed species, followed by *R. quelen*, which had an average mm and biomass. The species *T. striatulus* presented the smallest total length and biomass among the catfish.

Table 3. Fish/stomachs, average weight (W), and minimum and maximum length (TL) were examined for the catfish in the periods (dry and wet), and the downstream (D), intermediate (I), and upstream (U) stretch of the Guapi-Macacu River.

		D	I	U	Weight (g) (Mean)	TL-Min (mm)	TL-Max (mm)
Dry	<i>Clarias gariepinus</i>	3	2	17	1509.143	345	830
	<i>Rhamdia quelen</i>	-	7	1	216.0857	140	290
	<i>Trachelyopterus striatulus</i>	5	-	4	143.1449	186	225
Wet	<i>Clarias gariepinus</i>	-	1	7	1489	430	810
	<i>Rhamdia quelen</i>	-	-	3	373.8433	119	375
	<i>Trachelyopterus striatulus</i>	-	-	9	137	154	203

Regarding diet, the Index of Relative Importance (IRI%) revealed the species' food importance (Figure 11). *Trachelyopterus striatulus* was the only catfish that showed food preference, included in the zoobenthos category. Among the most present, we find Trichoptera, Ephemeroptera, Plecoptera, Hemiptera, Coleoptera, Diptera, and Odonata, with a predominance of terrestrial insects. *Rhamdia quelen* was the catfish that presented the highest percentage of ingestion of fish from the Loricariidae family, in addition to annelids, Coleoptera, molluscs, and decapods. *Clarias gariepinus* was the catfish that presented the highest percentage of detritus in its stomach; with a greater percentage of partially digested organisms evident, except for molluscs.

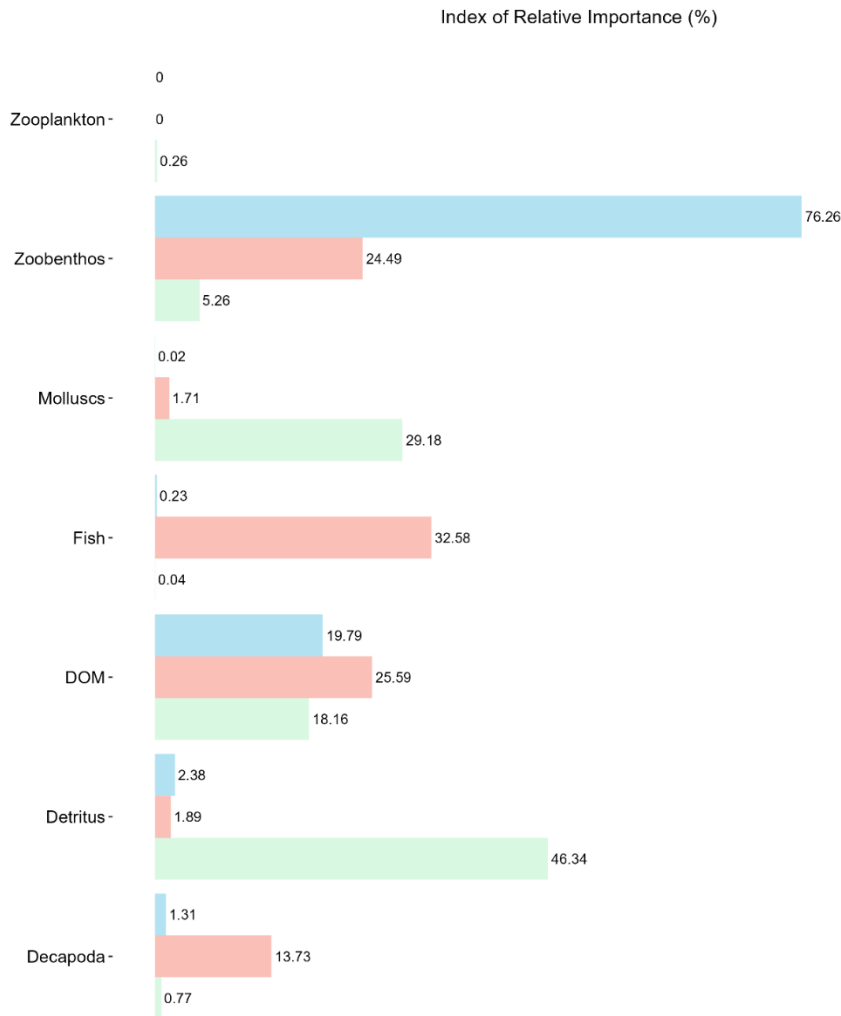


Figure 11. Food preference based on the Index of Relative Importance (IRI) of *Clarias gariepinus* (green), *Rhamdia quelen* (pink) and *Trachelyopterus striatulus* (blue) catfish in the Guapi-Macacu River. DOM = Digested Organic Matter.

PERMANOVA detected differences between the calculated indices for the three catfish species (Table 4). Niche width was the only index that did not show significant differences, where the three catfish species presented similar niche width variations (Figure 12c). Among the indices calculated for each species, *R. quelen* was the species that presented the best K, (Figure 12a). The highest RI values were found for *T. striatulus*, followed by *R. quelen*, which showed greater variability among catfish for RI (Figure 12b). The trophic position showed greater variability for *C. gariepinus* and was similar between *T. striatulus* and *R. quelen* (Figure 12d).

Table 4. PERMANOVA among of the calculated indices of Condition Factor (K), Repletion Index (RI), Niche Width and Trophic Position (TP) for the catfish in the Guapi-Macacu River, Brazil.

	R <sup>2</sup>	F	p-Value
Fulton Condition Factor	0.6691	53.584	<0.001*
Repletion Index (RI)	0.43789	20.644	<0.001*
Niche Width	0.01858	0.5017	0.6204
Trophic Position	0.16172	5.1123	0.006*

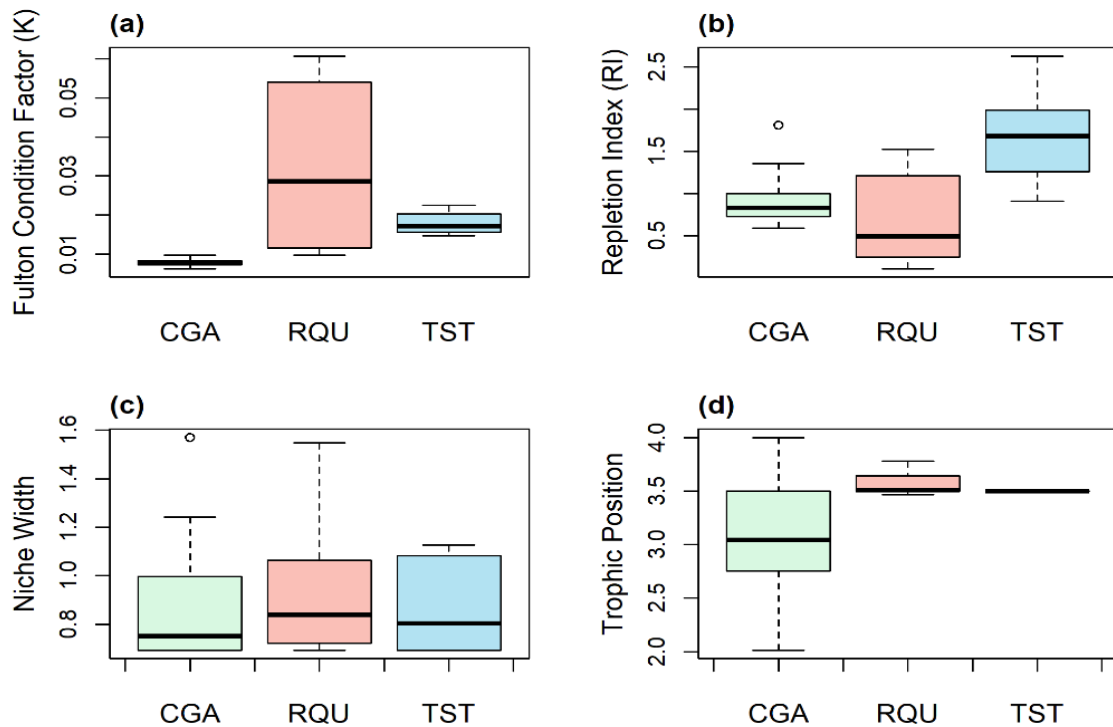


Figure 12. Boxplot with the distribution of the values of the calculated indices of Condition Factor (K), Repletion Index (RI), Niche Width and Trophic Position (TP) for the catfish: *Clarias gariepinus* (CGA), *Rhamdia quelen* (RQU) and *Trachelyopterus striatulus* (TST), in the Guapi-Macacu River.

Spearman's correlation detected relationships between RI x K, IR x TP, and RI x Niche width in the species analysed (Table 5). The condition factor (K) showed negative relationships with the amount of food ingested for *R. quelen*. *C. gariepinus* exhibited a positive relationship between RI and Trophic Position, with a low statistical significance. *T. striatulus* showed a positive relationship between the condition factor and the RI and a negative relationship between the trophic Niche Width and the amount of food ingested.

Table 5. Spearman correlation between the Repletion Index (RI) and the Condition Factor (K), Niche Width and Trophic Position indices for the catfish in the Guapi-Macacu River, Brazil.

	<i>Clarias Gariepinus</i>			<i>Trachelyopterus striatulus</i>			<i>Rhamdia quelen</i>		
	S	p-Value	rho	S	p-Value	rho	S	p-Value	rho
IR x Niche Width	3894.3	0.8335	0.040	1229.1	<b>0.03812*</b>	-0.506	259.29	0.08441	-0.571
IR x Trophic Position	2548.3	<b>0.0466*</b>	0.372	833.91	0.9334	-0.021	251.26	0.121	-0.522
IR x Fulton Condition Factor	5322	0.101	-0.310	284.67	<b>0.00463*</b>	0.651	284	<b>0.02419*</b>	-0.7212

The null model showed which items are partitioned by catfish in the different stretches of the Guapi-Macacu River (Figure 13) and in the dry and rainy seasons (Figure 14). The circles displayed in each figure represent the resource use for each species in a particular section of the river, as well as during different periods. In the model-simulated matrix for the upstream section, it can be observed that the circles of each species do not overlap for the majority of the prey items, except Decapoda. A similar result is found for the Zoobenthos category if there is no differentiation between the dry and rainy periods, yet the overlap is still minimal. Niche overlap between catfish species in the river stretches, where the Observed Index (0.035) was lower than the value obtained from the simulated matrices (Simulated Index = 0.043), revealed that catfishes tend to partition resources (P value of the lower tail = 0.02). We obtained a similar result in the null model for the periods (Figure 14), where the Observed Index (0.029) was lower than the value obtained from the simulated matrices (Simulated Index = 0.052), revealing that catfish tend to partition resources (P value of the lower tail = 0.02).

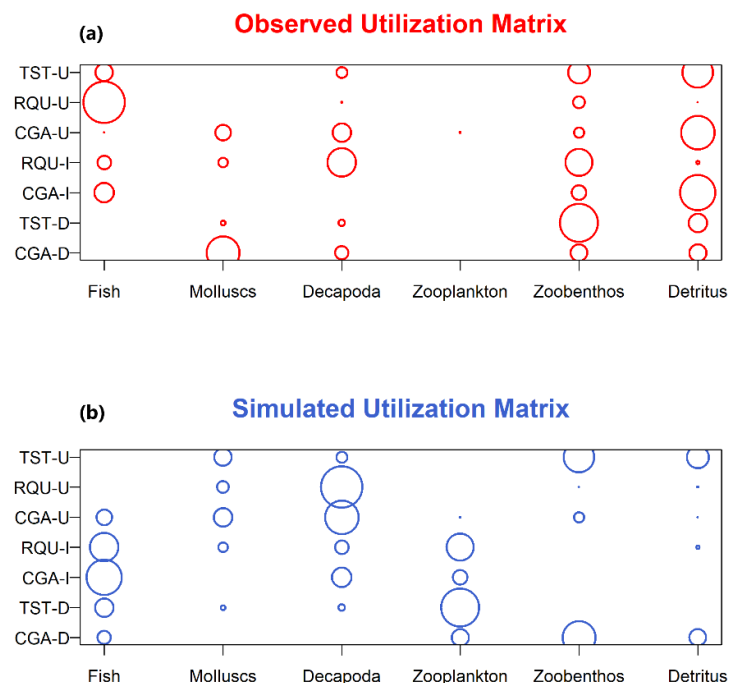


Figure 13. Niche overlap for observed (a) and simulated (b) matrices of trophic items consumed by *Clarias gariepinus* (CGA), *Rhamdia quelen* (RQU) and *Trachelyopterus striatulus* (TST) in the Guapi-Macacu River areas (Brazil): U- Upstream, I - Intermediate, and D- downstream. The area of each represented circle is proportional to the use of a specific item for each species.

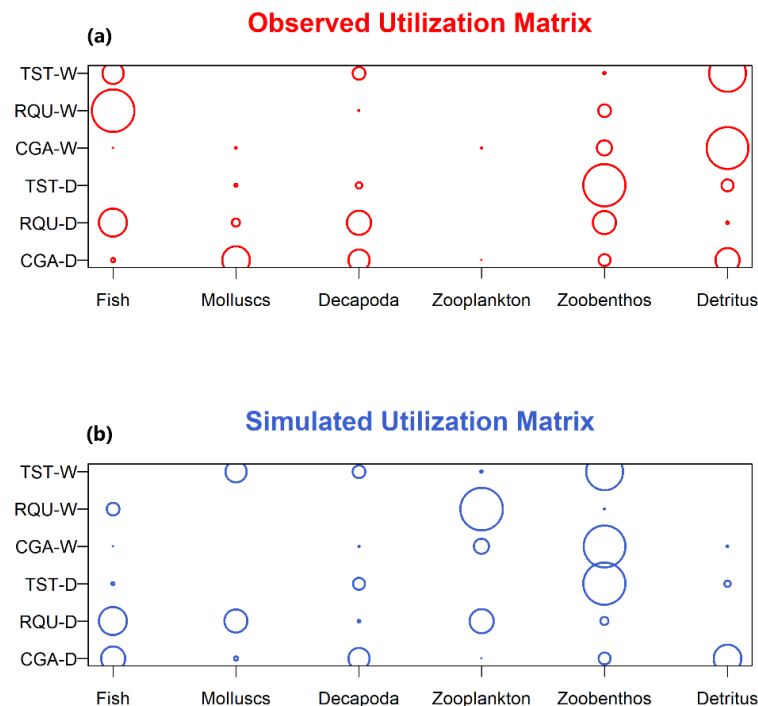


Figure 14. Niche overlap for observed (a) and simulated (b) matrices of trophic items consumed by *Clarias gariepinus* (CGA), *Rhamdia quelen* (RQU) and *Trachelyopterus striatulus* (TST) in the dry (D) and rainy (W) seasons in the Guapi-Macacu River, Brazil. The area of each represented circle is proportional to the use of a specific item for each species.

#### 2.4.2. Influence of Abiotic Variables

The variation between the samples of the abiotic variables was more evident between the periods than the stretches following the times of flood and drought of the river. The mean values ( $\pm$ standard deviation) of the environmental variables at the different points where the catfish specimens were obtained in the Guapi-Macacu River are shown in Table 6. The pH was higher in the dry period downstream. The temperature exhibited higher averages in the rainy season, probably due to the summer in the southern hemisphere. Dissolved oxygen presented higher values in the dry period, mainly upstream of the river. Transparency showed higher averages in the dry period. Pressure and turbidity exhibited similar behaviour, except for the dry period in the area upstream of the river, where turbidity presented the lowest values and pressure had the highest values. Turbidity showed higher values in the intermediate stretch in the rainy season.

Table 6. In the dry and wet periods, the mean and standard deviation of abiotic variables, pH, temperature, dissolved oxygen, transparency, pressure, and turbidity in the three areas of the Guapi-Macacu River, Brazil.

	pH		Temp (°C)		DO (mg/L)		Transp (cm)		Pres (mmHg)		Turb (FNU)	
Dry												
Downstream	7.2	±0.8	23.0	±0.5	5.1	±0.6	44.2	±1.7	757.8	±0.7	24.5	±3.5
Intermediate	6.4	±0.2	22.3	±0.6	4.9	±0.5	50.6	±2.6	757.9	±1.4	22.4	±3.6
Upstream	6.5	±0.5	22.6	±0.1	6.5	±1.0	56.9	±1.2	764.6	±3.3	12.7	±1.2
Wet												
Downstream	5.3	±0.4	25.5	±0.1	3.9	±0.4	16.1	±3.0	757.6	±0.5	216.2	±77.2
Intermediate	5.5	±0.1	25.3	±0.2	4.6	±0.9	35.2	±3.3	755.5	±0.3	150.6	±43.4
Upstream	5.6	±0.2	25.2	±1.2	4.6	±0.7	25.4	±2.5	729.5	±86.3	30.8	±6.2

The distribution of food categories about abiotic variables showed distinct patterns among the three species (Figure 15). On the first axis of the NMDS, *T. striatulus* and *R. quelen* are distributed more to the left of the graph, partitioning the zoobenthos category, and *C. garipepinus* is more to the right of the graph, utilizing items distributed in the categories of molluscs, zooplankton, and detritus. In the NMDS analysis, it is evident that DOM (Dissolved Organic Matter) is correlated with all three species. Due to the similarity in species distribution, they shared environments that exhibited similar variations in physicochemical factors. Therefore, there was only an indirect influence of these variables on the items selected by the catfish, as detected by the NMDS, which is related to the axes of the model. The higher samples of pH, transparency, and dissolved oxygen were directly correlated with the first axis of the NMDS, mainly associated with the mollusc category. Temperature was negatively associated with the second axis, being related to DOM samples. The differences detected by the model for each abiotic variable are shown in Table 7.

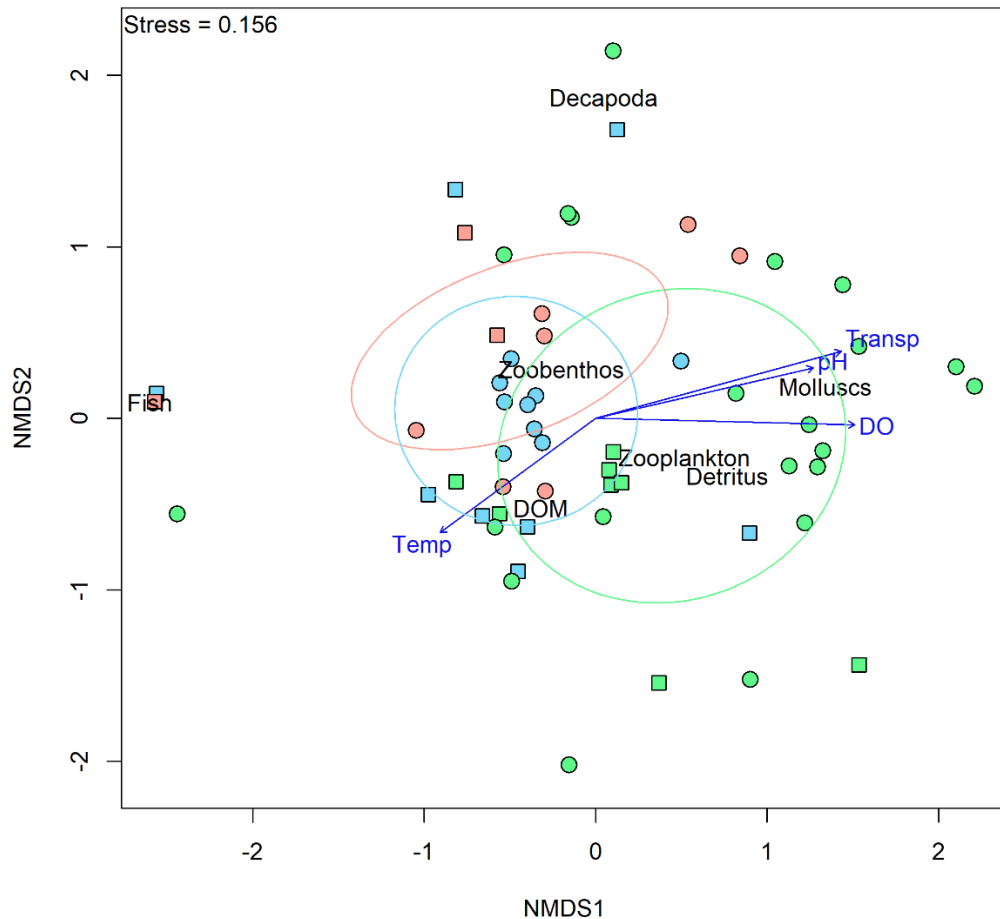


Figure 15. NMDS of the dietary composition of *Clarias gariepinus* (green), *Rhamdia quelen* (red) and *Trachelyopterus striatulus* (blue) and vectors of significant variables were obtained in the environmental model (see Table 7), with a representation of the dry season (square) and the rainy season (circle).

Table 7. Environmental factors adjustment: dissolved oxygen (DO), pH, temperature (Temp), transparency (Transp), turbidity (Turb) and pressure (Pres), at the collection points of each catfish specimen in the Guapi-Macacu River, Brazil.

	NMDS1	NMDS2	r <sup>2</sup>	Pr(>r)
Temp	-0.76819	-0.64023	0.1236	0.033
pH	0.94894	0.31546	0.1483	0.011
Turb	-0.99968	-0.02549	0.0403	0.371
DO	0.99491	0.10081	0.2040	0.001
Transp	0.96193	0.27330	0.1877	0.005
Pres	0.79042	0.61257	0.0207	0.520

The BRT model produced a satisfactory overall fit to the data, as indicated by the total mean deviation (6.344) and the mean residual deviation (4.069), demonstrating an ability to explain 35.85% of the total variability in the environmental gradient. This suggests that other variables or factors not considered in the model may be contributing to the remaining variation in the fullness index. The cross-validation (4.942±1.205) reasonably verified the generalization

of the data not used in the training, indicating that the model has some ability to make accurate predictions on new data sets. The variables of greatest importance in predicting the stomach fullness index (dependent variable) were identified as turbidity and pH, highlighting the significance of these factors for the food intake of the analysed catfish. Figure 16 (a and b) shows the fit of the Boosted Regression Tree (BRT) model for the abiotic variables, illustrating the relative influence of turbidity (58.28%), pH (22.84%), temperature (9.68%), dissolved oxygen (5.63%) and pressure (3.55%) on the variations in the fullness index of the collected catfish. However, it is essential to consider other possible influential factors, as enhancing the model can lead to better explanations of the investigated data.

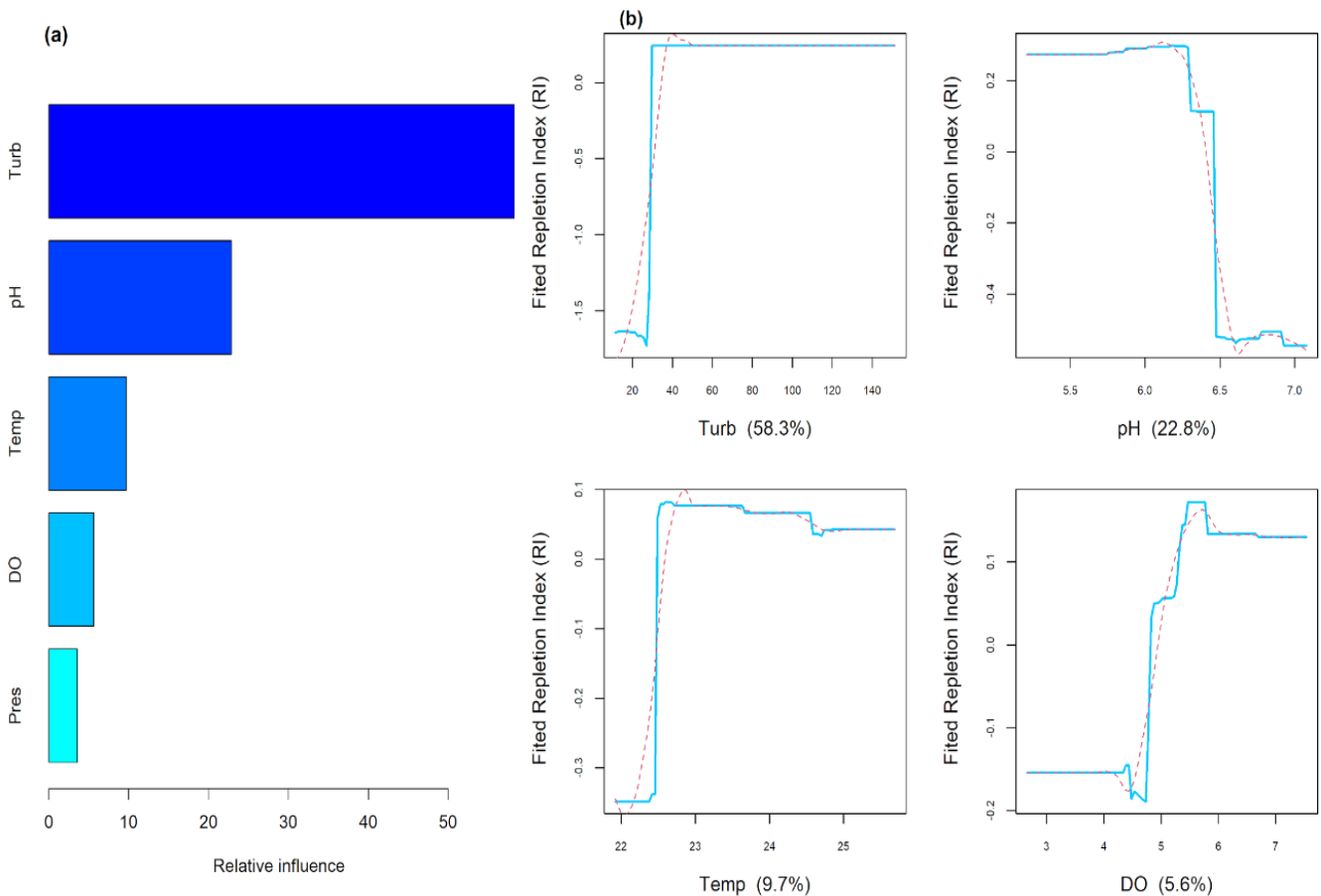


Figure 16. (a) Relative importance indicates the percentage contributions of predictors turbidity (Turb), pH, temperature (Temp), dissolved oxygen (DO) and pressure (Press) to predict variations in the repletion index (RI) for *Clarias gariepinus*, *Rhamdia quelen*, and *Trachelyopterus striatulus* using the BRT model. (b) Fitted functions centred by subtracting the mean (standard BRT) for each term in the model with main effects, ordered by the value of relative influence. Dashed red lines indicate 95% confidence intervals for the values estimated from predictions made from 1000 fitted models (blue line) for bootstrap samples of fish data.

## 2.5. DISCUSSION

Despite the similar distribution within the Guapi-Macacu River, among the three species of catfish, the native species showed low abundance for an area intended for environmental preservation. For example, in the Guandu River Basin (Rio de Janeiro - Brazil), *T. striatulus* exhibits higher abundance (Costa et al. 2013), and the same is observed for *R. quelen*, which shows greater abundance in natural environments (Araújo e Tejerina-Garro 2007; Fialho et al. 2008). The location of the Guapimirim APA, close to Guanabara Bay, must have contributed to this result, as it is a highly degraded ecosystem (Meniconi 2007). Therefore, omnivorous species, which can consume various resources from the environment, may struggle to obtain the necessary quantity of resources to sustain a larger population (Chase et al. 2002). According to Hooper *et al.* (2005), changes resulting from human activities lead to changes in the biotic structure and composition of ecological communities, with the reduction of species or the introduction of exotic species, which partly explains the more significant occurrence of the African catfish. The presence of the exotic species may also have contributed to the reduction in the abundance of the native species (Latini e Petrerre 2004; Moyle et al. 2007; Rayner et al. 2015), including its closest counterpart in this river, the *R. quelen*. Even if the species do not compete for food directly, this does not rule out the possibility that the exotic species may have competed for food when younger than the other catfish. However, the APA de Guapimirim does not have data before the invasion of the African catfish for comparison purposes. The anthropogenically impacted environment may also be related to the size and weight of the species, which are relatively smaller than those found in other settings (Albieri et al. 2014; Froese e Pauly 2021), probably related to the fact that eutrophication can affect species in terms of biomass variation, positively or negatively favouring some fish species (de Mutsert et al. 2016). However, even with the reduced number of captures, we were able to estimate the diet composition of the analysed species, compatible with those described in the literature.

The composition of *R. quelen*'s food items and categories indicates that the species is omnivorous (Gomes et al. 2000; Gomiero et al. 2007; Kütter 2009; Brandão-Gonçalves e Sebastien 2013) although tending towards carnivory (Gomes et al. 2000; Santos e Meurer 2020). The species' intestinal tract is similar to that of carnivores (Baldisserotto 2008), and the adaptations of its mouth apparatus allow it to select prey from the bottom of the river, and crush shells (Kütter 2009), justifying the higher percentage of fish in the stomachs. Even with a lower percentage of molluscs, insects, and decapods as recorded by Casatti & Castro (2006), these were still present in the diet of the species, showing the species' omnivory (Pedrotti et al. 2015).

*Trachelyopterus striatulus* is also considered omnivorous, encompassing zoobenthos, microcrustaceans, and plants (Silveira et al. 2013). The food preference found for the species in the present study is supported by the findings of Dias *et al.* (2005) and dos Santos *et al.* (2009), who identified insectivorous habits in lotic systems in the Southeast region. The presence of a higher percentage of terrestrial – and even some aquatic – insects may be related to the smaller size of the mouth opening, suggesting an adaptation to ingest smaller foods (dos Santos et al. 2015). However, their digestive tract is also adapted to carnivory (dos Santos et al. 2015), like *R. quelen* (Hernández et al. 2009), but a significant percentage of fish, like *R. quelen*, is not observed.

The analysis of the diet of the invasive species demonstrates that the African catfish can adapt to different environments. The high percentage of items such as detritus, mud, and decomposing materials confirms what was expected of the species (Ayinla 1988; Adewumi et al. 2014). Several studies have characterized the diet of *C. gariepinus* as a diet with a broad food spectrum (omnivore with a carnivorous tendency) for this species in natural environments (Bruton 1979a; Spataru et al. 1987; Mili e Teixeira 2006). The other diet items found in the stomachs of the African catfish demonstrated its generalist food strategy. This pattern was observed by Winemiller & Kelso-Winemiller (1996) when studying African catfish in their natural habitats, such as the Upper Zambezi River floodplain or Lake Sibaya in South Africa.

Although the ecological indices calculated individually for each species show better relationships when they are related to larger samples (Harley et al. 2001; Roset et al. 2007), our results seem to reflect well the feeding patterns for the three species. The best condition factor of *R. quelen* is related to the quality of food ingested, such as fish, which has a higher protein level compared to the other categories (Santos e Meurer 2020). In addition, this species' condition factor may have been directly favoured by temperature for digestion and better absorption of nutrients (Lermen et al. 2004), which is evident in Spearman's correlation, where *R. quelen* shows a negative relationship between RI and K, tending to absorb and metabolize food more quickly. The *T. striatulus* shows a negative relationship between the amount of food ingested and its niche width and a positive relationship between the RI and the physiological condition, of which these factors are directly related to its diet in the river. Furthermore, the small morphological differences in the digestive apparatus (e.g., mouth size and intestine length) of the three species of Siluriformes (Hernández et al. 2009; Ikpegbu e Nlebedum 2014; dos Santos et al. 2015) may have influenced the ecological indices reported here for these species.

With greater size and weight, the low condition factor of *C. gariiepinus* comes to attention. For Green (2001), body condition is determined by the ratio of the relative size of energy reserves, such as carbohydrates, proteins, and lipids, compared to structural components of the body that do not represent energy reserves, such as bones, cartilage, and scales (Camara et al. 2011). This result seems to be directly linked to a less-protein diet, with a lower percentage of insects, decapods, and fish. Lall & Tibbetts (2009) claim that protein is an essential component in the fish's diet to ensure proper growth and health. Another factor that may be contributing to this result is the already impacted environment (Getso et al. 2017), indicating that adverse environmental conditions in Guanabara Bay can negatively interfere with the condition factor, in addition to the reproductive period (Elliott e Bagenal 1979; Froese 2006), which was not evaluated in the present study.

Niche overlap between species was not confirmed when analysing the null model, which revealed low niche overlap, clearly indicating resource partitioning in different sections of the river and during the sampled periods. This indicates that species do not directly compete for resources; although dietary traits may create a false impression of intense competition for resources, the overlapping diets reveal the division (Albouy et al. 2011). However, Corrêa *et al.* (2011) suggested that null models can only indicate in which direction the observed patterns are, and additional data on resource availability and species interactions can provide more accurate answers regarding niche overlap and resource status (Maitra et al. 2020). There are many conditions inherent to the pattern shown here, but generally, the items were used in greater quantity or frequency (Bonato e Fialho 2014) in different ways between species. This result can also be attributed to other factors, such as differences in the size of individuals (Schoener 1968; Schückel et al. 2012; Garcia et al. 2018), the potential variation in diets caused by differences in foraging patterns, and other behavioural factors, which were not tested in the present study (Alexander et al. 1995; Davenport 1997; Albouy et al. 2011). Thus, the partition of resources between species indicates low competition, but it may have occurred at different stages of their life cycles. A similar result was found in a study conducted in the Intahém River Estuary - Bahia (Brazil), where the invasive species – *C. gariiepinus* – does not compete for resources with *Centropomus spp.* (Rabelo e Soares 2014).

Even though the species have similar morphological apparatus and omnivorous feeding habits (Gomes et al. 2000; Gamal et al. 2012; dos Santos et al. 2015), the catfish showed different ranges of trophic levels. This is due to the use of resources that vary in the quantity and quality of nutrients, directly influenced by the ecosystem they inhabit (Sánchez-Hernández

e Amundsen 2018). Variability in trophic position is also related to highly flexible and opportunistic feeding (Dill 1983), as is variation in the trophic position of ingested prey (Vander Zanden et al. 1997). This fact explains the variability of the trophic level of the *T. striatulus* which consumes more insects, related to foods with higher levels of phosphorus and nitrogen (Jardine 2016). On the other hand, *C. gariiepinus*, which consumes items with higher carbon content, such as vegetables and algae (Fernando e Suárez 2021), in addition to insects and fish, exhibits greater variability in trophic position, covering several levels.

Similarly, *R. quelen* exhibits a high frequency of items such as fish and insects and shows a trophic level slightly higher than *T. striatulus* and lower than *C. gariiepinus*. This may be correlated with the lower abundance of the population compared to the other species. In addition, the smaller volume of items for calculating its trophic position may also be related to the fact that *R. quelen* has greater digestibility and absorption of more protein foods (Santos e Meurer 2020). Due to these factors, the variability and unpredictability in the trophic position between species indicate that knowledge of the trophic position of a given population is not determinant or permanent for other populations of the same species, in different environments (Vander Zanden et al. 1997), varying according to the availability of high-quality resources.

The relationship between consumer size and trophic position, found for each species, is another factor that must be considered (Figure 17). Jardine (2016) notices that body size would lead to greater dependence of consumer biomass on allochthonous material in freshwater. This allochthonous material derives mainly from terrestrial plants and carbon-rich detritus (Hecky et al. 1993), and is found mostly in the diet of *C. gariiepinus*. Thus, it is likely that this species had a more predatory feeding habit in its juvenile phase, with lower biomass and body size. This also explains, in part, the feeding strategy identified for *T. striatulus* and *R. quelen*, with smaller sizes and lower percentages of detritus, both not showing a lower trophic position. In this aspect, the temperature must also have contributed to the food preference in detritus and plant material, and thus a reduction in trophic position (Dantas et al. 2019). For these authors, in warmer climates, the restrictions imposed by changes in the organism's physiology (Boersma et al. 2016) could impose an increase in metabolic demand, promoting greater carbon demands (Schmitz 2013), and thus increasing the consumption of plants and detritus by *C. gariiepinus*. Conversely, in the opposite manner, temperature may have exerted a lesser influence on the food choices of native species (which are smaller and lighter), making them less affected by high temperatures. This favours the ingestion of more protein-rich foods, contributing to their higher trophic position (Ou et al. 2017).

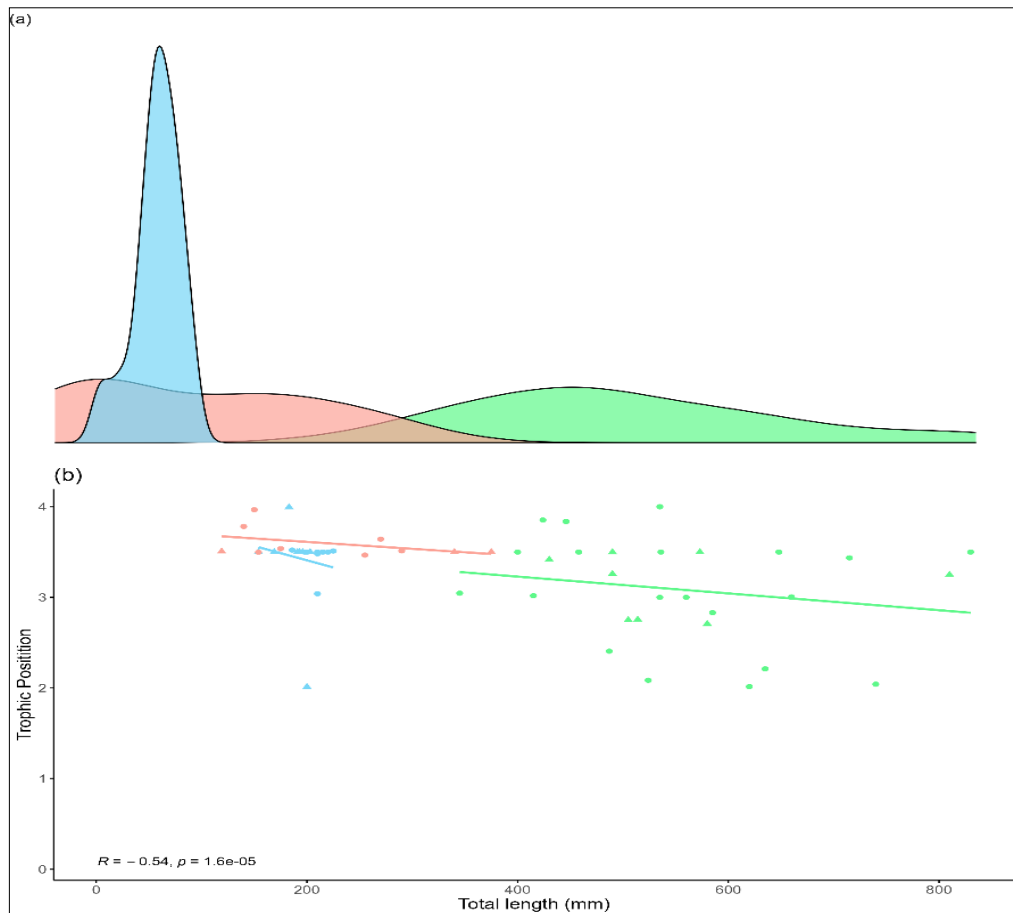


Figure 17. (a) Density of total length variation; (b) Spearman's correlations between trophic position (TP) and total length (TL) of catfish: *Clarias gariepinus* (green), *Rhamdia quelen* (red) and *Trachelyopterus striatulus* (blue) in the Guapi-Macacu River, Brazil.

As well as the trophic position, the index related to stomach fullness (RI) presented a significant variability among the analysed species. For Esposito *et al.* (2010), the capture of prey is related to predator satiation, probability of prey capture, and abundance of prey, in addition to search efficiency, while energy costs depend on the activity in which it occurs. For these authors, the capture decision relates local and global information about the net energy gain and the capture frequency. In this sense, we observed that *R. quelen* presented better energetic use in capturing prey, corroborating with the best condition factor presented. In contrast, *T. striatulus* seems to have a higher energy expenditure for prey capture, needing to maintain the frequency of food intake. On the other hand, the invasive species might have its RI affected by variations in size, age, sex (Adewumi *et al.* 2014), and feeding time (Ugwumba 1993; Adewumi *et al.* 2014). Additionally, the RI of the invasive species could be related to its foraging behaviour. When *C. gariepinus* assesses the environment suitable for its feeding through its barbels, which function as gustatory senses (Mukai *et al.* 2008), it exhibits suction time and flows through the mouth (proportional to cup size) (Van Wassenbergh *et al.* 2007), ingesting available food in the environment (Groenewald 1964; Drenner *et al.* 1978). However,

even with low energy expenditure for prey capture, *C. gariepinus* showed a low condition factor, which could be related to the quality of ingested food or other environmental factors such as temperature (Jamabo et al. 2015) and water level (Tesfahun 2018).

The absence of significant differences pointed out by the PERMANOVA regarding the width of the feeding niche confirmed the omnivorous nature of the three species, clearly indicating their potential to be highly plastic in response to different biotic and abiotic contexts (Dominguez Almela et al. 2021). The dietary niche width of a fish species can be an important factor in structuring aquatic communities, resource competition, and ecosystem functioning (MacArthur e Levins 1967). The similar and wide niche width indicates that these fish can consume a diverse range of resources within the system and, as these species diversify their diet, it can lead to prey depletion (Svanbäck e Bolnick 2007), resulting in trophic generalization (Dominguez Almela et al. 2021). Although the lack of niche overlap may suggest that the environment provides sufficient resources available for these species, resource partitioning can also reflect constraints on interspecific competition (Giller 1984). As our results indicate, these factors can contribute to slower energy intake (as observed in the invasive species) and lower abundance of native species (Chase et al. 2002), limiting the number of individuals that can coexist stably in the environment (MacArthur e Levins 1967; Giller 1984). However, environmental changes, such as alterations in food availability or the increase the invasive species, can affect the dietary niche width and disrupt niche partitioning (Wang et al. 2021).

The difference between the species' RI, related to their feeding habits, favoured the BRT model, demonstrating the influence of abiotic variables on food intake. In addition, the analysis of the abiotic variables denotes the characteristic environmental differences in the three stretches of the river and those related to the dry and rainy seasons (as seen in Table 4). However, these do not seem to directly influence these species' selection of resources. However, some of these factors, stand out for the influence they exert, including the turbidity, pH and temperature.

The BRT model exhibited a higher relative contribution to turbidity (58.28%). Turbidity is a factor that contributes to the protection of juveniles; on the other hand, it also serves as a facilitator for predators, allowing them to hide in high turbidity, and facilitating food intake (Ward e Vaage 2019), as indicated by our results. In addition to its connection with the catfish diet (Tesfahun 2018), turbidity is typically associated with the water's nutrient content (Damar et al. 2020). It is closely linked to algae proliferation in water bodies (O'Neil et al. 2012; Warren et al. 2021) and water quality, potentially indicating risks to fish health (Warren et al. 2021).

In this sense, the high turbidity that favoured catfish predation might also be linked to poor water quality. The BRT model also showed a relative contribution of pH (21.4%), with the larger samples of the downstream area of the river (more protected area). The pH variations from acidic to basic and vice versa have lethal consequences for aquatic life (Mushtaq et al. 2020), altering the availability of nutrients. For Lowe-McConnell (1999), rivers bordered by native vegetation provide a great variety of food items, mainly insects and higher plants. These nutrient sources, related to autochthonous resources, tend to increase pH (Mushtaq et al. 2020). Along with the higher nutrient input in this downstream section of the river, the seasonal factor during the dry period might have also contributed to the increase in pH. This is related to the fact that this area experiences the influence of salinity from the adjacent Guanabara Bay during the dry season (de Melo et al. 2014). It's also likely that the nutrients in the water during the dry period are related to pollutants (resulting from the same influx that increases salinity in the area), consequently raising the pH (Ogbonna 2014), negatively impacting the RI of these catfish.

In addition to these factors, the BRT model showed the influence of temperature on food intake. Temperature influences the metabolic rate in fish, directly affecting food consumption and the digestive process (Smith 1989). It also affects feeding rates, hydrolytic activity of digestive enzymes, and intestinal absorption rates (Silva e Araújo-Lima 2003), agreeing with the analysis shown by the NMDS, where the diet of the three species is directly correlated with temperature. Generally, the stomach fullness was favoured by the maximum values of this abiotic variable. In general, higher fullness indices are associated with higher temperature values, starting from 23°C and tending to decrease as the temperature rises, which are related to the catfish species.

Although the relative contribution of dissolved oxygen was low (5.63%) in food selection to BRT, it is another factor worth highlighting in our analyses, mainly because it is an intrinsic element of aquatic life. Despite having correlations with the distribution of some resources, such as molluscs for *C. gariepinus*, it does not seem to be correlated with other food categories of native species, according to the NMDS. Instead, this may be correlated to the fact that catfish are resistant to variations in dissolved oxygen concentrations in water (Adewolu et al. 2008; Montanha et al. 2011; Mattos et al. 2014), in addition to the fact that the highest dissolved oxygen samples are associated with greater transparency samples, making prey selection difficult (Tsunoda e Mitsuo 2018), thereby reducing the repletion index.

## 2.6. CONCLUSION

Our findings helped shed light on the trophic ecology of native and invasive species, as well as the abiotic mechanisms that facilitate or impact the development of these species. Our discoveries propose a potential pressure from the invasive species, which, being more robust, can occupy trophic niches of native species and likely intensify the invasion in adjacent rivers and throughout the Environmental Protection Area. In a broader sense, our results suggest that competition for ecosystem food resources might have occurred in the juvenile phase of the exotic species, acting synergistically in resource competition with native species, although this might have been mitigated by differentiation in dietary needs as they matured into the adult phase. Our findings also indicate that the omnivorous catfish species in the Guapi-Macacu River are physiologically well-adapted, likely due to their omnivorous nature, maintaining trophic ecology-related indices within expected ranges. On the other hand, key abiotic factors related to water quality are somehow linked to the stomach fullness of these catfish, which are relatively affected by variations in these factors. In this regard, environmental pressure resulting from environmental impacts related to Guanabara Bay was implicitly observed in the low abundance and body size of native species. However, *C. gariepinus*, despite its low body condition, maintained a more numerous and robust population, possibly favoured by the river's environmental conditions. Thus, we emphasize the urgent need to manage the spread and potential impacts of the invader *C. gariepinus* in the rivers and channels that comprise the Environmental Protection Area.

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### 3. CAPÍTULO 3

## **Genetic origin and morphometric traits of invasive African catfish (*Clarias gariepinus*) in a protected area in southeastern Brazil**

### **3.1. ABSTRACT**

The African catfish *Clarias gariepinus* is a freshwater species native to nearly all of Africa and a small part of Asia. Due to being a generalist species, highly fertile and able to survive in a wide range of environmental conditions, the African catfish has become invasive in many non-native systems where it was introduced, bringing negative impacts to wild ecosystems. In Brazil, *C. gariepinus* was introduced from Africa for aquaculture in 1986 and established itself in the wild within a decade, after escaping from aquaculture ponds and being further translocated to be used in recreational fishing. As further evidence of the expansion of this invasive species in Brazil, we sequenced the mitochondrial control region and cytochrome b markers to confirm the occurrence of *C. gariepinus* in a major river in a protected area in the southeastern region, and to assess its genetic diversity. Morphometric data was also collected to aid species identification and to compare to other invasive populations in Brazil. The analysis of 24 specimens revealed a single haplotype for each marker. The single cytochrome b haplotype is identical to a haplotype from the Democratic Republic of the Congo, unveiling the possible genetic origin of the *C. gariepinus* lineage that invaded Brazil. Morphometric data suggests consistency in the morphometric traits of *C. gariepinus* invasive populations in Brazil. The assessment and monitoring of the distribution of *C. gariepinus* in non-native areas are crucial for the maintenance of native freshwater biodiversity and ecosystem functioning worldwide.

**Keywords** Invasive fish, Clariidae, Morphometry, Mitochondrial DNA, Neotropical

### **3.2. INTRODUCTION**

Catfishes of the genus *Clarias* Scopoli 1777 (Siluroidei, Clariidae) are freshwater species with a wide geographical distribution. Native throughout the African continent and in a small part of Southeast Asia, it is one of the most widespread catfish genera in the world (Teugels 1996). *Clarias* species are fast-growing, omnivorous, resistant to diseases, withstand a wide range of salinity and pH, and tolerate hypoxic environments due to the suprabranchial organ that allows them to breathe atmospheric oxygen (Huisman and Richter 1987; Na-Nakorn

and Brummett 2009; Belão et al. 2011). These characteristics made them of great interest to the aquaculture industry, which resulted in their cultivation in many countries both inside and outside their native range (Na-Nakorn and Brummett 2009).

The African catfish *Clarias gariepinus* Burchell 1822 is the second most economically important freshwater fish species in Africa and has already been introduced for aquaculture in at least 37 countries on almost all continents (Barreras and Zapata 2019; Elberri et al. 2020). Some of the most productive countries are Nigeria, Cameroon, Kenya, the Netherlands, and Hungary (Dauda et al. 2018). In its native range, *C. gariepinus* can be found in a wide variety of environments, being adapted to lentic and lotic ecosystems, such as lakes, dams, rivers, floodplains, and estuaries (Weyl et al. 2016). As a result of being widely introduced for aquaculture and adapted to various environments, escapees have settled in many non-native systems, causing negative impacts on native communities (Kadye and Booth 2012; Khan et al. 2021). It should come as no surprise that impacts of *C. gariepinus* invasions have already been recorded, as they feed more frequently, exhibit faster growth rates, and reach larger sizes than *Clarias batrachus* (Low et al. 2022), a similar congeneric recognized as one of the 100 worst invasive species in the world (Lowe et al. 2000). Alarming, native populations of *C. batrachus* are being threatened by introduced populations of *C. gariepinus* in Singapore (Low et al. 2022). Therefore, the assessment and monitoring of the distribution of *C. gariepinus* in non-native areas are crucial for the maintenance of native freshwater biodiversity and ecosystem functioning worldwide.

According to the Food and Agriculture Organization of the United Nations (FAO) database, *C. gariepinus* was introduced to Brazil in 1986 for aquaculture purposes (FAO 2023a). Within a decade after its introduction to Brazil, *C. gariepinus* became established in the wild after escaping from aquaculture ponds and being further translocated to be used in recreational fisheries (Cambray 2005; Weyl et al. 2016). Since then, *C. gariepinus* has spread rapidly to multiple watersheds, mainly in the south and southeast regions, where it is classified as fully invasive (Weyl et al. 2016). Although there are no direct assessments of the impacts of *C. gariepinus* in non-native environments in Brazil, there is anecdotal evidence that it is already negatively impacting the abundance of native fish species in freshwater systems where it has been introduced (Vitule et al. 2006). The expansion of this invasive species could be a threat to Brazilian freshwater ecosystems, which harbor some of the most biodiverse ecosystems in the world (Abell et al. 2008).

Freshwater ecosystems in southeastern Brazil are composed of small, highly isolated coastal systems and exhibit a large number of endemic species (Abilhoa and Bastos 2009;

Nogueira et al. 2010). The Guapi-Macacu River, located in the Guapimirim Environmental Protection Area, close to the Guana- bara Bay, is home to several freshwater and marine species, which use this area for refuge and reproduction (Dumith and Santos 2022). In order to confirm the invasion of *C. gariepinus* in the Guapi-Macacu River, we analyzed two mitochondrial loci, allowing for the correct identification of this invasive species, as well as aiding the reconstruction of their invasion history in Brazil. In addition, morphometric traits were analyzed, to aid in species identification and examine ecomorphological hypotheses.

### **3.3. MATERIALS AND METHODS**

#### ***3.3.1. Ethical Statement***

Sampling was previously authorized by the System of Authorization and Information in Biodiversity - SIS- BIO, in accordance with Brazilian environmental protection legislation (SISBIO number 59329-1).

#### ***3.3.2. Study Site and Sampling***

The Guapi-Macacu River, located in the southeastern region of Brazil, plays a crucial role in maintaining the ecological balance of the Guanabara Bay, Rio de Janeiro. As part of the Guapimirim Environmental Protection Area (Guapimirim EPA), it represents a key refuge for the remaining fragments of the vital mangrove ecosystem. Recent research has highlighted the river's substantial drainage basin and its characteristic low average depth, which contribute to the unique ecological features of the region (Dias Gonçalves et al. 2019). This dynamic river system exhibits a diverse range of landscapes, serving as a transitional zone from the coastal lowlands to the elevated plateau (Cabral and Fiszon 2004). However, despite its protected status, the Guapi-Macacu River has been subject to substantial anthropogenic pressures stemming from the neighboring metropolitan region of Rio de Janeiro state. These human activities have severely compromised the water quality within the river (Pereira et al. 2016).

We sampled 32 points established along the entire river (Figure 18) in February and again in August of 2018. Specimens were caught with gillnets of different meshes (60 × 1.5 m, with mesh sizes of 15, 30, and 45 mm), cast nets (60 × 1.5 m, with mesh- sizes of 0.5 cm), and traps and through angling. All fish were euthanized on ice. Environmental data was also collected; however, a detailed description of the data collection methodologies, along with the analysis and results derived from this dataset, is documented in a separate publication (Dumith

and Santos 2022). Sampling points coordinates are available as Supplementary Information (Supplementary Table 1).

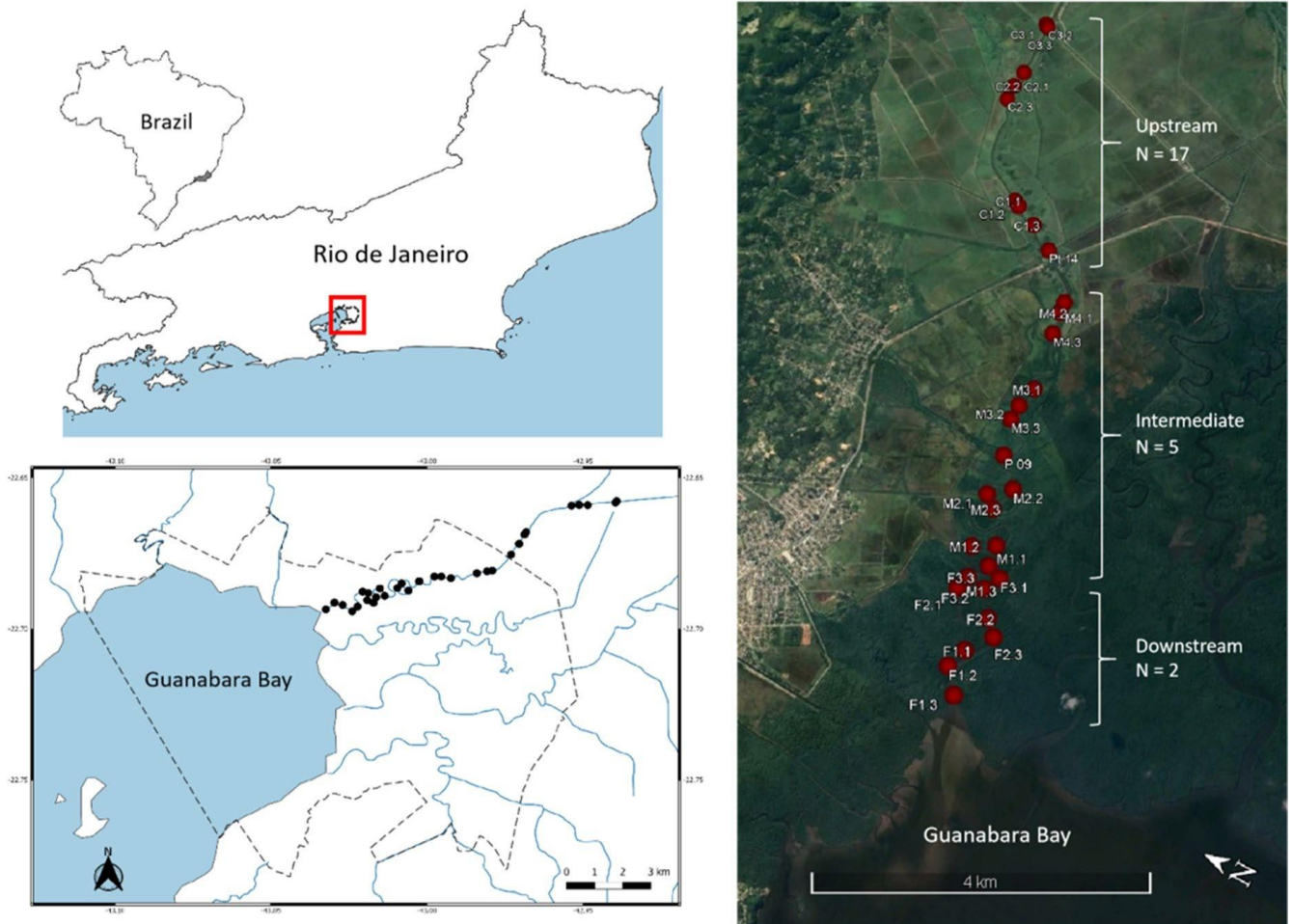


Figure. 18 Geographic location of the Guapi-Macacu River and sampling sites of *Clarias gariepinus*. The dashed line represents the boundaries of the Guapimirim Environmental Protection Area. In satellite view, the number indicate the number of specimens collected in each section of the river.

### 3.3.3. Dna Extraction, Amplification, and Sequencing

Total genomic DNA extractions were performed from 25 mg of muscle tissue using the NucleoSpin Tissue Kit (Macherey-Nagel GmbH & Co.; <http://www.mn-net.com>), following the manufacturer's instructions. The mitochondrial control region (CR) and the cytochrome b region (cytb) were selected for analysis. Both markers can distinguish between *Clarias* species and have several sequences available in the Gen- Bank database. Specifically, cytochrome b (cytb) is widely recognized for its utility in phylogeny, and the control region (CR) was chosen due to its high mutational rate, facilitating the detection of genetic variability. The CR was amplified with primers tPro2-5 (5'-ACCCTAACTCCCAAAGC-3') and HN-20-3 (5'-GTGTTATTGCTTTAGTTAAGC-3'), while cytb was amplified with the pair of primers GLUDG-5' (5'-CGAAGCTTGACTTGAARAACCAAYCGTTG-3') and Cytb3-3' (5'-

GCCAAATAGGAARTATCATTC-3') (Lee et al. 1995; Palumbi 1996). For both loci, the amplification reaction mixture (25 µl) contained 1 unit (U) Taq DNA polymerase (Thermo Scientific Inc.; <http://www.thermofisher.com>), 1× NH<sub>4</sub>SO<sub>4</sub> reaction buffer, 25 mM MgCl<sub>2</sub>, 0.16 mM deoxynucleotide triphosphate (dNTP) (Thermo Scientific), 8 pmol of each primer, and 5 ng of genomic DNA. For CR amplification, the PCR cycle consisted of an initial heating step of 5 min at 94 °C, followed by 40 cycles of 94 °C for 30 s, 45 °C for 30 s, and 72 °C for 1 min and a final extension at 72 °C for 5 min. The cytb amplification cycle consisted of an initial heating step of 5 min at 94 °C, followed by 40 cycles of 94 °C for 30 s, 45 °C for 30 s, and 72 °C for 1 min and a final extension at 72 °C for 5 min. To confirm amplification of the fragments of interest, an aliquot of the amplicons was stained with GelRed and analyzed on a 1% agarose gel in 0.5X TAE. The amplicons were then purified and sequenced by Macrogen Inc (Seoul, South Korea) in both directions, using the same PCR primers, through the traditional capillary sequencing method.

Sequences were manually edited and aligned in MEGA7 (Kumar et al. 2016) by the ClustalW algorithm with others sequences of *Clarias* spp. available on GenBank. The following species had available cytb sequences and were included in the analysis: *C. meladerma*, *C. intermedius*, *C. fuscus*, *C. gabonensis*, *C. theodora*, *C. werner*, *C. alluaudi*, *C. liocephalus*, *C. jaensis*, *C. camerunensis*, *C. anguillaris*, *C. gariepinus*, *C. ebriensis*, *C. macrocephalus*, *C. dussumieri*, *C. batrachus*, and *C. magur*. As for the CR analysis, *C. gariepinus*, *C. dussumieri*, *C. liocephalus*, *C. macrocephalus*, *C. fuscus*, *C. batrachus*, and *C. magur* were included (Supplementary Table 2). To confirm the taxonomic identification of the sampled specimens, a phylogenetic reconstruction in MEGA7 was performed for each marker using the maximum likelihood (ML) method, with 1000 bootstrap replicates to test the reliability of the branches. The nucleotide substitution models selected were TN93 + G for the cytb and HKY + G for the CR, as suggested by MEGA7 as the best fit based on the Bayesian information criterion. Sequences of *Ictalurus punctatus* were used as outgroup in both analyses. We also used BEAST v1.10.4 (Suchard et al. 2018) to conduct a Bayesian analysis to compare topology with the ML analysis. A strict clock was selected, and a Yule speciation process was imposed for the analysis. Run of MCMC for  $1.0 \times 10^6$  steps sampling every 100 under the HKY model. BEAST default values for all the other parameters were kept. All sequences accession numbers are available as Supplementary Information (Supplementary Table 2).

To infer the possible origin of the Brazilian line- age, we constructed a ML phylogenetic tree based on the cytb marker including all *C. gariepinus* haplotypes from native populations available on Genbank (Supplementary Table 2). For this analysis, the K2 + G model

was selected as it was indicated by MEGA7 as the best fit based on the Bayesian information criterion.

### 3.3.4. Morphometric Analysis

The morphometric measurements of each fish were obtained using a Mitutoyo digital caliper (model n° CD – 6” C-B) and a metric ruler (precision of 1 mm), to obtain five morphometric indices, shown in Table 8. The total length, standard length, head length, peduncle length, eye diameter, open mouth width, and open mouth height were recorded for each specimen collected during the study. In addition, the animals were weighed and their sex was determined based on external observations of the gonads. Our findings were also compared to other morphological studies for *Clarias* spp. in other invasive populations in Brazil.

Table 8 Morphometric indices calculated from specimens of *Clarias gariepinus* in the Guapi-Macacu River.

Attribute	Formula	Relationship
Relative eye diameter	Eye diameter/head length	Attribute directly related to the importance of vision in feeding, and may also indicate the vertical position in the water column, as species that inhabit deeper waters tend to have smaller eyes (Wikramanayake 1990).
Relative head length	Head length/standard length	Fish with relatively larger heads have been found to capture relatively larger prey (Gatz 1979). Therefore, the relative size of the head should be larger for predatory species (Watson and Balon 1984).
Relative caudal peduncle length	Caudal peduncle length/standard length	Fish with long caudal peduncles are considered good swimmers (Watson and Balon 1984).
Relative mouth width	Mouth width/standard length	The dimensions of the mouth would indicate, like the size

## 3.4. RESULTS

### 3.4.1. Genetic Analysis

Our genetic analysis focused on 24 individual fish specimens collected from the Guapi-Macacu River, sampled at six specific points along the upstream section of the river. The sequences obtained from the control region (CR) and cytochrome b (cytb) regions were submitted to the GenBank database under accession numbers OP935939–OP935986. Notably, our analysis revealed the presence of a single CR and cytb haplotype among the studied specimens, indicating a limited genetic diversity within the sampled population. The maximum likelihood phylogenetic analysis of cytb (427 bp) and CR (335 bp) markers revealed that sequences of *C. gariepinus* captured in the Guapi-Macacu River clustered with other *C. gariepinus* sequences available in the GenBank database (Figure. 19 A and B), confirming the taxonomic identification of these individuals. Bayesian analysis in BEAST yielded a tree with

similar structure and topography to the ML (Supplementary Information). In the cytb analysis (451 bp) including all *C. gariepinus* haplotypes available on GenBank, the Guapi-Macacu haplotype clustered with other *C. gariepinus* sequences from the ichthyofaunal province of Congo, being identical to a haplotype identified by Van Steenberge et al. (2020) in the Democratic Republic of the Congo (Figure 20).

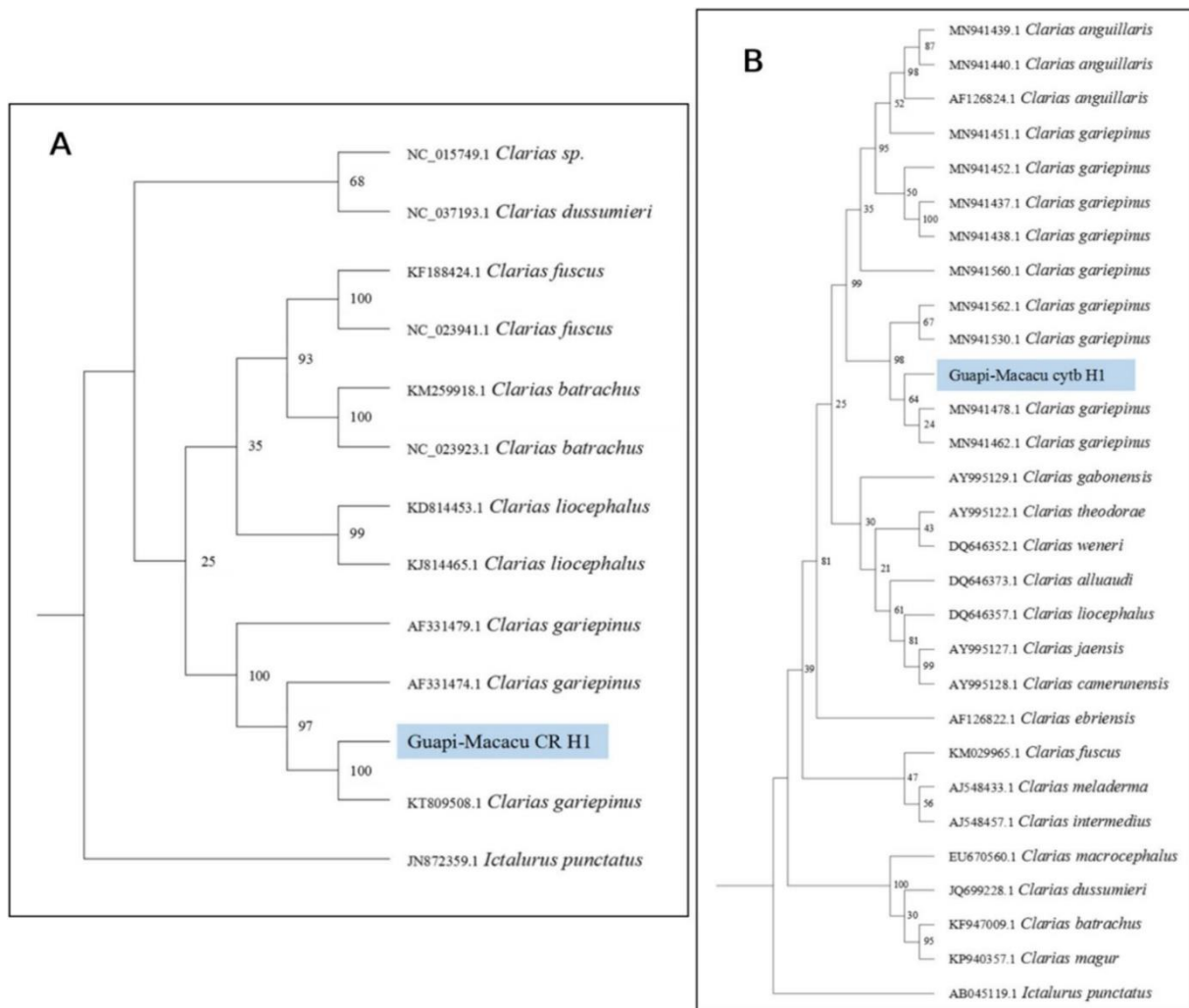


Figure. 19 Maximum likelihood trees based on *Clarias* (A) control region and (B) cytochrome b sequences. Numbers on nodes represent bootstrap support values after 1000 replications.

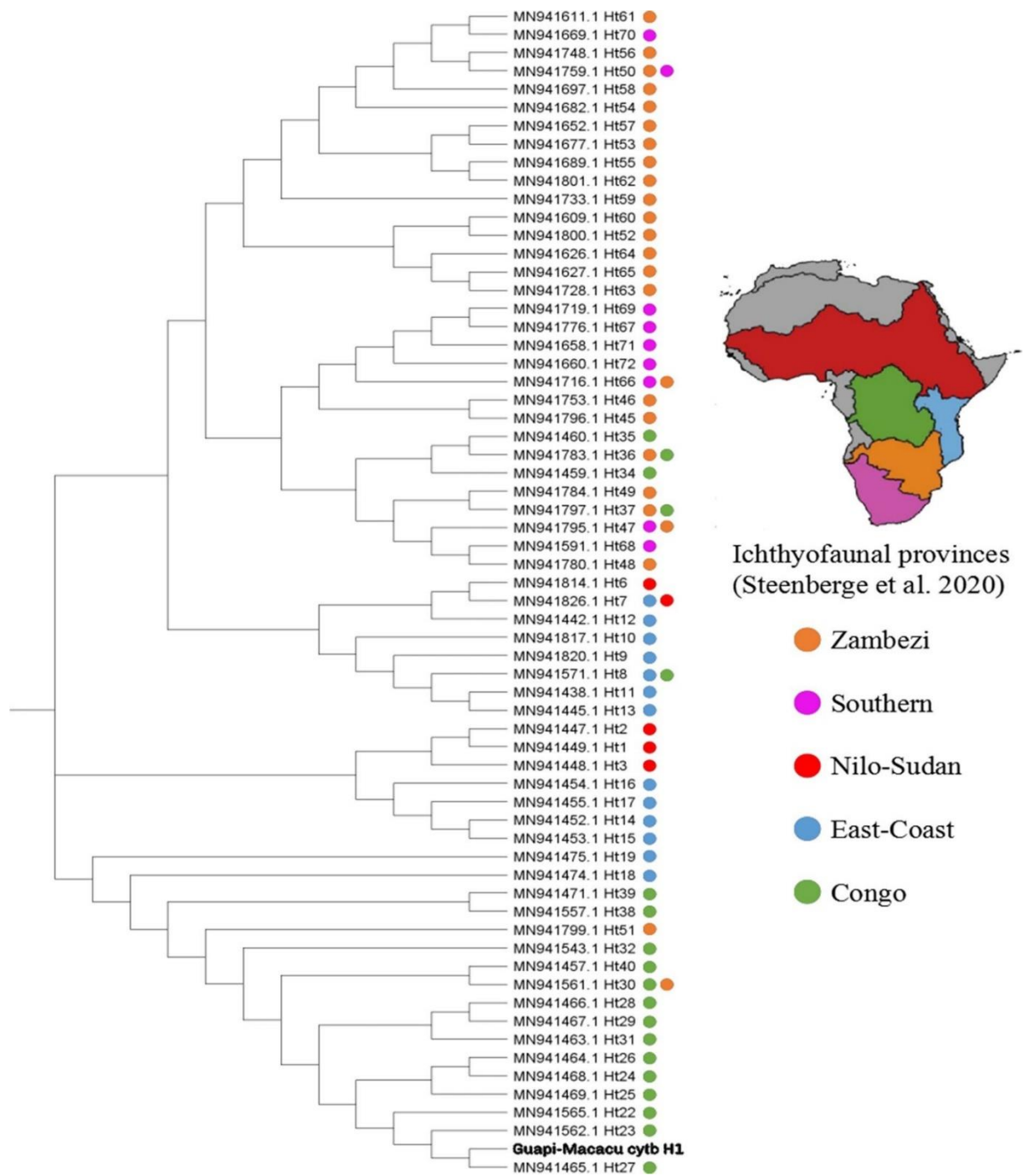


Figure. 20 Maximum likelihood tree based on *Clarias gariepinus* cytochrome b haplotypes. All haplotypes from Steenberge et al. (2020) available in GenBank were included. Colored circles represent where each haplotype was found. Map figure indicating localities of the African ichthyofaunal provinces adapted from Steenberge et al. (2020).

### 3.4.2. Morphometric Analysis

The observed characteristics of *Clarias gariepinus* included the distinct positioning of the eyes in the dorsal region and the presence of barbels, which are well-documented features of the species. Analysis of the 24 specimens indicated a sex distribution of lected specimens ranged from 345 to 830 mm, while total weight spanned from 334 to 4030 g. Most individuals were collected in the upstream segment of the river; this area was observed to accommodate specimens with the highest and lowest recorded sizes for both male and female individuals. Conversely, the downstream and intermediate segments of the river also hosted individuals of both sexes, exhibiting an average length of approximately 500 mm and an average weight of around 1000 g. Morphometric indices calculated for the population can be found in Tables 9 and 10. The total length ranged from 830.00 to 345.00 mm with an average of 552.13, while total weight ranged from 334.00 to 4030.00 with an average of 1495.42.

Table 9. Minimum, maximum and average values (standard deviation) of the morphometric indices (%) calculated for the population of *Clarias gariepinus* captured in the Guapi-Macacu River

Attribute	Maximum	Minimum	Average sd
Total length (mm)	830.00	345.00	552.13±133.42
Total weight (g)	4030.00	334.00	1495.42±1017.79
Standard length (mm)	720.00	310.00	478.13±117.18
Relative eye diameter	10.21	4.87	7.30±1.24
Relative head length	28.23	21.92	24.38±1.52
Relative caudal peduncle length	6.18	1.28	2.92±1.13
Relative mouth width	15.68	6.86	11.74±1.56
Relative mouth height	13.35	6.72	9.22±1.56

The ANOVA results for total length including individuals from the other two invasive populations in Brazil (Guaraguaçu River basin and Encantada lagoon) revealed a non-significant *p*-value of 0.1638 (Supplementary Information). Contrastingly, the ANOVA for total weight revealed a significant *p*-value of 0.0299. However, Tukey's HSD test further pinpointed a significant total weight difference only between the invasive populations in Guaraguaçu River basin and Encantada lagoon.

Table 10. Description of head length percentage of *Clarias gariepinus* in the Guapi-Macacu River and in other ecosystems.

Collection area	Head length %	Author
Guapi-Macacu-BR	24.51	Current study
Senegal River-SE	31.60	Agnèse et al. 1997
Göksu-TR	27.00	Turan et al. 2005
Asi-TR	26.00	Turan et al. 2005
Seyhan-TR	27.00	Turan et al. 2005
Ceyhan-TR	27.70	Turan et al. 2005
Aksu-TR	26.70	Turan et al. 2005
Sakarya-TR	25.30	Turan et al. 2005
Congo River-CO	19.40	Hanssens 2009
Rivers Mezan-Nkam-Mungo-CA	30.36	Nkongho et al. 2019
Ero Dam (Ikum Ekiti)-NI	26.62	Ola-Oladimeji et al. 2017

### 3,5 DISCUSSION

The genetic analyses based on two mitochondrial markers confirmed that the catfish specimens captured in the Guapi-Macacu River are *C. gariepinus*. As mtDNA markers are unable to detect hybridization due to uniparental inheritance, we cannot rule out the possibility of hybridization, either with native species or before translocation of these individuals. Stocks of congeneric and even intergeneric hybrids of *C. gariepinus* are widely disseminated due to their value in aquaculture (Na-Nakorn and Brummett 2009). However, all specimens analyzed in the present study carried mtDNA haplotypes from *C. gariepinus*, suggesting a limited likelihood of hybridization.

The use of molecular data allows us to infer the geographic origin of invasive populations when potential source populations are thoroughly sampled and sufficiently differentiated (Fitzpatrick et al. 2012). Van Steenberge et al. (2020) analyzed cytb sequences from 443 individuals of *C. gariepinus* from 97 native localities in Africa, where they identified a clear geographical signature. The authors identified 67 cytb haplotypes distributed in five ichthyofaunistic provinces, which were defined using similarities between fish faunas. The single cytb haplotype found in individuals sampled from the Guapi-Macacu River is identical to a haplotype that occurs in the province of Congo (Van Steenberge et al. 2020). This haplotype (Ht 22 in Van Steenberge et al. 2020) is unique to the Democratic Republic of the Congo (DRC) and was the most common haplotype found in that country. Therefore, our results suggest that the *C. gariepinus* lineage found in the Guapi-Macacu River originated from individuals from a native population from the DRC. This same haplotype can also be found in

introduced populations in Hungary and the Philippines (GenBank accession numbers KT809508.1 and KJ533252.1).

As noted by Cambray (2005), when *C. gariepinus* arrives in a new country, there is a tendency for it to “leapfrog” to more countries. A notable example is the introduction of fish from Laos to Thailand, then from Thailand to Bangladesh, Myanmar, the Philippines, Malaysia, and India (FAO 2023b). In addition, many countries are subjected to introductions from more than one country, such as fish from Thailand and Taiwan being introduced to the Philippines. Therefore, it is not possible to determine whether the invasive population of the Guapi-Macacu River came directly from the DRC or indirectly via another non-native population. Most likely they were translocated from other Brazilian systems with older *C. gariepinus* invasions, such as the Paraná River basin, which is the most invaded river basin by this species in Brazil (Weyl et al. 2016). Molecular data from invasive *C. gariepinus* populations in Brazil is lacking, which prevents a direct assessment of this hypothesis. Until now, there has been no information available regarding the specific potential source populations of the invasive African catfish found in Brazil. To better understand the history of introduction of this species throughout Brazil, other invasive populations must be analyzed.

Both mitochondrial loci analyzed in the present study were monomorphic, which is representative of a strong founder effect, indicating a single introduction event, especially when considering that the CR is a hypervariable region of mtDNA in many taxa, including fish (Brown et al. 1993). Several studies have analyzed the genetic diversity of native and cultivated populations of the African catfish (Barasa et al. 2016; Van Steenberge et al. 2020; Alal et al. 2021). However, despite being recognized as a global threat to freshwater biodiversity, little is known about the genetic diversity of the invasive populations. The successful establishment, despite the low levels of genetic diversity, may be explained by *C. gariepinus* hardy characteristics (Estoup et al. 2016).

The African catfish is a highly fecund and resistant species (Roodt-Wilding et al. 2010) and able to tolerate a wide range of temperature, pH, and oxygen concentration, in addition to presenting high phenotypic and reproductive plasticity, which also facilitates its establishment in a wide variety of habitats (Cambray 2003; Solomon et al. 2015; Singh 2021). They are also known as the walking catfish due to their ability to walk on land, which facilitates their dispersion. These factors make the African catfish a resistant invader with a high potential for proliferation and dispersion.

The medium total length and weight of *C. gariepinus* from the Guapi-Macacu River (810 mm and 3644 g) were found to align closely with measurements recorded in other

introduced populations in Brazil, such as the Guaraguaçu River basin (Paraná state) (578 mm and 1655 g) and Encantada lagoon (Bahia state) (551 mm and 1024 g) (Vitule et al. 2006; Rocha 2008), which was supported by the ANOVA. These findings suggest a degree of consistency in the morphometric traits of *C. gariepinus* invasive populations in Brazil. The lack of genetic sampling and other morphometric trait measurements from specimens from the other invasive populations in Brazil hinders more in-depth analysis and highlights the necessity for more studies. Comparisons of specific attributes, such as relative head size and mouth dimensions, revealed consistency with previous research highlighting the importance of these traits in understanding feeding behavior and physiological adaptations (Motta et al. 1995; Van Wassenbergh et al. 2006).

The prevalence of *C. gariepinus* in the upstream section of the Guapi-Macacu River was previously associated with the increased dissolved oxygen levels and temperature compared to downstream sections (Dumith and Santos 2022). The fact that most individuals were sampled just outside of the Guapimirim Environmental Protection Area may also be an indication of its effectiveness, as a lower number of invaders inside the protected area is predicted by the biotic resistance hypothesis (Beaury et al. 2019). Nevertheless, *C. gariepinus* were found throughout the entire river, indicating its establishment and raising concerns about possible impacts.

*Clarias gariepinus* is ecologically similar to *C. batrachus* (Low et al. 2022), a congeneric listed as one of the world's 100 worst invasive species (Lowe et al. 2000), although the former feeds more frequently, exhibit faster growth rates, and reach bigger sizes than *C. batrachus* (Low et al. 2022). Therefore, it is to be expected that invasive *C. gariepinus* also impose risks to non-native ecosystems. The concerns over its potential threat to native aquatic biodiversity led to the banishment in 1997 of the culture or import of *C. gariepinus* in India (Chatterjee 2019). Field studies revealed impacts of *C. gariepinus* invasions on native invertebrate communities (Weir 1972; Kadye and Booth 2012), and modelling approaches demonstrated that invasive *C. gariepinus* have a considerable impact on the food web of tropical reservoirs (Khan and Panikkar 2009; Khan et al. 2021). The integration of predictive modeling techniques, like Ecological Niche Modeling, can provide valuable insights into the potential future range expansion of *C. gariepinus*, aiding in the identification of areas at heightened risk.

There are multiple anecdotal accounts that the presence of invasive *C. gariepinus* reduces the abundance of native fish species, which could pose significant threats to biodiversity (Cambray 2003; Vitule et al. 2006). The presence of *C. gariepinus* in the Guapi-Macacu River is especially problematic, as it is located in the Guapimirim Environmental

Protection Area, an important site for the protection of the mangrove remnants of the Guanabara Bay (Fries et al. 2019), which, therefore, warrants careful monitoring and management strategies to safeguard the local biodiversity and ecosystem integrity.

### 3.5. CONCLUSION

Our results provide further evidence of the expansion of the African catfish in Brazilian inland waters, as highlighted by Weyl et al. (2016). Molecular data indicates the Democratic Republic of the Congo as the genetic source of the invasive population in the Guapi-Macacu River. Morphometric data suggests consistency in the morphometric traits of *C. gariepinus* invasive populations in Brazil, possibly indicating the same source of introduction. The use of molecular data can contribute to a better understanding of the introduction history of this species in all of Brazil and aid the development of management strategies, for example, by the identification and tracking of different stocks. Therefore, to better understand the expansion of *C. gariepinus* in Brazil, it is important to sample other introduced populations. Future studies should address the genetic diversity and the ecological impacts of invasive *C. gariepinus* populations on freshwater biodiversity in Brazil.

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## 4. CAPÍTULO 4

### **The Rustivity of the African Catfish (*Clarias gariepinus*): Reproductive Aspects of the Invader in the Environmental Context**

#### **4.1. ABSTRACT**

Invasive alien species (IAS) can proliferate unchecked, causing significant impacts on the ecosystem and posing a significant threat to biodiversity. *Clarias gariepinus* (African catfish), introduced to Brazil for aquaculture in the 1980s, now inhabits various natural environments in the South and Southeast regions of the country. This catfish demonstrates rapid adaptation to various environments, establishing itself as a noteworthy IAS. The Guapi-Macacu River, located within the Guapimirim Environmental Protection Area in Rio de Janeiro, harbors a population of *C. gariepinus* that may threaten the existing fish community. This study aimed to investigate the reproductive dynamics of this non-native population, analyzing reproductive stages and indices, and physicochemical variables related to water quality and reproduction. Consequently, we identified a predominant population of adults in various stages of maturation, mainly located upstream during the dry season. Continuous reproduction was observed regardless of the population's origin, with the Dobriyal index emerging as an effective indicator for assessing gonadal maturation. Analysis of reproductive indices underscores the catfish's preference for environments with superior environmental quality, shaping its distribution and reproductive patterns. Furthermore, our results highlight the importance of seasonal variation, especially during the rainy season, in the environmental conditions and reproductive behavior of *C. gariepinus*. This study emphasizes the ongoing need to monitor alien species, reducing the impact of IAS on the aquatic ecosystem.

**Keywords:** *Brazil*; bioinvasion; reproduction; environmental conditions; reproductive indices.

#### **4.2. INTRODUCTION**

Invasive alien species (IAS) can proliferate unchecked, causing significant impacts on the ecosystem (Santos et al. 2018). These invaders can directly compete with native species for resources, feeding on them and contributing to the impoverishment and homogenization of ecosystems (Clavero e García-Berthou 2006). This represents one of the greatest threats to the reduction and degradation of habitats, negatively impacting biodiversity and ecosystem

processes (Katsanevakis et al. 2014; Colin et al. 2018; Gallardo et al. 2019; Erarto e Getahun 2020a). In aquatic environments, the presence of IAS is associated with human activities (Gallardo et al. 2015; Havel et al. 2015), with aquaculture being one of the vectors for the introduction and spread of these species (Gozlan et al. 2010; Lee et al. 2017). Effective control and management of these invaders are essential to preserve the integrity of aquatic ecosystems and minimize adverse effects on native fauna and flora (Carey et al. 2012; Woodford et al. 2016).

*Clarias gariepinus* (Burchel, 1822) (Siluriformes, Clariidae) is one of the most globally cultivated species, being the primary catfish species produced and consumed in Africa and Asia, and some parts of Europe (Dauda et al. 2018). With tasty flesh, boneless, and high protein content (Oladipo e Bankole 2013; Adebayo et al. 2016; Karim et al. 2017), these nutritional characteristics are important for consumption in the aquaculture market (Papuc et al. 2019). From a commercial perspective, African catfish exhibit rapid and easy growth can tolerate low concentrations of dissolved oxygen in the water (Moussa 1956; Belão et al. 2011) and can be stocked at high densities in aquaculture (Kucharczyk et al. 2019). However, in captivity, it loses its natural reproductive ability (Romanova et al. 2018, 2020), requiring ongoing research to supply larvae and juveniles for the industry. This is because the environmental and physicochemical characteristics of the water also interfere with its reproduction, which is essential for its reproductive cycle. (Legendre et al. 1996; Santi et al. 2017; Ariole et al. 2021).

In Brazil, the African catfish was introduced for aquaculture in the 1980s in many states (Cambray 2003; Vitule et al. 2009). However, inadequate management of cultivation systems favored its bioinvasion into various natural environments, bringing numerous damages to the ecosystem and significant consequences (Vitule et al. 2006, 2009; Weyl et al. 2016). A Guapimirim Environmental Protection Area (Guapimirim EPA) in the Rio de Janeiro state is an important protected area for fish species that utilize it as a nursery and breeding ground. It provides shelter and maintenance for fish stocks in adjacent areas and Guanabara Bay (Teixeira-Leite e Vianna 2023). This significant area also houses the alien species *C. gariepinus*, originating from discontinued aquaculture in the region, with this species not finding favor in the domestic market (Gutierre et al. 2014), facilitating the abandonment of fish farming and the subsequent escape of the African catfish. However, little is known about its reproduction in this area, where the species already dominates the fish community of the main river in the Guapimirim EPA (Dumith e Santos 2022).

However, the Guapimirim EPA still faces numerous issues related to the environmental quality of the water (Fistarol et al. 2015; Vicente et al. 2016; Costa et al. 2018). This is because

there are industrial hubs in its vicinity, a lack of sanitary sewage treatment, deforestation of riparian forests, river siltation, and the proximity to Guanabara Bay, all of which have contributed to a deterioration in water quality (Hernández e Hiram 2011; Monteiro et al. 2012; Fistarol et al. 2015). Water quality is an important factor in the distribution and survival of various fish species, directly impacting reproduction and development (Bhatnagar e Devi 2013; Heath 2018), including IAS. Due to the rusticity and easy adaptation of *C. gariepinus*, the reproduction of this bio-invader has been poorly studied in invaded environments in Brazil.

The rusticity of the African catfish is one of the most important characteristics that associate its name with an IAS, bringing negative consequences in bio-invaded ecosystems (Jarić et al. 2015; Oyebola et al. 2022; Sreekanth et al. 2022). Therefore, its characteristics such as feeding tactics, size, and aggressiveness promote competition for resources and have impacts on biodiversity (Parvez et al. 2022; Low et al. 2022). Additionally, the reproductive capacity is favored when it escapes from the cultivation system into the natural environment (Krishnakumar et al. 2011). In this sense, it is crucial to understand and identify the environmental factors that promote its establishment and reproductive success in bio-invaded environments, highlighting the interaction with abiotic variables as facilitators of its reproductive capability.

The objective of the research was to assess the reproductive aspects of the population of *C. gariepinus* inhabiting the Guapi-Macacu River (within the Guapimirim EPA), revealing the composition of reproductive stages, as well as indices related to ecological condition (condition factor), hepatosomatic index, and reproducibility. We also sought to assess some physicochemical variables in the water-related to environmental quality, as well as the reproduction and development of the species. We aim to indicate how much these abiotic factors can affect its distribution and reproductive capability, evaluating its dominance and potential risk to the aquatic community in the Guapi-Macacu River.

### **4.3. MATERIAL AND METHODS**

#### ***4.3.1. Study Area and Sample Design***

The Guapimirim EPA encompasses several rivers and canals, which become inundated during the rainy season. Among the various rivers, the largest and most voluminous is the Guapi-Macacu River, which also serves as a water supply for part of the State of Rio de Janeiro (Brazil). Upstream, the river has a steep slope, with the riverbanks exhibiting greater vegetative cover. Before reaching the river mouth, in a transitional area, the river's velocity decreases due

to increased sinuosity (Ferreira et al. 2019). The downstream region features a slower velocity, with a broader bed and vegetation consisting of mangroves and marshes (Fries et al. 2019).

The collection of African catfish was conducted in two field samplings covering the dry and rainy seasons of the year 2018. Thus, the two most significant seasonal periods (rainy and dry) in the Neotropical region and the Southern Hemisphere (da Silva et al. 2009, 2018; Henrique et al. 2009) were considered. Various fishing gear was employed, including gill nets (60m x 1.5m) with different mesh sizes (15, 30, and 45mm adjacent knots), cast nets, fyke nets (nylon trap with 40cm height and 35cm diameter), hooks, and lines. The fishing equipment was set up and used with three replicates (one hour for each replicate) at each point, during both day and night, in two campaigns (February and August), totaling 64 sampling points (Figure 21). Encompassing the physiographic diversity of the Guapi-Macacu River from the mouth to the upstream, seven abiotic variables were measured to assess some parameters related to water environmental quality and necessary for the reproduction of the alien species. For this purpose, the YSI Multiparameter Sonde (Model: 6600) was used, which collected information on temperature, pH, turbidity (NTU), dissolved oxygen (mg/L), salinity (PSU), phycocyanin (RFU), and chlorophyll (RFU) at the collection points concurrent with the positioning of fishing gear. After collection, the fish were labeled, kept refrigerated in ice (still in the field), and transferred to the Laboratory of Applied Ecology (Federal Fluminense University).

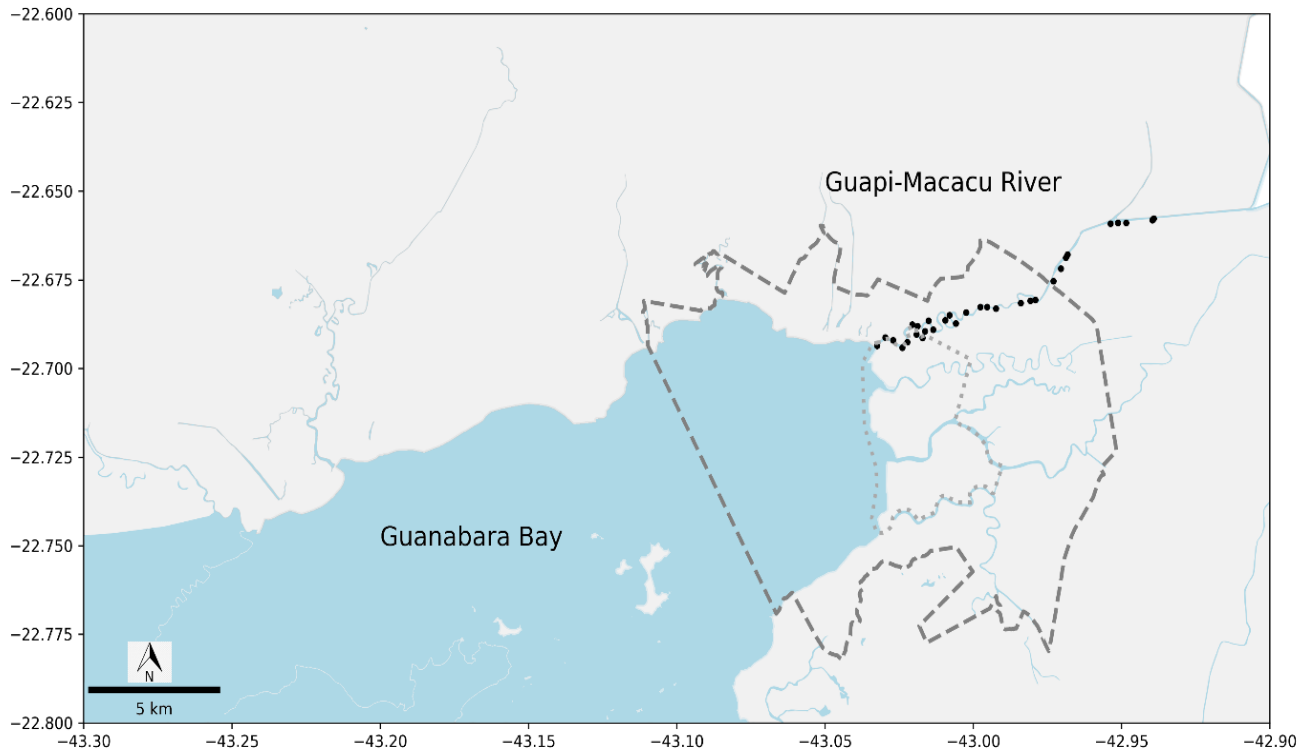


Figure 21 – Sampling area map (collection points) in the Guapi-Macacu River (black points along the river), with the delineation of the Guapimirim Environmental Protection Area (dashed line) in Rio de Janeiro, Brazil.

#### 4.3.2. Laboratory Procedures

In the laboratory, thirty fish were identified (Froese and Pauly, 2021), weighed (precision balance), and measured (total and standard length) in millimeters. Subsequently, the fish were eviscerated to remove the liver and gonads, which were weighed and assessed for sex and macroscopic gonadal maturation stage. Sex determination was made through observation under a stereoscopic microscope, based on the macroscopic morphology of the gonads (Vazzoler 1996; Souza e Chaves 2007). Six maturation stages were determined for females and males:

- **Immature (A):** females - filiform, translucent ovaries, very small in size, without signs of vascularization; macroscopically, oocytes are also not observed; males - small and filiform testicles.
- **Early maturation (B1):** females - ovaries with increased size and circumference, slightly yellowish color, poorly vascularized, and the presence of small oocytes; males - testicles increased in size compared to the previous stage, flatter compared to ovaries and whitish.

- **Final maturation (B2):** females - ovaries much larger than the previous stage, with dark yellowish color, very close in size to mature ovaries, but with the presence of opaque and small oocytes; males - well-developed, lobulated testicles, whiter in appearance, with a membrane that ruptures with slight pressure.
- **Mature (C):** females - size slightly larger than the previous stage, occupying almost the entire coelomic cavity; oocytes are yellowish, with a hemorrhagic appearance, and quite cylindrical; males - they appear turgid, whitish, occupying a significant portion of the coelomic cavity, reaching their maximum size, with a milky white coloration.
- **Empty (D):** both decrease in size; they exhibit varying degrees of flaccidity, with stretched membranes, and few oocytes are observed in the case of females.
- **At rest (E):** both occupy less than 1/3 of the coelomic cavity, reverting to the characteristics of the immature or early maturation stages.

Juveniles, when found, were determined when the gonads encountered could not be assigned to either the male or female genders (Vazzoler 1996).

#### 4.3.3. Data Analysis

The stages of maturation were evaluated macroscopically, and the gonadosomatic index (GSI), Dobriyal index (DI), and modified gonadosomatic index (MGSI) were estimated to assess which one is more appropriate for the sampled population of *C. gariepinus* through SPEARMEN correlation with total length. Total length is used as a reference for gonadal maturation (Binohlan e Froese 2009). The GSI was calculated using the formula  $GW \times 100/EW$ , where GW is the gonad weight and EW is the eviscerated fish weight (Vazzoler 1996). The MSGI was calculated using the formula  $MGSI = 100 \times GW/BW - GW$  (Nikolsky 1963) and the Dobriyal index given by  $DI = \sqrt[3]{GW}$  (Dobriyal et al. 1999), where BW is the fish weight, and GW is the gonad weight of the fish. We also calculated the hepatosomatic index (HSI) using the formula  $LW / EW \times 100$ , where LW is the liver weight and EW is the eviscerated fish weight (Vazzoler 1996). Fulton's condition factor is referred to as the "K factor," assuming the isometric growth of the fish calculated as  $K = BW/TL^3$ , where K is Fulton's condition factor; 3 is the exponent indicating isometric growth, TL is the total length, BW is the total fish biomass (Cren 1951). The SPEARMAN correlation was also used to correlate the hepatosomatic index and the most suitable reproductive index for the entire population, as well as to correlate it with the condition factor. According to Htun-Han (1978), these three indices can provide insights into the nutritional status of the fish and may interfere with its reproductive cycle.

The abiotic variables measured were assessed through Principal Component Analysis (PCA), which was applied to the environmental data matrix at the catfish capture points to address the temporal distribution patterns of the population. These abiotic factors were also modeled using Boosted Regression Trees (BRT) with the index that best characterizes the community (GSI, DI, or MSGI). This allows us to assess the potential influences of these variables on the reproductive index of *C. gariiepinus*. According to the procedure outlined by Elith et al. (2008), the BRT model was fitted with a tree complexity of 10, a learning rate of 0.001, a bag fraction of 0.75, and a Gaussian error distribution (for more details on these parameters, refer to Elith et al. 2008). Ten-fold cross-validation was applied to address the non-independent structure of the data (Fabricius e De'ath 2008; Buston e Elith 2011). Although BRT models are complex and used for machine learning, even with large datasets, they can be summarized to provide good ecological insight. Furthermore, their predictive performance is superior to traditional modeling methods (Elith et al. 2008). Therefore, BRT models result in clear graphics and are suitable for studying ecological questions (Cappo et al. 2005; Fabricius e De'ath 2008).

#### **4.4. RESULTS**

The specimens of *C. gariiepinus* were captured, predominantly males (63,3% M and 36,7% F), all adults at different stages of maturation (Figure 22 a and b), mainly upstream of the Guapi-Macacu River, the dry season contributed to the majority of the collections (73.3% during the dry period and 26.7% during the rainy season), as shown in Table 11. The weights of the fish varied between 366g and 2432g, with a total length ranging from 385mm to 660mm for females. As for males, the total length ranged from 345mm (smaller) to 830mm (larger), while the weight varied between 334g and 4030g. The hepatosomatic index exhibited higher means for females at smaller sizes, remaining higher than males in all size ranges except between 466mm and 588mm, where males had higher averages of this index (Figure 23a). Comparatively between sexes, smaller-sized females exhibited a lower condition factor related to males. However, males showed a single peak in the condition factor between the size ranges of 466mm to 527mm, therefore low in all other samples (Figure 23b).

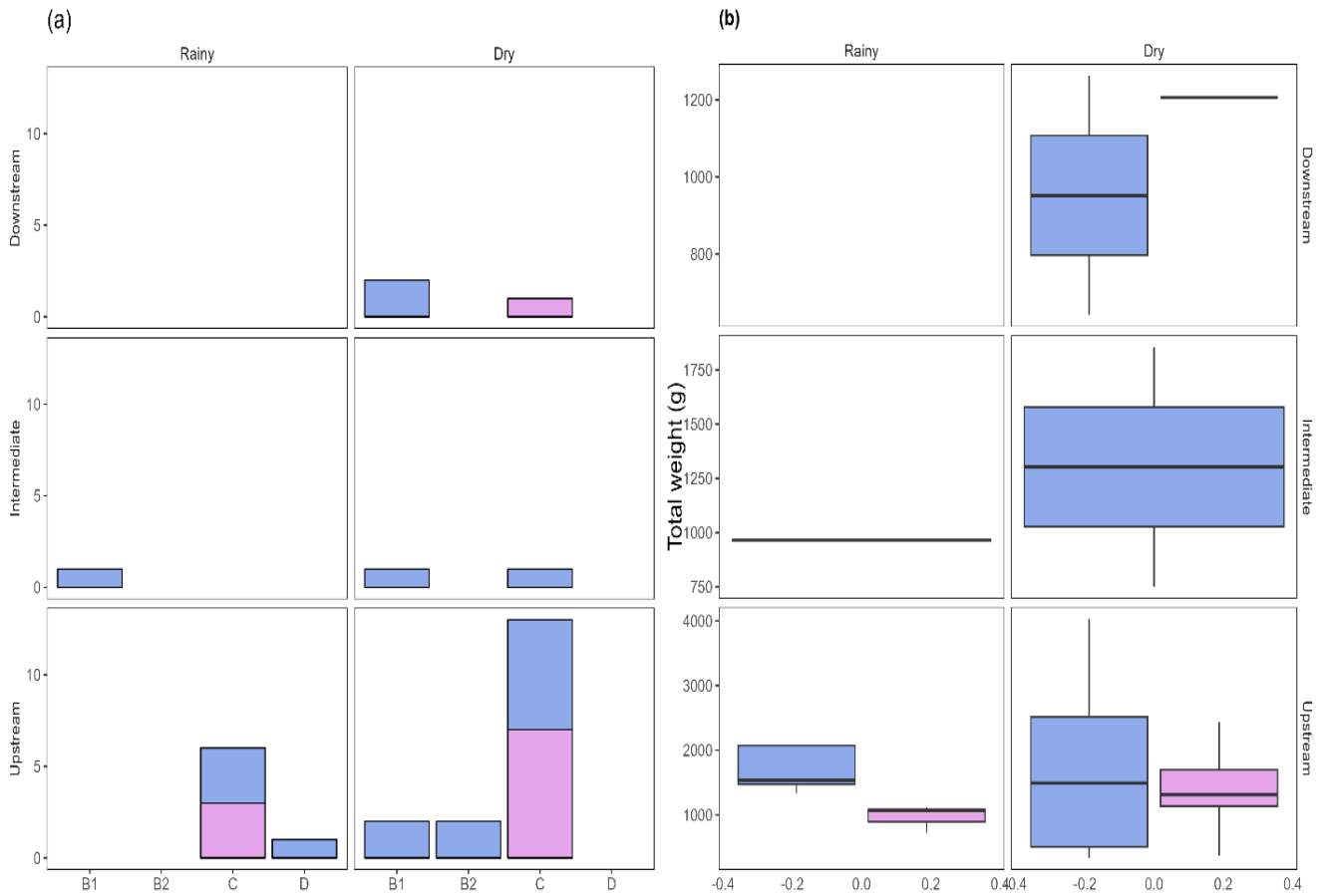


Figure 22 – Maturation stages (a) and box plot of the total weight of *Clarias gariepinus* specimens during the rainy and dry seasons in the three stretches (downstream, intermediate, and upstream) of the Guapi-Macacu River.

Table 11 – Gonadal maturation stages, early maturation (B1), final maturation (B2), mature (C), and empty (D), of male (M) and female (F) specimens collected during the dry and rainy seasons in the upstream (U), intermediate (I), and downstream (D) sections of the Guapi-Macacu River.

Zone	Sex	B1	B2	C	D
D	M	2	-	-	-
	F	-	-	1	-
I	M	2	-	1	-
U	M	2	2	9	1
	F	-	-	10	-

Among the indices related to gonad weight, only the DI index showed a significant Spearman correlation with the total length of the collected specimens. The Dobriyal index consistently showed higher values for females in all size ranges (Figures 23 a and b). During the dry season, which recorded the highest number of samples, a significant correlation ( $p < 0.05$ ) was observed between Dobriyal indices and hepatosomatic indices for 51% of the analyzed data, along with a similar trend in correlation with the condition factor (Table 13). Conversely,

during the rainy season, no significant results were obtained ( $p>0.05$ ), and the explanatory capacity of these correlations was limited. Overall, there appears to be a more notable correlation between DI and the calculated condition factor during advanced stages of gonadal development.

Table 12 – Spearman's rank correlation between reproductive indices gonadosomatic index (GSI), Dobryal Index (DI), modified gonadosomatic index (MSGI), and the total length of *Clarias gariepinus* specimens in the Guapi-Macacu River.

Index	<i>Rho</i>		<i>p-value</i>	
	Males	Females	Males	Females
GSI	0.5701	0.2727	0.001	0.4182
DI	0.8368	0.6545	<0.001	0.0383
MSGI	0.5877	0.3	0.009354	0.3711

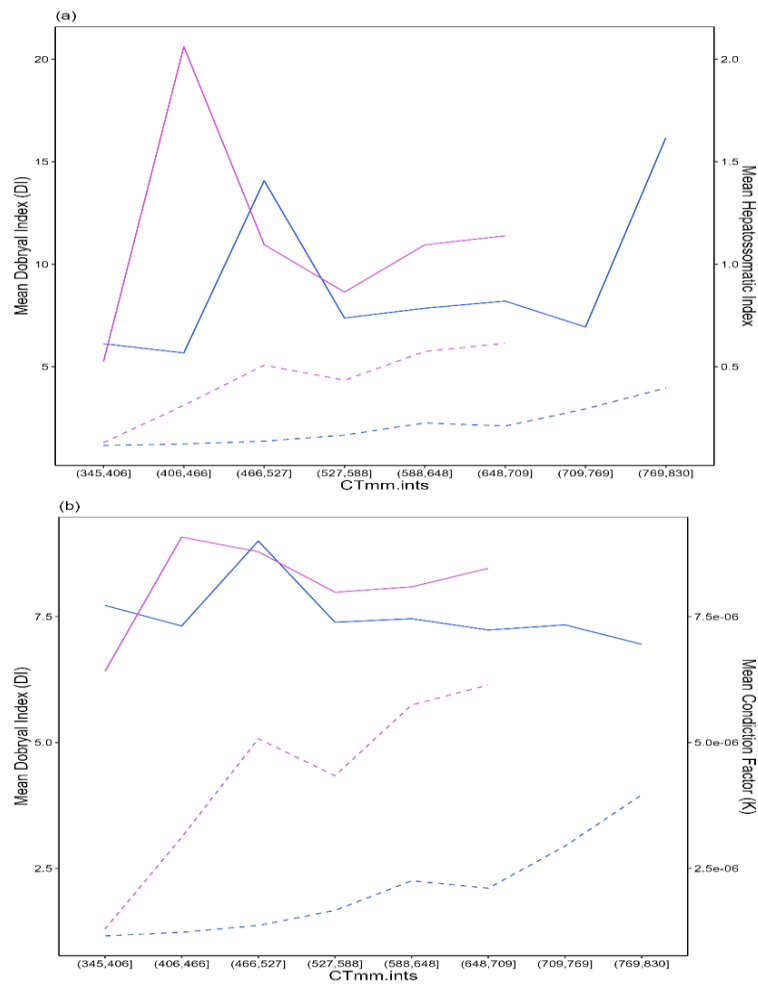


Figure 23 – Variation of the hepatosomatic index (solid line, figure a) and condition factor (solid line, figure b) about the Dobryal index (dotted line) for male (blue) and female (pink) individuals of the *Clarias gariepinus* population in size classes (closed interval: parentheses, open interval: square brackets).

Table 13 – Spearman's rank correlation between the Dobriyal index and the hepatosomatic index (HSI) and condition factor (K) of collected *C. gariepinus* specimens during dry and rainy seasons in the Guapi-Macacu River.

	<i>Rho</i>		<i>p-value</i>	
	Dry Season	Rainy Season	Dry Season	Rainy Season
HSI	0.5787	0.4285	0.0055	0.2992
K	0.5019	-0.0476	0.0185	0.9349

In the abiotic variables measured at the collection points along the river, higher averages for temperature were evident during the rainy season, associated with the warmer period in southeastern Brazil (Figure 24a). pH behaved oppositely to temperature, favoring more acidic waters during the warmer and rainy periods (Figure 24b). Turbidity exhibited higher averages during the rainy season, although the upstream stretch showed little variability between periods (Figure 24c). The rainy season was responsible for displaying the lowest averages of dissolved oxygen in the intermediate and downstream sections of the river (Figure 24d). Low salinity was characteristic in all three collection sections, except during the dry period downstream, where salinity intrusion was recorded in this section of the river (Figure 24e). Phycocyanin and chlorophyll can infer the quality of the Guapi-Macacu River and be correlated with turbidity, reaching maximum values during the rainy season downstream (Figures 24f and 24g). Principal Component Analysis (PCA) highlighted the complex relationships between environmental variables in the distribution of the African catfish (Figure 24h), with the first axis having major contributions from pH (20.58%), dissolved oxygen (21%), temperature (17.19%), and chlorophyll (16.12%). On the second axis, the abiotic variables with major contributions were turbidity (19.52%) and phycocyanin (50.71%). The physiographic characteristics collected in the stretches of the Guapi-Macacu River during field sampling are shown in Table 14.

Table 14 – Average percentage (%) of vegetation physiography within each of the spatial areas corresponding to the upstream (U), intermediate (I), and downstream (D) sections of the Guapi-Macacu River.

Zone	Tree	Mangrove	Grass	No_Veg
D	35.00%	50.00%	---	15.00%
I	23.33%	48.33%	18.33%	10.00%
U	0.08%	16.96%	59.00%	23.96%

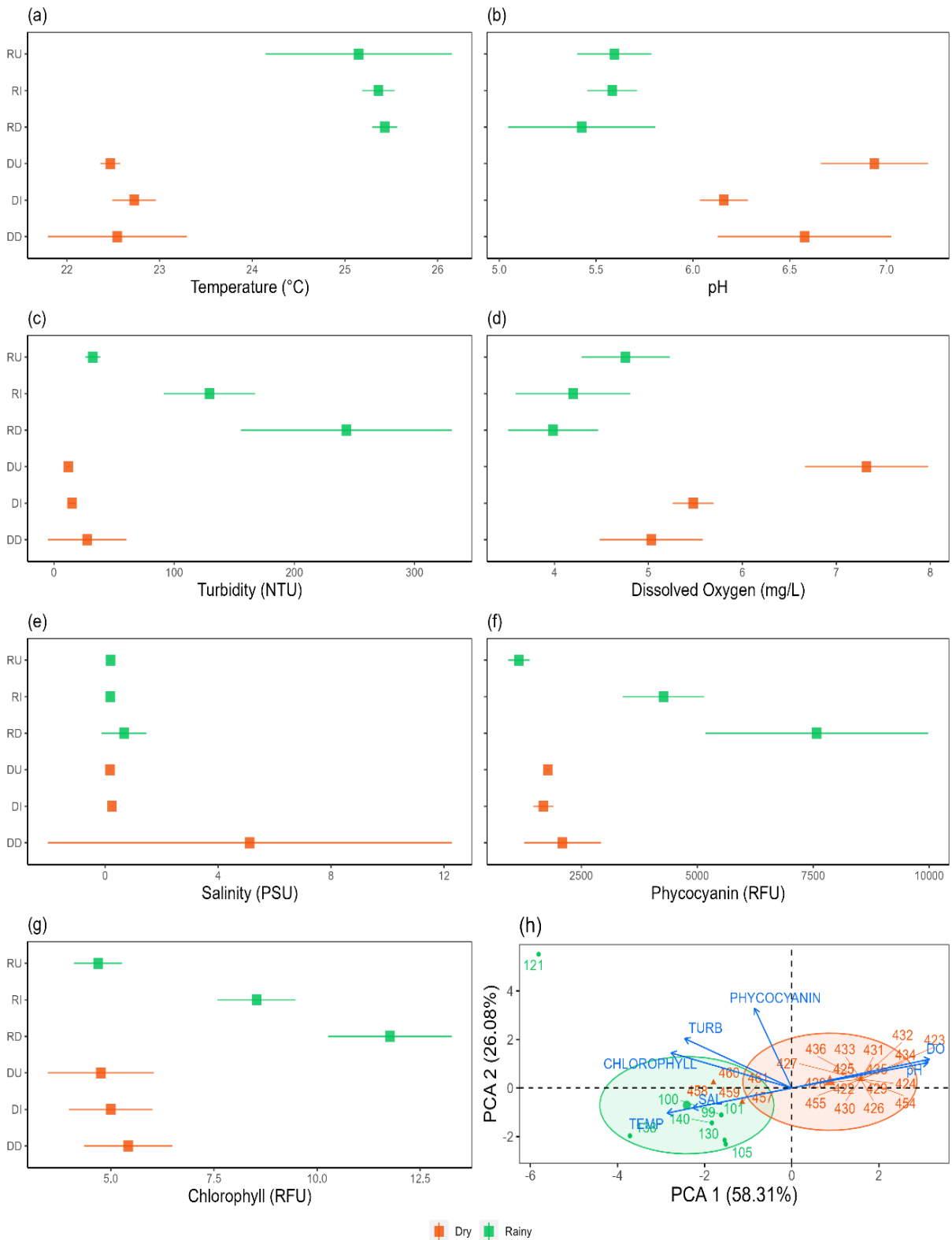


Figure 24 – Mean (central square) with standard deviation of temperature (a), pH (b), turbidity (c), dissolved oxygen (d), salinity (e), phycocyanin (f), and chlorophyll (g) during dry and rainy periods along the river. Principal Component Analysis (PCA) and the percentage of inertia explained by the two axes with explanatory variables for the distribution of *C. gariepinus* in the Guapi-Macacu River (h). Dry Downstream (DD), Dry Intermediate (DI), Dry Upstream (DU), Rainy Upstream (RU), Rainy Intermediate (RI) and Rainy Downstream (RD).

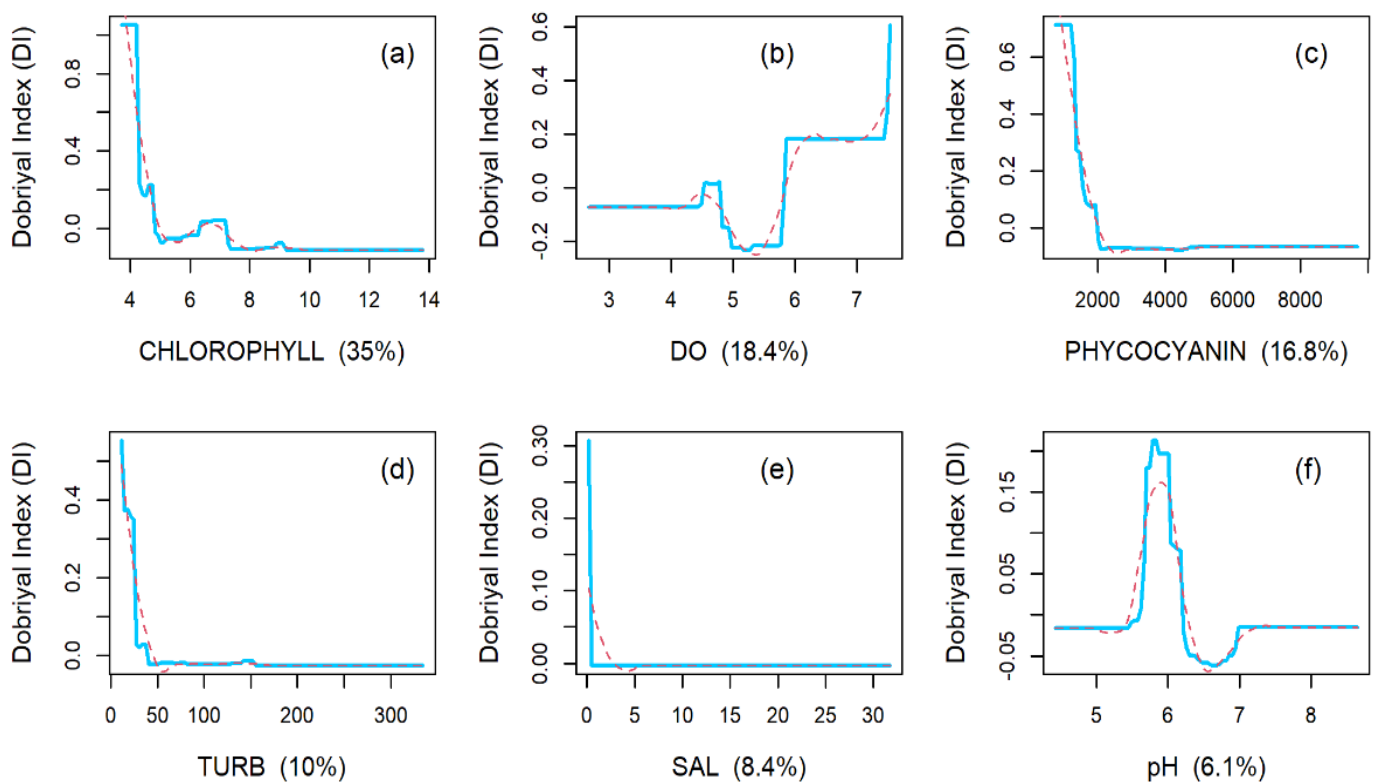


Figure 25 – Relative importance indicating the percentage contributions of the predictors chlorophyll (a), dissolved oxygen (b), turbidity (c), and pH (d), to predict variations in the Dobriyal index for *C. gariepinus* using the BRT model. Fitted functions for each term in the model with main effects of BRT sorted by relative influence value. Dashed red lines indicate 95% confidence intervals for values, estimated from predictions made from 1000 models fitted to bootstrap samples of fish data.

The Boosted Regression Trees (BRT) model demonstrated an overall satisfactory fit to the data, as evidenced by the absence of overfitting, indicated by the average total deviance (3.249) and average residual deviance (1.247). This suggests a capacity to explain approximately 61.6% ( $R^2$ ) of the total variability in the Dobriyal index. However, it is important to note that other factors may not be considered in the model contributing to the remaining variation. Cross-validation (2.316;  $SE=0.47$ ) supported this adequate generalization to new datasets not used during training, explaining 57.2% (adjusted  $R^2$ ) of the cross-validated data. This indicates that the model can make reliable predictions. Figure 25 illustrates the model fit for the relative influence of six abiotic variables. The Dobriyal index showed a decreasing trend with higher values of chlorophyll (35.27%). Dissolved oxygen (18.44%) exhibited an increasing trend in the Dobriyal index with higher values. Phycocyanin (16.79%) showed a decreasing influence on the Dobriyal index, as did turbidity (10.04%) and salinity (8.43%). The pH, with a relative influence of 6.10%, exhibited peaks in the Dobriyal index at pH values

below 7. However, the model showed a low percentage of interaction between variables (< 1.7%).

#### 4.5. DISCUSSION

In general, the reproductive population dynamics of fish are more accurately assessed when greater capture effort is applied over time (Singh 2021). However, due to the constant difficulty in capturing the exotic species *C. gariepinus* in various bioinvaded areas (Erarto e Getahun 2020b), a more intensive sampling effort was chosen in the largest river of the region's watershed (Rangel et al. 2016). Thus, the 64 established collection points along the river allowed for the collection of 30 *C. gariepinus* samples in just two campaigns.

The upstream area of the river was the section where the majority of the collected specimens were captured, mainly using traps, unlike the species' native habitat, where it is easily caught using cast nets (Turan et al. 2005; Ola-Oladimeji et al. 2016; Decru et al. 2016). However, in Brazil, the sampling of juveniles in bio-invaded ecosystems has been comparatively lower than that of adult specimens (Weyl et al. 2016), a trend also reported in India (Tilahun et al. 2016). In this context, the absence of juveniles suggests that the alien species may also benefit from the APA, which, with the presence of mangroves, serves as a nursery and breeding ground for fish, providing shelter for the juveniles. Another factor related to the challenging capture of the catfish in the APA may be associated with the rainy season, during which the increased river volume and high turbidity further contribute to sheltered areas for the species. Consequently, it was not possible to determine the length at first maturation. Most collected specimens were mature, in the C stage of gonadal maturation, indicating that the species finds a conducive environment for reproduction. *Clarias gariepinus* exhibits a more favorable reproductive period in its natural habitat during the rainy season and with an extended photoperiod (Bruton 1979b; Olaleye 2005; Romanova et al. 2018). On the other hand, the species is also observed with mature individuals throughout the year in various ecosystems (Nwadukwe e Ayinla 1993; Legendre et al. 1996), consistent with our findings.

Despite appearing numerically low, the capture of the African catfish is quite prominent compared to other Brazilian ecosystems (Weyl et al. 2016), where the alien species has significantly impacted the aquaculture community (Alves et al. 2007), and become an IAS. Indeed, by examining the abundance of the alien species and native species (Dumith e Santos 2022), we observe that the IAS shows a significant level of abundance. According to the Brazilian Ministry of the Environment, the fish fauna of the Guapimirim EPA and Guanabara Bay hosted around 167 fish species (81 marine and 86 freshwaters) in 2006 (ICMBio). In

addition to the poor water quality which may be related to the concentrations of chlorophyll and phycocyanin observed mainly in the downstream section, the alien catfish may also contribute to this decline, potentially impacting native fauna (Vitule et al. 2009; Weyl et al. 2016) maintaining its population since the 1980s.

The African catfish population showed a higher percentage of male individuals, which is not suitable for reproductive index calculations (Ataguba et al. 2012). However, due to its widespread use in aquaculture (Lisachov et al. 2023), many studies have been conducted to characterize its reproductive biology (Olaleye 2005; Arome Ataguba et al. 2013; Al-Deghayem et al. 2017; Nazneen et al. 2021), revealing distinct characteristics between captive and wild populations (Olaleye 2005; Müller et al. 2019) and facilitating reproductive characterization in both sexes (Adebayo et al. 2012; Arome Ataguba et al. 2013). In this regard, our findings show that, although in a smaller proportion, reproductive indices tend to follow the same pattern for both males and females. Regarding gonadal maturation stages, the study conducted by Singh et al. (2021), observed greater variability in gonadal maturation stages for males than for females, in natural environments, which confirms our findings. Additionally, our studies also agree that the GSI of females is higher than that of males in natural environments where the is considered IAS.

Among the calculated indices to assess the alien species, the Dobriyal index showed the strongest correlation with fish length, likely due to its better accuracy, considering only gonad weight (Rayal et al. 2021). This is in contrast to other indices that take into account the total weight of the fish. Indeed, there was a better correlation between DI and the condition factor for females, which can be explained by the fact that eggs constitute 15 to 20% of the body weight (Megbowon e Fashina-Bombata 2013), due to advanced stages of gonadal development. The IGS is widely employed in various studies, often utilized for calculating the size at first maturation. However, discrepancies exist in its calculations. Conversely, the MSGI is infrequently employed, much like the Dobriyal index, yet it appears to reflect similar patterns to the IGS. The study conducted by Rayal et al. (2021) compared the Dobriyal index and the IGS of Barbo Ticto (*Puntius ticto*), an ornamental fish found in the Aasan River in India. The study demonstrated that the Dobriyal index was the most appropriate for the species, corroborating the findings of Esmacili and Shiva (2006). In their study (Esmacili e Shiva 2006) compared the IGS, MSGI, and Dobriyal Index to determine the gonadal first maturation length of Pupfish (*Aphanius persicus*). This suggests in this sampled population, the reproductive index of *C. gariepinus* may be determined solely by gonad weight (Table 12).

In this manner, the Dobriyal index was compared to other indices that infer the fish's energy reserves (glycogen) and ecological condition. It is observed that glycogen peaks precede the gonadal development phase. However, the condition factor tends to be lower during the peak of gonadal development. This may indicate that, despite a slightly lower ecological condition, the African catfish can store sufficient energy to sustain its gonadal development. The relationships between condition factors and HSI are well-established (Nwadukwe e Ayinla 1993). Our findings suggest that variations between peaks in ecological condition and HSI are more prominent in smaller and likely younger individuals. As the fish grows, the ecological condition tends to decrease, but the peaks in HSI, which relate to the fish's energy reserves, persist (Abdel-Warith et al. 2014). These peaks favor those in the DI, sustaining continuous reproduction regardless of size class and for both sexes. Thus, the results observed for the calculations of reproduction indices (DI, GSI, and MSGI) for *C. gariepinus* denote continuous reproduction, unaffected by past pressures from aquaculture production propagule pressure (Colautti et al. 2006). Therefore, environmental conditions may play a determining role for the species, such as variations in river levels and other abiotic factors (Abdel-Latif et al. 2021). Nevertheless, the species' resilience characteristics were also evident in these growth relationships, even with a low condition factor. In aquaculture systems, it tends to maintain growth under conditions where abiotic factors are unfavorable (Damar et al. 2020), where many fishes could otherwise perish.

The characteristics of the abiotic factors measured in the Guapi-Macacu River indicate that the African catfish population has greater affinities to pH, dissolved oxygen, salinity, and temperature, which are shaped by the influence of dry and rainy periods and the physiographic characteristics of the river (Table 14). The percentage decrease in trees and mangroves in the upstream section may have contributed to the increase in pH and better water oxygenation (Shapiro 1990), improving the environmental conditions of the water. In this context, it is likely that the migration of the African catfish (Mbalassa e Nshombo 2020), occurred in the Guapi-Macacu River to seek optimal environmental conditions for reproduction. However, the alluvial plains of the environmental protection area, which are flooded during the rainy season, tend not to offer optimal oxygenation due to the extensive mangrove vegetation cover. Furthermore, the mangrove also adds to the capture challenge in both periods.

Thus, when we correlate DI with abiotic variables, we observe that these differ somewhat from the relationships indicating the relative influence of these same factors. The most significant variations in pH, found to align with the greatest variations in dissolved oxygen during the dry season, were selected for the distribution of specimens. These variations were

preferred in sections where better conditions were observed, particularly upstream (Pokharel et al. 2018), highlighting the higher percentage of specimens encountered (Pease et al. 2012). The pH is a crucial abiotic factor for fishes the variation of which can influence the regulation of ammonia excretion (Wilkie e Wood 1996). For the BRT model, despite its low relative influence, our findings suggest that values close to a pH of 6 may favor its reproductive peak, aligning with previous studies described for the species (e.g: Aigbogun et al., 2015; Marimuthu et al., 2019). However, dissolved oxygen exhibited a more pronounced relative influence on DI than pH. *Clarias gariepinus* is often found in conditions of deficient dissolved oxygen levels in the water (Oké e Goosen 2019; Ariole et al. 2021; Davies Ibienebo Chris et al. 2022; Nwizugbo et al. 2023), and even in mud at times (Papuc et al. 2019), owing to its specialized aerial respiratory organ (Jimoh et al. 2020; Monteiro et al. 2021). It is possible, therefore, that the African catfish may require improved concentrations of dissolved oxygen for optimal gonadal development and reproduction.

Temperature is a critical factor for the reproduction of *C. gariepinus* (Ukwe e OM Abu 2016; Müller et al. 2019; Romanova et al. 2020), however, our BRT model exhibited a low relative influence of this factor (5.14%), likely due to the already elevated temperature in the Guapi-Macacu River throughout the year. The characteristics of temperature variations and the low amplitude of salinity variation were more pronounced during the rainy season, favoring the bioinvasion and dispersion of this alien species in the river (Gutierre et al. 2014). This is linked to the fact that the African catfish triggers its migratory and reproductive cycles with an increase in water temperature (Kadye e Booth 2013). Moreover, the period of intense rainfall is also associated with an increase in temperature in southeastern Brazil. Thus, the combination of these factors not only promotes the migrations and reproduction of the species but may have influenced the relationship with temperature in the BRT model. Additionally, the effects of climate change, already more pronounced in smaller water bodies (Pachauri e Reisinger 2007; Rahel e Olden 2008) may have contributed to the maturation of individuals, favoring early sexual differentiation (Bruton 1979b; Santi et al. 2016; Romanova et al. 2020). In this context, the relationship between the seasonal period and the reproductive season of the species is well associated with temperatures above 26°C (Romanova et al. 2018), indicating sexual differentiation above 28°C (Santi et al. 2017) mainly found in the upstream area of the Guapi-Macacu River. This may have favored the higher number of male individuals in our findings. In contrast to temperature, higher salinity values hurt the reproduction of this alien species (Borode et al. 2002; Olumuji e Mustapha 2012; Amachree et al. 2018; Zidan et al. 2022), proving lethal for *C. gariepinus* at salinities close to that of seawater (Gutierre et al. 2014).

Thus, it is likely that the increase in salinity downstream has contributed to the lower catch in this area.

Parameters such as phycocyanin, chlorophyll, and turbidity, directly related to water quality (Damar et al. 2020) and catfish feeding habits (Tesfahun 2018), allowed inference about environmental aspects influencing the environmental quality of the Guapi-Macacu River and were also correlated in the PCA. Turbidity is directly associated with the proliferation of algae in water bodies (O'Neil et al. 2012). However, this factor also serves to facilitate the feeding of *C. gariepinus* (Dawah e Gomaah 2005; Tesfahun 2018), enhancing its ability to capture prey (Ward e Vaage 2019). Chlorophyll measures the concentration of algae in the water body (Sadeghian et al. 2018; Watanabe et al. 2018). However, the characteristics of water quality depend on the species of algae present. In many cases, when there is rapid growth of blue-green algae, the environment may already be contaminated (Yan et al. 2018; Wang et al. 2022; Xu et al. 2022). Phycocyanin is the exclusive accessory pigment of freshwater blue-green algae in productive inland waters (Randolph et al. 2008), requiring phosphorus and nitrogen dissolved in water to reproduce (Pérez-González et al. 2021). However, eutrophic bodies tend to increase the supply of these nutrients, favoring the proliferation of such algae (Carmichael 1994). These factors may be correlated and also contribute to the feeding habits of the alien species (Dumith e Santos 2023), but appear to be contrary to his reproduction.

Indeed, among the rivers of the Guapimirim EPA, the Guapi-Macacu presents the best environmental condition, although it has experienced a decline in water quality over many periods (Costa et al. 2018). In this regard, the BRT model indicates an inverse relationship in the relative influence of environmental conditions associated with these three abiotic factors (phycocyanin, chlorophyll, and turbidity), showing a less favorable tendency towards the DI of the African catfish. On the other hand, conditions with better-dissolved oxygen and pH are more conducive to the optimal development of the species' gonads. Meanwhile, temperature and salinity contribute to suitable conditions for the species' maintenance in the river. Thus, it is likely that parameters related to water quality negatively impact the species' reproduction, suggesting that it may be vulnerable to a deterioration in the environmental quality of the Guapi-Macacu River (Dumith e Santos 2023).

#### **4.6. CONCLUSION**

Our findings indicate that the Guapi-Macacu River provides favorable conditions for the reproduction of the African catfish, with a good flow amplitude between dry and rainy periods and the necessary photoperiod for gonadal development. The abundance observed for the

species, comparatively higher than in other regions of Brazil, signifies a potential risk to native species. Additionally, the species seems to benefit from constant elevated temperatures, recorded throughout the year and expected to intensify due to climate change. On the other hand, water quality is a determining factor in the development of the species' gonads, highlighting the need for better conditions for reproduction. Overall, analyses of the African catfish population suggest that the species has adapted well to the environment over 40 years, selecting areas where environmental parameters are more conducive to reproduction. As a result, we suggest that public policies and environmental agencies encourage the capture of the catfish, especially in the upstream area. This action would enable the control and monitoring of the reproductive activities of the IAS, as well as reduce its abundance. Therefore, constant studies of the *C. gariepinus* population in the Guapi-Macau River, as well as in the other rivers that compose the Guapimirim EPA, are necessary.

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## CONCLUSÃO GERAL

No rio Guapi-Macacu a distribuição e abundância da ictiofauna, denotam fortes influências dos fatores físico-químicos da água, que variam conforme os períodos chuvoso e

seco. Os trechos do rio estão segregados, de acordo com as variáveis abióticas: trecho a Montante – maior oxigênio dissolvido; Intermediário - maior transparência da água; e a Jusante - maior concentração de pH.

O bagre africano, habita preferencialmente áreas a montante do rio e sua participação foi notória e preocupante, principalmente em relação à sua abundância e seu grande porte, além de ser considerado um predador voraz, de elevada capacidade de deslocamento e resistência a permanecer fora da água, representando assim, uma ameaça significativa para esse ecossistema. A baixa abundância e riqueza das espécies nativas dulcícolas, na APA de Guapimirim é menor, quando comparadas com trabalhos anteriores, a implantação da piscicultura desativada na região. Percebe-se na prática que o rio, já está sofrendo com a bioinvasão e a degradação ambiental, que vem da Baía de Guanabara. Neste sentido, o bagre africano pode ser considerado, um bioindicador do rio Guapi-Macacu, podendo ser futuramente apresentado como um monitoramento ambiental da área.

A baixa abundância de *R. quelen* e *T. striatulus*, espécies de bagres nativas e equivalentes, que apresentam a mesma distribuição do bagre africano, indica competição por alimento e habitat entre elas. Apesar das três espécies serem onívoras, não houve sobreposição de nicho entre elas. O bagre africano apresenta maior plasticidade na dieta, sendo mais generalista, já o *T. striatulus* apresenta uma dieta mais insetívora e o *R. quelen* uma tendência a piscivoria. Embora as duas espécies nativas, apresentem maior fator de condição, quando comparadas ao bagre africano, elas se encontram com menor abundância nesse ecossistema.

Dentre os nossos resultados inéditos, está a comprovação da origem de *C. gariepinus* que habita o Rio Guapi-Macacu. Os dados moleculares indicam que a população invasora tem origem na República Democrática do Congo e podem ajudar a traçar o histórico de introdução da espécie. Aliado ainda as características morfométricas (diâmetro dos olhos, comprimento da cabeça, comprimento do pedúnculo caudal e tamanho da boca), os resultados sugerem que as populações invasoras no Brasil são consistentes com as de origem de cativeiro, confirmando as informações colhidas de pescadores e residentes locais, sugerindo única fonte de introdução.

O rio Guapi-Macacu evidencia, o período de seca e cheia independente da época do ano, sendo crucial para a reprodução do bagre africano. Além disso, entre as características ambientais propícias a sua reprodução, a elevada temperatura é um fator determinante. O rio Guapi-Macacu e sua localização geográfica, mantêm temperaturas mais constantes, e ainda aliados aos fatores de aquecimento global, ambiente de mangue e vegetação densa, proporcionam um local adequado para a reprodução da espécie, durante todo o ano. Há também evidências que, a qualidade da água, pode ser considerada crucial para o desenvolvimento de

gônadas, sendo esta, inversamente proporcional a fatores como clorofila, ficocianina e turbidez, indicativos de perturbações adversas no corpo hídrico.

Diante desses desafios, é urgente implementar políticas públicas e ações ambientais que incentivem a captura do bagre africano, principalmente na área a montante do rio Guapi-Macacu, bem como, nos demais rios que compõem a APA de Guapimirim. Além disso, a implementação de programas de monitoramento contínuo, são necessários para avaliar o impacto dessa espécie invasora e adaptar as estratégias de manejo. A colaboração entre cientistas, gestores ambientais e formuladores de políticas públicas é crucial, para desenvolver e implementar estratégias eficazes de manejo e conservação. Uma das ações mais eficazes para esta medida, seria a sinergia entre regulamentações do setor privado, atividades educacionais, e principalmente alternativas sustentáveis para a criação de espécies nativas, além de investir mais amplamente na capacitação e ação para o manejo de espécies invasoras não nativas (ver Dechoum<sup>1</sup> et al. 2024), com a adequada disseminação de informações de alta qualidade para a sociedade, o que no momento, tem sido incompatível, com a realidade encontrada no Rio Guapi-Macacu.



## Ichthyofauna Structure at Risk due to the Bio Invasion of *Clarias gariepinus* in a River at Guapimirim Environmental Protection Area, Southeastern Brazil

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### ABSTRACT

The African sharp-toothed catfish (*Clarias gariepinus*) is the most important catfish species for aquaculture. It has a great capacity to endure several stressing factors, such as adverse environmental conditions, besides having broad feeding flexibility. Notwithstanding, the presence of this alien fish species in the Guapimirim Environmental Protection Area, in southeast Brazil, may have negative impacts on the community of native fish. In 2018, during dry and rainy seasons, samples from the fish community were collected in 32 sites of the Guapi-Macacu River, in addition to abiotic variables (salinity, pH, temperature, turbidity, dissolved oxygen, and transparency) to diagnose which factors influence the distribution of the alien fish along the river. Moreover, species were evaluated as bio indicators in the ichthyofauna to identify potential alterations in the community. Multivariate analyses indicated that the African sharp-toothed catfish dominates the buffer zone of the environmental protection area, benefiting from higher levels of dissolved oxygen and temperature. However, *C. gariepinus* still does not dominate the most protected area of Guapimirim, where a higher percentage of native fish species dwell. Alterations in abiotic factors, related to the increase in the temperature, can significantly contribute to the dominance of this invasive alien fish in this protected area, requiring constant monitoring of some key species, as well as the population of the invasive species in this environmental preservation area.

**Keywords:** Invasive alien fish; Overlapping habitats; Abiotic variables; Bioindicators; Climate change

### INTRODUCTION

Invasive Alien Species (IAS) is considered one of the main threats to biodiversity and an important component of global environmental change [1]. Once present and acclimated, the IAS becomes difficult to control and results in marked changes in the native fauna, compromising the balance and stability of the ecosystem by reducing their stocks and causing extinctions, competition, pathogen transmission, and hybridization [1-5].

The African sharp-toothed catfish - *Clarias gariepinus* is a native species to much of the African continent and parts of southwest Asia, such as Israel, Syria, and southern Turkey [6,7]. It has the exceptional ability to migrate to the terrestrial environment and breathe atmospheric air through pseudolungs, which allows it to endure adverse conditions of temperatures and low oxygen concentrations [7]. *Clarias gariepinus* was introduced in Brazil in the 1980s for aquaculture and fish-and-pay lakes [8]. The escape of the African catfish from aquaculture systems to the natural environment has threatened several species of native fish, making it a potent IAS for these ecosystems [9]. The potential invasions of *C. gariepinus* have shown that the species can progressively establish itself depending on the source of the invader's dispersion, causing a reduction in

food availability and pressure on the ecosystem's trophic chain [10-12]. Added to this factor, the invasiveness of the African catfish can happen in a short period, resulting in malnutrition, low growth, or even elimination of some native species [13]. In other cases, unplanned hybridization, with an escape into the natural environment, can cause the irreversible loss of native biodiversity [14].

The Guapimirim Environmental Protection Area (Guapimirim APA) is located northeast of the Guanabara Bay, one of the most environmentally critical coastal areas of the Brazilian coastline, in Rio de Janeiro, Brazil. The Guapimirim APA is a Federal Conservation unit, created in 1984 to protect the remaining mangrove and to ensure the survival of human populations that still depend on this environment. The Guanabara Ecological Station (Guanabara ESEC) is located within the APA. The ESEC is a fully protected Conservation Unit, with no admittance of people within its borders, except for scientific or educational purposes. The Guapimirim APA has several rivers and canals; among them, the Guapi-Macacu River stands out, due to its water output, supplying drinking water to most municipalities in its eastern portion [15]. In this river, in particular, the riverine population is aware of the presence of the African catfish; however, we know little about its

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# Use of trophic ecology of omnivorous fish and abiotic factors as supporting tools for assessing environmental impacts in a neotropical river

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## Abstract

The study of diet is one of the mechanisms by which competition for resources between species that cohabit in the same ecosystem can be inferred. Therefore, the relationships of the indices that measure specialization in the diet of fish species are necessary to characterize the nutritional quality of these populations and the ecosystem's environmental health. Three species of catfish were selected: one invasive (*Claris gariepinus*) and two natives (*Trachelyopterus striatulus* and *Rhamdia quelen*), with similar distribution along the Guapi-Macacu River, in the Guapimirim Protection Area (Rio de Janeiro). Fifty-nine catfish of the three species were collected in total, along 32 collection points in the Guapi-Macacu River in two periods (dry and rainy) in 2018. Non-parametric statistics showed the partition of resources between species and the influence of abiotic factors (temperature, pH, transparency, and dissolved oxygen) contributing to the selection of available resources in the environment. Diet-related indices—repletion index (RI), condition factor (K), niche width, and trophic position (TP) of the specimens collected—contributed to measuring the nutritional status of each of these catfish species, showing that *R. quelen* has a relationship between RI and K, tending to absorb and metabolize nutrients faster than other species. In addition, the invasive species occupies a wide range of TPs compared to native species, confirming its feeding plasticity. On the contrary, *T. striatulus* needs large amounts of terrestrial insects to maintain its poor condition factor. Also, the RI showed direct influences of abiotic variables, with the temperature being the most prominent. Our results suggest that the invasive species can benefit from this environment that shows signs of environmental degradation.

## KEYWORDS

environmental impacts, invasive alien fish, null model, repletion index, Siluriformes, trophic position

## 1 | INTRODUCTION

Fish is one of the main bioindicators in aquatic ecosystems (Chovanec et al., 2003; Duarte et al., 2020; Kılıç & Yücel, 2022), being effective for assessing various types of environmental contamination (Araújo et al., 2018; Authman, 2015; Freitas & Siqueira-Souza, 2009). Thus,

the assessment of trophic ecology is a powerful tool for describing the dynamic interactions between organisms and their environments (da Silveira et al., 2020). And studying the diet of fish species in preserved areas also serves as a tool to assess the environmental and especially aquatic health of these areas (da Silva et al., 2008). Such information provides subsidies to understanding the mechanisms that



## Genetic origin and morphometric traits of invasive African catfish (*Clarias gariepinus*) in a protected area in southeastern Brazil

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**Abstract** The African catfish *Clarias gariepinus* is a freshwater species native to nearly all of Africa and a small part of Asia. Due to being a generalist species, highly fertile and able to survive in a wide range of environmental conditions, the African catfish has become invasive in many non-native systems where it was introduced, bringing negative impacts to wild ecosystems. In Brazil, *C. gariepinus* was introduced from Africa for aquaculture in 1986 and established

itself in the wild within a decade, after escaping from aquaculture ponds and being further translocated to be used in recreational fishing. As further evidence of the expansion of this invasive species in Brazil, we sequenced the mitochondrial control region and cytochrome b markers to confirm the occurrence of *C. gariepinus* in a major river in a protected area in the southeastern region, and to assess its genetic diversity. Morphometric data was also collected to aid species identification and to compare to other invasive populations in Brazil. The analysis of 24 specimens revealed a single haplotype for each marker. The single cytochrome b haplotype is identical to a haplotype from the Democratic Republic of the Congo, unveiling the possible genetic origin of the *C. gariepinus* lineage that invaded Brazil. Morphometric data suggests consistency in the morphometric traits of *C. gariepinus* invasive populations in Brazil. The assessment and monitoring of the distribution of *C. gariepinus* in non-native areas are crucial for the maintenance of native freshwater biodiversity and ecosystem functioning worldwide.

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**Keywords** Invasive fish · Clariidae · Morphometry · Mitochondrial DNA · Neotropical

### Introduction

Catfishes of the genus *Clarias* Scopoli 1777 (Siluroidei, Clariidae) are freshwater species with a