

**WADMILTON MANUEL DA TRINDADE FERNANDES**

**ANALYSIS OF MACROFAUNAL DIVERSITY IN FLUVIAL HABITATS  
OCCUPIED BY *PEIXINHO* IN SÃO TOMÉ: AN APPROACH TO  
UNDERSTANDING BIODIVERSITY**



**UNIVERSITY OF ALGARVE**  
Faculty of Science and Technology

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**Master's Degree in Biodiversity, Fisheries, and Marine Conservation**

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**Declaração de autoria de trabalho/ Declaration of work authorship**  
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I hereby declare to be the author of this work, which is original and unpublished. Authors and works consulted are properly cited in the text and included in the reference list.

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*“Education is the most powerful weapon which you can use to change the world.” - Nelson Mandela*

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## **Abstract**

River ecosystems provide various ecosystem services, such as support, provision, and regulation. In São Tomé and Príncipe, the services provided by rivers contribute to the food security of many families. However, little is known about the biodiversity in these habitats; for example, there is still no checklist of species. In this study, a biodiversity survey was conducted in the rivers of São Tomé Island to describe their macrofaunal composition. Various techniques, including traps and snorkeling, were used to record species. Sixteen species belonging to 11 families were documented, including six fish species, five crustaceans, three insects, and two mollusks. The macrofaunal composition varied between and along the rivers. This study enhances our understanding of the biodiversity in these ecosystems, providing a foundation for future research and conservation efforts.

**Keywords:** *Sicydium spp.*, rivers, ecosystems, biodiversity indexes, beta diversity, São Tomé and Príncipe

## Resumo

Os ecossistemas fluviais fornecem vários serviços ecossistêmicos, tais como, suporte, provisão e regulação. Em São Tomé e Príncipe os serviços prestados pelos rios contribuem para a segurança alimentar de muitas famílias. Entretanto, pouco se sabe sobre a biodiversidade nesses habitats, por exemplo, ainda não existe um *checklist* das espécies. No presente estudo, fez-se o levantamento da biodiversidade nos rios da ilha de São Tomé para descrever a sua composição macrofaunística. Para tal, foram usadas várias técnicas, tais como, armadilhas e snorkeling para registrar as espécies. Foram registadas 16 espécies pertencentes a 11 famílias. Os peixes (seis espécies) da diversidade, crustáceos, (cinco espécies), insectos representam (três espécies) e moluscos representam (duas espécies). A composição macrofaunística variou entre e ao longo dos rios. Este estudo enriquece o conhecimento sobre biodiversidade desses ecossistemas criando uma base para investigações futuras bem como para acções de conservação.

**Palavras-chave:** *Sicydium spp.*, rios, ecossistemas, índices de biodiversidade, São Tomé e Príncipe.

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## **List of abbreviations**

GGOI – Gulf of Guinea Oceanic Islands

INE- Instituto Nacional de Estatística

IUCN- International Union for Conservation of Nature

SD – Standard Deviation

RPF- Rio Paga Fogo

RLM- Rio Lembá

RIO – Rio Iô Grande

RMM – Rio Martim Mendes

## **1. Introduction**

### **1.1. Biodiversity: concept and importance**

Biodiversity, or biological diversity, is a fundamental concept in ecology and nature conservation. It refers to the diversity of life, including diversity within species, between species and ecosystems (Magurran, 2010). But they also include species richness and genetic diversity (Maclaurin & Sterelny, 2009). It represents the web of life from microorganisms to plants and animals (Barbero & Maffei, 2016). Biodiversity plays an important role in maintaining ecosystem stability, provision of ecosystem services and resilience to environmental challenges (Balvanera et al., 2006; Szövényi, 2011). It also allows recording temporal changes in species composition (Hooper et al., 2005).

Ecosystem services provided by biodiversity can be supportive, provisional, regulatory, and cultural (Booi et al., 2022). Aquatic environments provide various ecosystem services, including livelihood benefits, irrigation water, improved soil fertility, and habitat provision for aquatic animals and plants (Phukan, 2021). In addition, they include resistance to biological invasion, disease regulation, seed dispersal, pollination, and water purification (Alahuhta et al., 2013). These services are vital, providing natural resources essential to our survival and well-being.

### **1.2 The main factors that influence biological diversity**

Recent studies have highlighted several abiotic and biotic factors that influence biological diversity in aquatic ecosystems. Hydrodynamic exposure, water temperature, depth, flow velocity, climate the benthic community and primary productivity are some of these factors (Baptista & Leitão, 2014; Floeter et al., 2007; Baptista et al., 2014).

Aquatic fauna faces more significant threats compared to terrestrial fauna (Wiens, 2016). Between 1970 and 2016, there was a decline in freshwater biodiversity, with an estimated reduction of  $\approx 84\%$  (Maasri et al., 2021). Several factors contribute to this reduction, many of which are attributed to human activities as demonstrated by the study conducted by Reid et al., (2019)

according to this work, the main threats were, over-exploitation, pollution, flow regulation, land use change, invasion of exotic species and climate change.

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Chemical pollution, including organic micropollutants such as pharmaceuticals, pesticides, increased salinization, high nutrient loads, eutrophication process, and increased infiltration of organic waste (Dallas & Day, 2004). The dilution capacity of these pollutants by rivers is being compromised due to changes in water flow and overexploitation of water resources. In addition to the significant reduction in water flow and the resulting dilution capacity, a notable decrease in the abundance of microorganisms such as algae, fungi, and bacteria has been observed in many river systems, despite their importance in removing harmful pollutants and as a food source for primary consuming organisms, such as some macroinvertebrates (Burgis, 1999). In addition, agricultural and industrial activities also have a significant impact on water quality, influencing the rate of sedimentation, turbidity and introducing pollutants such as fertilizers, hormones, antibiotics and biocides into aquatic ecosystems (Burgis, 1999)..

According to Dudgeon et al. (2005), the invasion of exotic species increases the effects of physical and chemical impacts caused by humans on freshwater ecosystems due to the ability of these species to invade and thrive in degraded or modified systems. Invasive species may include animal, plant, or microbial organisms that have the ability to profoundly alter aquatic environments by competing, preying, and displacing native species (Dudgeon et al., 2005). Although not all alien species become invasive, they pose a serious threat to aquatic ecosystems (Hulme, 2009; Martin & Coetzee, 2011; Padilla & Williams, 2004).

### 1.3 The biodiversity of the oceanic islands of the Gulf of Guinea

The Gulf of Guinea region is composed of four main islands: Ano Bom, São Tomé, Príncipe and Bioko, the latter being considered a mainland island. The island of Príncipe, São Tomé and Annobón, have never been linked to each other or to the mainland. As a consequence, they present high levels of endemism, particularly in plants, birds, reptiles and amphibians (Jones, 1994).

Bioko Island is an island with an area of 2027 km<sup>2</sup>, approximately 35 km by 72 km in dimension. It is located on the continental shelf at a distance of 32 km from the coast of Cameroon, separated by a sea with a depth of 60 meters. During the most recent glacial periods, Bioko has undergone repeated isolation and connection cycles (Ali, 2018) and more recently has been connected for about 11,000 years (Lambeck & Chappell, 2001). The highest point on the island, Pico Basilé, rises 3011 meters above sea level.

Consequently, Príncipe Island, which has a total area of 139 km<sup>2</sup> (approximately 17 km by 8 km), is situated at a distance of 210 km southwest of Bioko and 220 km west of the coast of the African continent (Ceríaco et al., 2022). This island has six main islets: Pedra da Galé, Mosteiros, Bom-Bom, Carço, Tinhosa Grande and Tinhosa Pequena. The highest point on the island, Pico do Príncipe, rises to 942 meters above sea level.

The Island of São Tomé, which covers a total area of 857 km<sup>2</sup> with dimensions of approximately 47 km by 28 km, is situated at a distance of 150 km southwest of Príncipe Island and 255 km west of Gabon (Ceríaco et al., 2022). This island is surrounded by several islets, such as Cabras (located in the north), Santana (in the east), and Sete Pedras e Rolas (located in the south). The highest point on the island is Pico de São Tomé, with an altitude of 2024 meters above sea level.

Annobón Island, with an area of 17 km<sup>2</sup> (6 km by 3 km), is the smallest and most isolated of the islands in the Gulf of Guinea. Located 180 km southwest of São Tomé and approximately 340 km from the mainland, this island has the highest peak, namely Santa Mina, with an altitude of 610 meters above sea level.

The islands of São Tomé, Príncipe and Annobón are widely recognized as global priorities for biodiversity conservation due to their unique characteristics (Roberts et al., 2002). Studies to understand biodiversity on these islands have been conducted for centuries, but have intensified in recent years (da Costa et al., 2022; Jones, 1994; Soares et al., 2022). On the islands of São Tomé and Príncipe, biodiversity is characterized by low species diversity, but associated with a high rate of endemism (Floeter et al., 2007; Hachich et al., 2015).

The relationships between species are often complex, but they constitute the structure that sustains biodiversity, making the study of these interactions fundamental to understanding the functioning of ecosystems (Thébault & Fontaine, 2010). However, due to the need for a detailed knowledge of the basic aspects of species ecology and the difficulty of quantifying them, few interactions between species have been investigated in depth on these islands (Rodrigues et al., 2017).

Recent studies on the ichthyofauna of the Oceanic Islands of the Gulf of Guinea (GGOI), revealed the presence of 268 species of coastal fish, approximately 12% of which are endemic to these islands. Wirtz et al. reported in 2007, 59 new records of coastal fish for the islands of São Tomé and Príncipe, including 10 new species that have not yet been described. Among the endemic species of São Tomé and Príncipe are *Clepticus africanus* and *Scorpaena annobonae* (Wirtz, 2017; Wirtz et al., 2007).

Several studies followed in the same area. A new species of *Gorogobius*, *Gorogobius stevcici*, was described in São Tomé (Kovaić & Schliewen, 2008). A new species of gobids *Didogobius amicuscaridis* spec. nov. was discovered on the island of São Tomé (Schliewen & Kovačić, 2008), Parrotfish (Labridae: Scarinae), *Sparisoma choati* (Rocha et al., 2012), *Serranus pulcher* is described on the islands of São Tomé and Príncipe (Wirtz & Iwamoto, 2016). Additionally, ten new records of marine fish, including three condrictes and seven teleosts, were documented for the first time in São Tomé (Rodrigues et al., 2016), five more records (Reiner & Wirtz, 2016), reports of three new species *Lecanogaster* n. sp., *Enneacampus kaupi* and *Monochirus atlanticus* (Wirtz, 2017).

A study conducted by Maia (2018) with the aim of describing reef fish assemblages and the benthic community in São Tomé and Príncipe identified a

variety of habitats at depths ranging from 10 to 30 meters. In addition, of the seven species of sea turtles, five of them are found in São Tomé and Príncipe and all are threatened (Ferreira-Airaud et al., 2022).

On the other hand, a study conducted in the São Tomé and Príncipe estuaries reported four new occurrences, *Pomadasys jubelini*, *Bostrychus africanus*, *Dalophis cephalopeltis* and the introduced species *Oreochromis mossambicus* (Félix et al., 2017). In the mangrove of the island of Príncipe, 1533 birds were observed, including 26 species, 10 of them endemic (Huettmann, 2003). In the case of São Tomé, 1942 individuals of 29 species were observed (Félix et al., 2017).

#### **1.4 Impacts of hydrological and climate change on biodiversity and functioning of river ecosystems**

Changes in precipitation and temperature patterns have resulted in extreme weather events such as prolonged heat waves, reduced water flow, or intense flooding. These changes are significantly affecting the temporal patterns of water flow in river networks in various regions of the world (Messenger et al., 2021) including the islands of the Gulf of Guinea.

An increase in the duration of periods of low water flow is observed, leading to a gradual transition to slow-flowing phases in river systems (Sabater, 2008), or even complete interruption of flow (Messenger et al., 2021). These hydrological changes also have impacts on sedimentological processes (Inman & Jenkins, 1999) and contribute to the increase in water temperature (Kaushal et al., 2010). On the other hand, major flood events have become more frequent (Arnell & Gosling, 2016). In São Tomé there was in 2019, the largest flood in the last thirty years with significant damage including loss of human life, landslide and destruction of infrastructure.

These floods can cause changes in the geomorphological configuration of river networks and, when they occur catastrophically, have lasting impacts on river biodiversity (Woodward et al., 2015). Therefore, it is evident that extreme hydrological and climatic events have the potential to affect biodiversity and the functioning of river ecosystems. However, a comprehensive synthesis is needed

to better understand the magnitude and direction of these impacts (Arnell & Gosling, 2016).

Rivers are highly dynamic environments that house a wide variety of microhabitats and support a wide diversity of species, from microorganisms to the most complex such as fish and mammals. The functional diversity of the communities present in the rivers is crucial for the performance of their functions and for the benefits they provide to society. In addition, the biological diversity of rivers is an indicator of ecosystem health, and their preservation is key to ensuring the functioning of the ecosystem services they provide.

Unfortunately, freshwater biodiversity is threatened by climatic factors and other significant challenges. In addition, the limited dispersal capacity associated with endemism makes river organisms highly vulnerable to anthropogenic impacts (Dudgeon, 2014). Therefore, it is crucial to understand variations in biodiversity trends in response to biotic and abiotic threats (Dudgeon, 2014; Tickner et al., 2020).

### **1.5 The hydrographic network and the faunal community of the river habitats of São Tomé and Príncipe**

The hydrographic network of the island of São Tomé extends throughout the national territory. The rivers rise in the center of the island and flow into the slopes. More than ten correspond to large rivers (Figure 1). On the other hand, the island of Príncipe has less than 10% of the rivers of the national territory. These basins are linked to rivers that have lengths ranging from 5 to 27 km and are located at altitudes of 1000 to 1500 meters (de Oliveira & Lima, 2017).



Greeff, 1884. Little is known about the bio-ecology of the São Tomé *peixinho*. It is known that the diet of the post-larvae of *Sicydium bustamantei* includes zooplankton and macroalgal detritus as the main sources of organic matter (Baptista et al., 2020). They are most common on oceanic islands with steep rivers, due to their ability to disperse and colonize newly formed or highly disturbed habitats (Keith et al., 2011; McDowall, 2007; Thuesen et al., 2011). Adults have a herbivorous diet, facilitating access to abundant food in impoverished environments (Benstead et al., 2009).

### **1.7 The habitats of *peixinho***

The *peixinho* is an amphidrous species that needs the connection between different ecosystems (Myers, 1949). The fish's habitats include estuaries (Haroun et al., 2018), ocean, and rivers. Newly hatched larvae reproduce in rivers and depend on water currents to reach pelagic habitat (Luton et al., 2005; McDowall, 2009). During this phase, they feed on zooplankton, undergo physical transformations, and become juveniles (Kondo et al., 2013). When they reach an adequate size, these juveniles perform a second migration back to the rivers (Boehlert, 1988; Keith, 2003).

These migrations provide artisanal and recreational fisheries, with patterns related to the lunar phase and seasons (Baptista et al., 2020; Teichert et al., 2012; Valade et al., 2009). The post-larvae are caught using mosquito nets or traditional traps constructed from vegetable fibers called tchangas. This fishing activity is not regulated, the volume of catch at each stage is unknown, which may lead to overexploitation.

In this way, it is of utmost importance to conduct comprehensive studies of the rivers in order to document all the biological diversity that these systems support. At the same time, it is critical to understand and ensure the security of our water resources for future generations. Despite recent advances in biodiversity research, there are still knowledge gaps to be filled. To date, there is no checklist of the species that occur in the rivers of São Tomé and Príncipe. It is necessary to continue investigating the interactions between species, the ecology of different habitats and the impacts of human activities on the conservation of these ecosystems. In this context, the present work is intended to contribute to

increasing knowledge about the biology and ecology of *peixinho*. It discussed the macrofaunistic diversity of the rivers occupied by the *peixinho* on the island of São Tomé aiming at a better understanding of its biodiversity.

Aware of the lack of scientific research in the rivers of São Tomé, the following question was defined: What is the macrofaunistic diversity in the fluvial habitats occupied by the *peixinho* in São Tomé?

### **1.8 Objectives**

The main objective of this study is to contribute to the understanding of biodiversity in fluvial habitats occupied by *Sicydium spp.* in São Tomé, from a macro faunal approach. To achieve this main objective, the following specific objectives were outlined: (i) to describe the biological community that interact in these habitats, (ii) to verify whether this community differs in the different habitats, (iii) to physically characterize the habitats of *Sicydium spp.*, (iv) to create the pattern of distribution or occurrence of the species along the river and (v) to test the influence of environmental factors on the population density in the different habitats.

### **1.9 Hypotheses**

The following hypotheses were defined for the present study:

I. The macrofaunal diversity in the river habitats occupied by the São Tomé *peixinho* varies significantly between the different rivers.

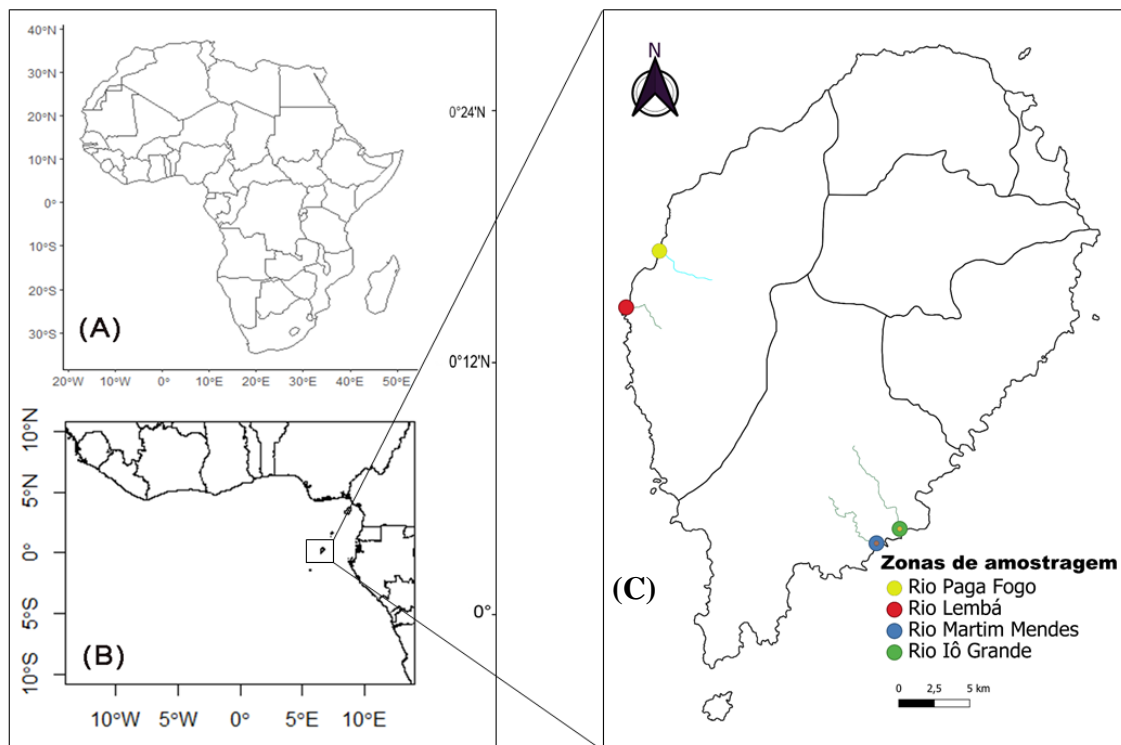
II. The macrofaunal diversity in the occupied river habitats of São Tomé fish presents variations along the rivers

## 2. Materials and Methods

### 2.1 Description of the Study Area

The Democratic Republic of São Tomé and Príncipe is one of the youngest and smallest countries in Africa, it is composed of two main islands and several islets. The islands of São Tomé and Príncipe are located at equatorial latitudes (between the meridians 1044'N and 0001'S, and the parallels of 6028'E and 7028'E) (Maia et al., 2018). São Tomé is on the Cameroon volcanic line in the Gulf of Guinea to western Africa with the coordinates (0°20'94"N; 6°62'10"E) (Maia, et al., 2018) (Fig.2).

Sampling was carried out in four rivers of São Tomé (Figure 2), two in the West region (Paga Fogo and Lembá rivers) and two in the South (Iô grande and Martim Mendes rivers).



**Figure 2:** Location map of the island of São Tomé in the Atlantic Ocean: (A) African continent; (B) Gulf of Guinea; (C) São Tomé Island with its sampling points.

## 2.2 Data collection

The selection of rivers was based on two aspects. (1) the rivers needed to be colonized by the *peixinho*, (2) a river with a larger watershed than the other was selected for each region and its relevance in relation to possible impacts and sources of pollution, aiming to represent different ecological conditions. The sampling campaigns were carried out in the dry season (May 2022) and rainy season (January 2023). Four different methods were used to evaluate the macrofaunistic diversity (Figure 3): traditional traps made from palm fiber called txangas, snorkel (direct observation), hand net (shrimp) and hand trawl net (redinha).



**Figure 3:** Sampling methods used in the sampling of rivers in São Tomé: A) txangas, B) hand trawl net (redinha), C) snorkel (direct observation) and D) hand net.

The sampling points were marked with the help of a GPS, from the mouth of each river, at regular intervals of 500 meters. This approach allowed the coverage of different parts of the rivers, encompassing possible variations in macrofaunal diversity along each watercourse. At each sampling point, three tchangas were placed to capture the local macrofauna. The traps were carefully positioned along the banks of the rivers, choosing places where the current is milder, favoring the ascent of *Sicydium* spp. This distribution of the traps allowed to capture representative specimens of the river habitats occupied by these organisms. The traps remained set at each sampling point for an average period of 4 hours, ensuring sufficient time for the capture of a variety of species present in the environment.

The other sampling method used was the hand trawl. This technique consists of dragging a net along the bottom of the river to capture the aquatic fauna present in different water columns. The trawl was used in addition to the other methods to obtain a more comprehensive sampling and capture organisms that may be present in deeper layers of the river or where the flow of the current is greater. Trawling with the net was performed at each sampling point, covering an area of approximately 10 meters along the riverbed. During trawling, the net traveled to different depths, allowing the sampling of organisms that inhabit both the bottom of the river and the upper layers of the water column. The sampling net was placed against the current while the substrate was moved, directing the macroinvertebrates into the network.

In the appropriate places, the snorkel technique was adopted to perform direct observations of the aquatic macrofauna. Using an underwater still and video camera, submerged images were recorded to complement the information collected. This approach allowed a more detailed observation of the aquatic environments and the diversity of species present, contributing significantly to the characterization of river habitats. The integration of visual data, combined with the other methodologies, provided a more complete and comprehensive analysis of the macrofaunal community in these rivers.

Additionally, a hand net with a mesh of 0.05 cm and a width of 25 cm was used to capture macroinvertebrates. During sampling, all habitat types were considered, including lentic and lotic areas, margins and center of the channel,

as well as different types of sediments, such as gravel, sand, clay and macrophytes. Overall, the combination of the four methods allowed sampling to be covered for various habitat types (e.g., lentic and lotic areas, margins and center of the channel) and different types of sediments.

The organisms were stored in the field in labeled vials containing alcohol (70%) for further screening and identification up to the family level. The classification of all organisms was performed using identification guides (Haroun et al., 2018; Pezold et al., 2006; Wirtz, 2003). To assist in the identification of the organisms, the researcher Dr. Peter Wirtz (CCMAR) was assisted.

All sampling and data analysis procedures followed ethical standards and environmental preservation standards. The samples were captured with due respect for the environment and the local fauna, seeking to minimize the impact of field activities. Individuals were identified up to the species level unless identifiable features were absent, as is the case for certain juvenile stages, due to lack of adequate descriptions or insufficient information for these phases. To characterize the environmental conditions, water parameters were measured, including temperature, salinity and TDS (Total Dissolved Solids). A VWR CO310 multiparametric probe was used to perform in situ measurements at all sampling points. During the rainy season it did not collect turbidity data on the Iô Grande River due to a technical problem in the multiparametric probe. To collect temperature data in that river, the digital thermometer was used.

To complement the analysis of river environments, a structured questionnaire was applied to collect information on the physical characterization of the habitat at each sampling point according to (Calisto et al., 2002). The questions covered aspects such as the morphology of the river, types of substrate, presence of surrounding vegetation.

## 2.3 Data Analysis

The stages of data analysis included the following procedures: (i) identification of the diversity of macrofauna in the different rivers, (ii) characterization of variations in environmental indicators; (iii) establishment of relationships between macrofauna and environmental parameters.

The checklist approach was adopted to record the species found. This methodology allowed documenting the presence of different species without the need for precise quantification of abundances. The list of species identified during sampling provides an initial view of the biological diversity present in river habitats. Given the non-standardized nature of the sampling during the rainy season, for the analysis of the macrofaunal composition between the rivers and along the rivers, only the data from the dry season were used.

The collected data were submitted to appropriate statistical analyses for the comparison of macrofaunistic diversity in the different rivers and sampling points. Diversity indices, such as the Shannon-Wiener Index and the Simpson Dominance Index, were calculated to assess species richness and evenness. The Shannon-Wiener index is described by the equation:

$$H' = -\sum_{i=1}^S p_i \ln(p_i)$$

where 'H' is the Shannon Diversity Index, S is the total number of species in the community,  $p_i$  is the ratio of the abundance of the species to the total abundance of the species..

The formula for calculating the Simpson Index is:  $D = \frac{1}{\sum_{i=1}^S p_i^2}$

where D is the Simpson Index, S is the total number of species in the community, and  $p_i$  is the ratio of the abundance of the species to the total abundance of the species.

Species richness can be presented as the number of different species in the graph, that is, how many species have been classified. The relative abundance of fish species was calculated as the number of specimens of a given species. Dominance was also expressed as the percentage of corresponding species in the total number of individuals captured. The evaluation of the diversity of the fish community among the different habitats was conducted with statistical rigor, taking into account the particularities of our data.

An important component of biodiversity is beta-diversity, which provides an estimate of taxa substitution along a gradient (e.g., along a river corridor) (Ricklefs 1990). Beta-diversity is often overlooked, although it can be an important indicator for assessing the ecological integrity of rivers (Ward and Tockner 2001). We used the degree of dissimilarity as a beta-diversity index (Sørensen index; Sørensen 1948). To start the process, three dissimilarity distance matrices were generated based on species incidence data. This results in three matrices based on paired comparisons of each site: the Sørensen dissimilarity index ( $\beta_{sor}$ ) expresses the total compositional variation with values ranging between 0 and 1, the Simpson dissimilarity index matrix ( $\beta_{yes}$ ) changes the composition due to the rotation species, and  $\beta_{sor}$  minus  $\beta_{yes}$  is the resulting nesting component ( $\beta_{sne}$ ). The calculation was conducted using the "beta.pair" function of the R betapart package, employing the "Sorensen dissimilarity index" (Baselga & Orme, 2012). To estimate the sampling efficiency, a collector curve is developed for the diversity of the four rivers. The degree of turnover and nesting was calculated using the betapart package in R Statistical software version 4.3.1 (R Core Team, 2023).

### **3. Results**

#### **3.1 Macrofaunistic composition in the fluvial habitats occupied by the *peixinho***

16 species belonging to 11 families were recorded (Table 1): fish represent 37.5% (six species) of the diversity, crustaceans (shrimps and crab) represent 31.3% (five species), insects represent 18.8% (three species) and molluscs represent 12.5% (two species). The Gobiidae family was more dominant representing 28.6% (four species) of the diversity, followed by Palaemonidae 18.2% (two species), and the others, Atyidae, Eleotridae, Potamonautidae, Neritidae, Gerridae, Nemertea, Dytiscidae, Pandalidae and Singnathidae individually represent 9.1% (one species). Macrofaunal diversity varied slightly between seasons. The species *Syngnathus fuscus* and *Pandalus nipponensis* were recorded only in the dry season. On the other hand, the species *Awaous lateristiga*, *Nematogobius maindroni*, *Dytiscus* sp., *Gerris* sp., Ephemeroptera

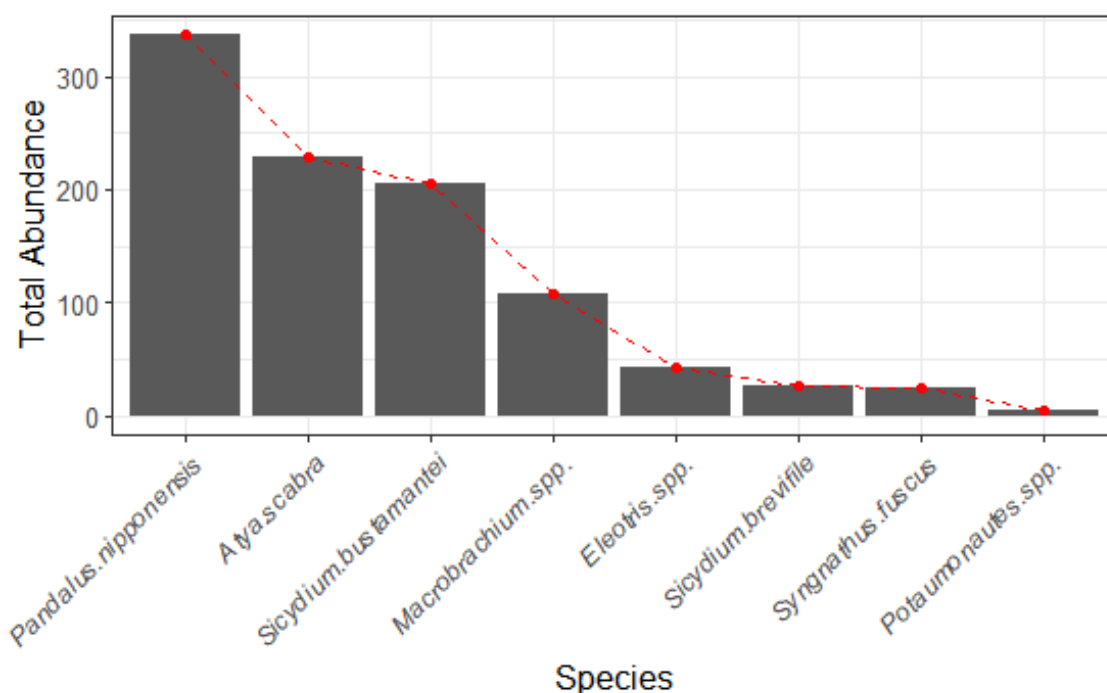
(unidentified species) and Nemertini (unidentified species) were recorded only in the rainy season (Table 1).

**Table 1:** Composition of the macrofauna sampled in the four rivers of São Tomé Island, Iô-Grande, Martim Mendes, Paga Fogo e Lembá, in January 2023 (Wet season) and June 2022 (Dry season): (X) present, (#) Absent, (?) identified up to genus, IUCN Red List Classification: (LC) Least concerned, (VU) Vulnerable, (DD) Data deficient

Order	Family	Scientific name	Local name	Dry season	Wet season	IUCN
Decapoda	<i>Atyidae</i>	<i>Atya scabra</i>		x	x	LC
Decapoda	<i>Palaemonidae</i>	<i>Macrobrachium vollenhovenii</i>	Mangolô	?	x	LC
Decapoda	<i>Palaemonidae</i>	<i>Macrobrachium zariquieyi</i>	M.Plamina	?	x	-
Decapoda	<i>Potamonautidae</i>	<i>Nesonautes saotome</i>		?	x	-
Decapoda	<i>Pandalidae</i>	<i>Pandalus nipponensis</i>		x	#	DD
Perciformes	<i>Gobiidae</i>	<i>Sicydium bustamantei</i>	Peixinho	x	x	DD
Perciformes	<i>Gobiidae</i>	<i>Sicydium brevifile</i>	Peixinho	x	x	VU
Perciformes	<i>Gobiidae</i>	<i>Awaous lateristriga</i>	Xarroco	#	x	LC
Perciformes	<i>Gobiidae</i>	<i>Nematogobius maindroni</i>		#	x	LC
Perciformes	<i>Eleotridae</i>	<i>Eleotris annobonensis</i>		?	x	DD
Syngnathiformes	<i>Syngnathidae</i>	<i>Syngnathus fuscus</i>		x	#	LC
Cycloneritida	<i>Neritidae</i>	<i>Nereina afra</i>		#	x	DD
Hemiptera	<i>Gerridae</i>	<i>Gerris sp.</i>		#	x	-
Palaeonemertea	<i>Nemertea</i>	Unidentified species.		#	x	-
Coleoptera	<i>Dytiscidae</i>	<i>Dytiscus sp.</i>		#	x	-
Ephemeroptera	-----	Unidentified species		#	x	-

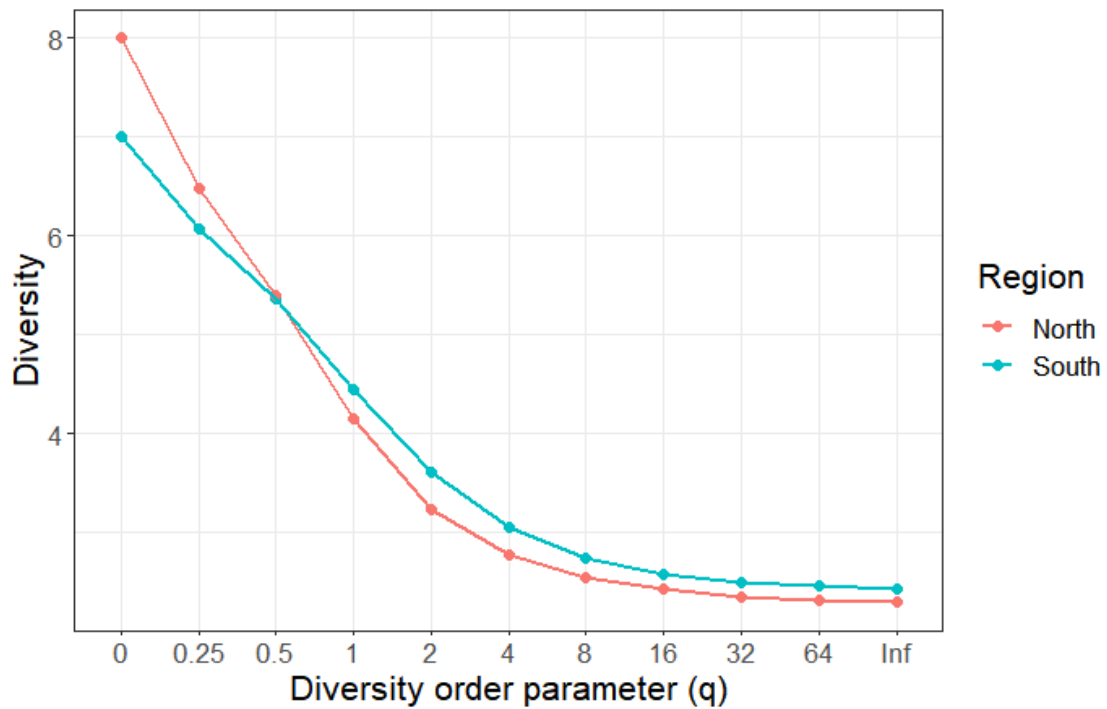
### 3.1. 2 Variation of macrofaunal composition between rivers

In total, 973 samples were captured to assess the abundance of macrofauna. The total number of samples captured varied between sampling sites. The Martim Mendes River had a higher relative abundance of captured species (307). On the other hand, there was a lower relative abundance of the species caught in the Lembá River (191). The species *Pandalus nipponensis* and *Atya. scabra* were the most abundant, demonstrating a pattern of dominance within the ecosystem (Figure 4). On the other hand, *Potamonautes spp.* and *S. fuscus* exhibited a more restricted presence.



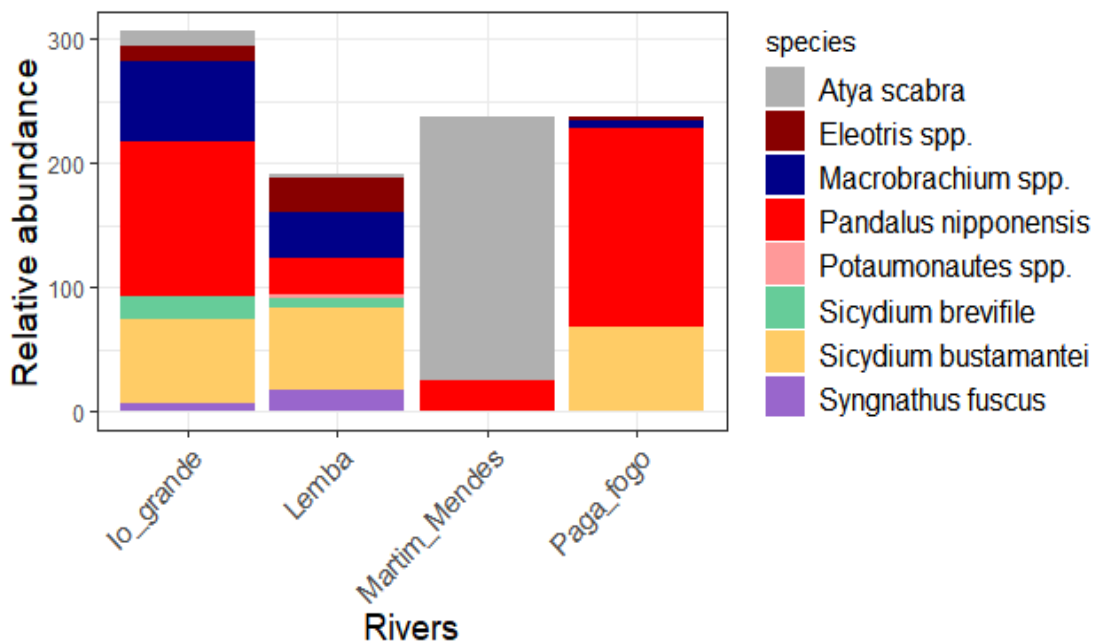
**Figure 4:** Total abundance of species sampled in the four rivers of São Tomé Island, Iô-Grande, Martim Mendes, Paga Fogo and Lembá, in June 2023 (dry season).

The alpha diversity analysis indicated that the northern rivers (Lembá and Paga Fogo) have a higher species richness, while the southern rivers (Iô-Grande and Martim Mendes) have a slightly more equitable diversity (Figure 5). The total species richness recorded was eight, with a distribution of seven species in the South and eight species in the North. The total Shannon index was calculated at 1.63 and suggested that the southern rivers exhibit a slightly higher species diversity compared to those in the north (1.49 ~ 1.42). The Simpson index indicated that the rivers of the south have a greater dominance of some species compared to those of the north. In addition, the Pielou index suggested that the distribution of abundances in the rivers of the south is more equitable than in the north.



**Figure 5:** Species diversity profile sampled in the southern (Iô Grande and Martim Mendes) and northern (Paga Fogo and Lembá) rivers in June 2022 on the island of São Tomé; (q=0): Simpson dominance index, (q=1): Shannon diversity index, (q=3): Pielou evenness index

The analysis of the relative abundance of the species showed that *Potamonautes* spp. occurred only on the Lemba River. On the other hand, the domain of *Atya scabra* in the Martim Mendes River was verified (Figure 6).

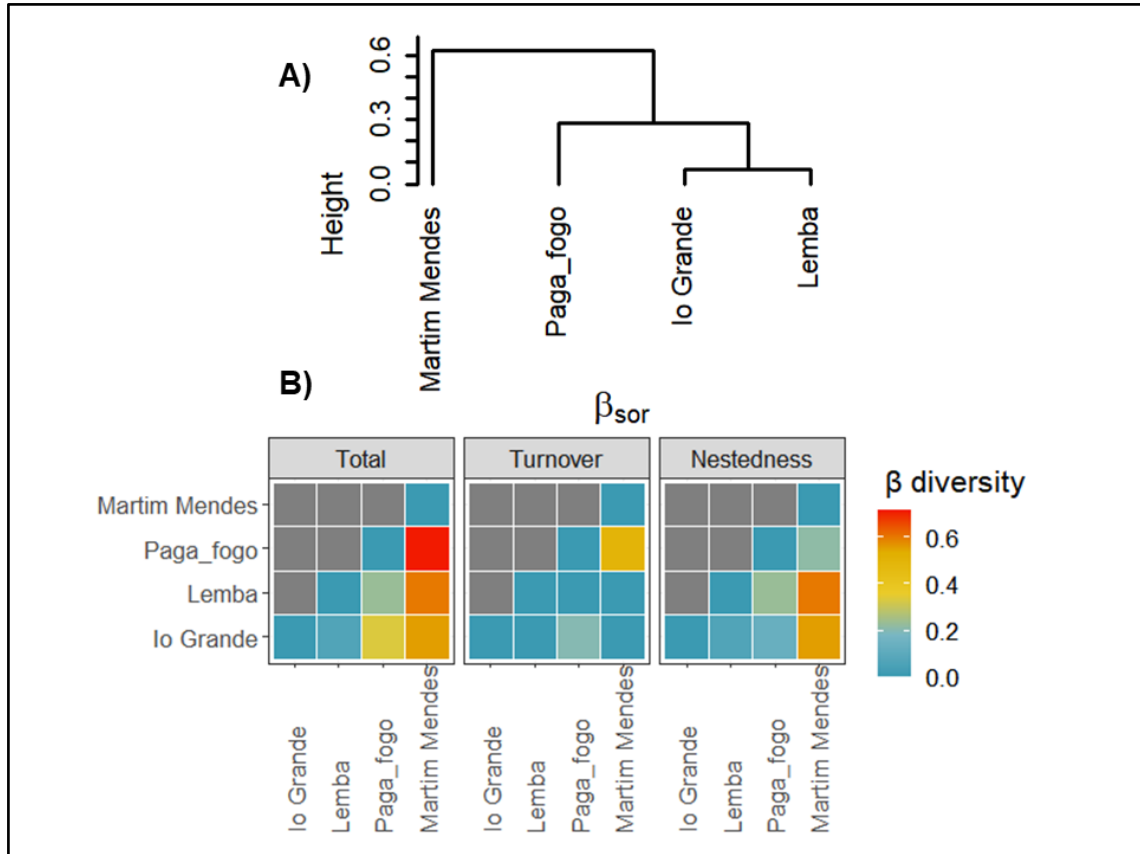


**Figure 6:** Composition of the macrofaunal community. in the four rivers of São Tomé Island, Iô-Grande, Martim Mendes beta, Paga Fogo and Lembá, in June 2022.

An analysis of the average dissimilarity of the components  $\beta$  sor (Sørensen dissimilarity) and  $\beta$  sim (Jaccard similarity) revealed differences in the composition of the macrofaunal community of the rivers (.SOR = 0.462) (beta.SIM = 0.125). The variation in the macrofaunal community was most pronounced in the Martim Mendes river (75%), followed by the Paga Fogo river (63%). No significant differences were found between the macrofaunal communities of the Lembá River and the Iô Grande River (22%).

Dissimilarity between the sampled locations was assessed by calculating the dissimilarity matrix based on the Sorensen index, which highlights differences in species composition or environmental characteristics between the sampled locations. Higher values indicate greater dissimilarity. The results showed a more striking dissimilarity between the macrofaunal communities of Paga Fogo and Martim Mendes (0.714), followed by Lembá and Martim Mendes (0.60) (Figure 7). On the other hand, the dissimilarity between Iô Grande and Paga Fogo was approximately (0.333), while between Lembá and Paga Fogo it was approximately (0.231).

Furthermore, a pattern of species replacement, known as "turnover", was identified in the communities of the Martim Mendes and Paga Fogo rivers.



**Figure 7:** (A) Grouping using mean binding of the  $\beta_{sor}$  (Sørensen dissimilarity) components of species dissimilarity between the different sampling points. (B) Components of the Beta diversity among the rivers of São Tomé, Iô-Grande, Martim Mendes, Paga Fogo and Lembá, with sampling done in June 2022.

### 3.1.3 Environmental characteristics of River habitats

As médias de temperatura variaram entre 23.3 °C e 25.7 °C, com um desvio padrão de 0.1 a 0.9, indicando uma pequena variação nas temperaturas dentro de cada ponto de amostragem na época seca. Por outro lado, as médias de temperatura na época chuvosa variaram entre 23.3 °C e 27.2 °C, com um desvio padrão de 0.1 a 0.8.

The temperature averages ranged from 23.3 °C to 25.7 °C, with a standard deviation from 0.1 to 0.9, indicating a small variation in temperatures within each sampling point in the dry season. On the other hand, the mean temperatures in the rainy season ranged between 23.3 °C and 27.2 °C, with a standard deviation of 0.1 to 0.8.

In the dry season, the maximum and minimum water temperature was recorded in the Lembá River ( $25.2 \pm 0.6$  °C) (mean  $\pm$  standard deviation) and Martim Mendes River ( $23.3 \pm 0.5$  °C), respectively following the same pattern in the rainy season Lembá River ( $27.2 \pm 0.3$  °C) and Paga Fogo River ( $23.3 \pm 0.5$  °C) (Table 2). Regarding the TDS (Total dissolved solid) values, a mean variability was observed, with means ranging from 63.7 mg/L to 87.9 mg/L, and standard deviation ranging from 1.3 to 18.7 mg/L during the dry season. In the rainy season, turbidity ranged from 46.2 to 87.3 mg/L. In the dry season, the maximum average and minimum average turbidity (TDS) was recorded in the Paga Fogo ( $87.9 \pm 1.3$  mg/L) and Martim Mendes ( $63.7 \pm 18.7$  mg/L) rivers, respectively (Table 2). In the rainy season, turbidity data on the Iô Grande River were not collected. The maximum mean turbidity (TDS) and minimum mean was recorded in the Lembá River ( $87.3 \pm 3$  mg/L) and Martim Mendes ( $46.2 \pm 5.9$  mg/L), respectively. On the other hand, the results revealed little significant variations in salinity levels along the rivers ( $< 0.3$ ).

**Table 2:** Summary of the descriptive analysis of temperature parameters and TDS (Total dissolved solid) at the sampling points in June 2022 (Dry season) and January 2023 (Wet season) on the island of São Tomé: (RIG) Rio Iô Grande, (RLB) Rio Lemba, (RMM) Rio Martim Mendes, (RPF) Rio Paga Fogo.

Site	Dry season								Wet season							
	Temperature				TDS				Temperature				TDS			
	mean	sd	min	max	mean	sd	min	max	mean	sd	min	max	mean	sd	min	max
RIG	25.1	0.9	23.5	26.1	87.4	10.9	69.5	111.1	25.2	0.1	25.1	25.3	---	---	---	---
RLB	25.2	0.6	24.2	25.9	87.0	10.9	67.7	106.6	27,2	0.3	26.7	27.4	87.3	3	82.1	89.8
RMM	23.3	0.5	22.9	24.5	63.7	18.7	5.3	78.9	23.3	0.5	22.7	23.7	46.2	5.9	41.5	52.8
RPF	24.8	0.5	24.3	25.6	87.9	1.3	85.3	89.7	25,5	0.8	24.9	26.6	86.4	1.6	84.2	88.1

The analysis of habitat quality revealed that, on average, the 18 sampling points present minimal disturbance (Figure 8). This assessment covered several factors, including vegetation cover, water quality, land use, and river sedimentation.



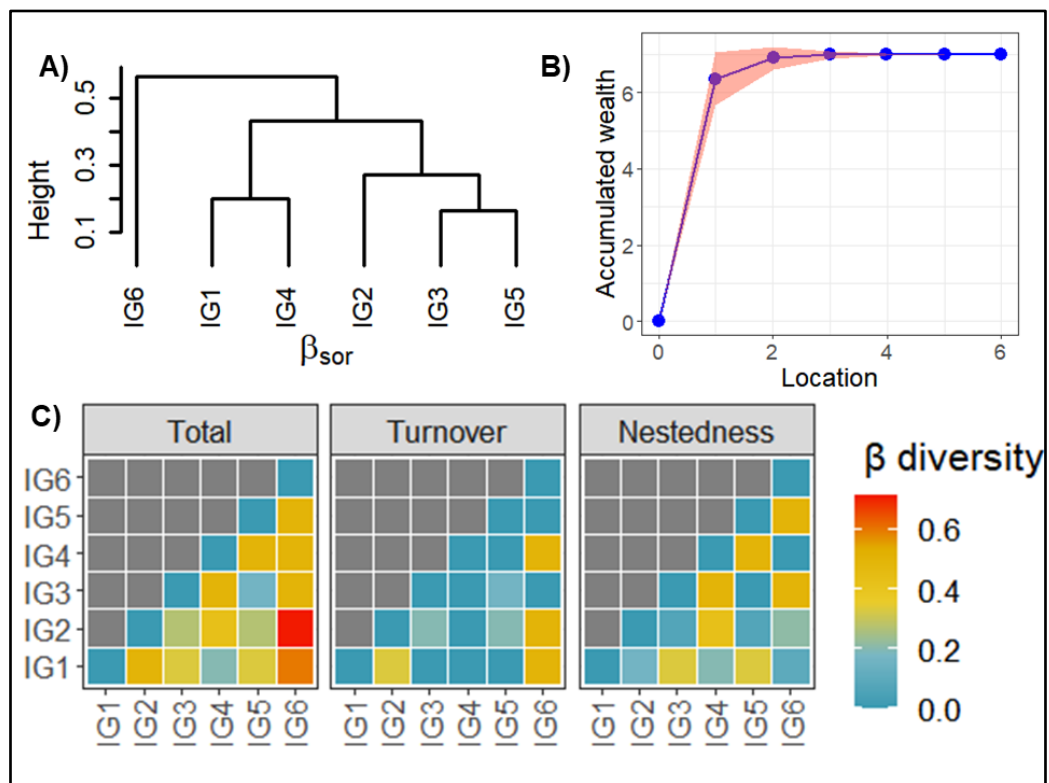
**Figure 8:** Graphic representation of the four rivers studied on the island of São Tomé in January 2023, showing the conservation status of the natural habitat (A) Paga Fogo, (B) Lembá, (C) Iô-Grande and (D) Martim Mendes.

### 3.2. Variation of macrofaunal composition along rivers

#### 3.2.1 Variation in species composition between different sampling points in the Iô Grande River

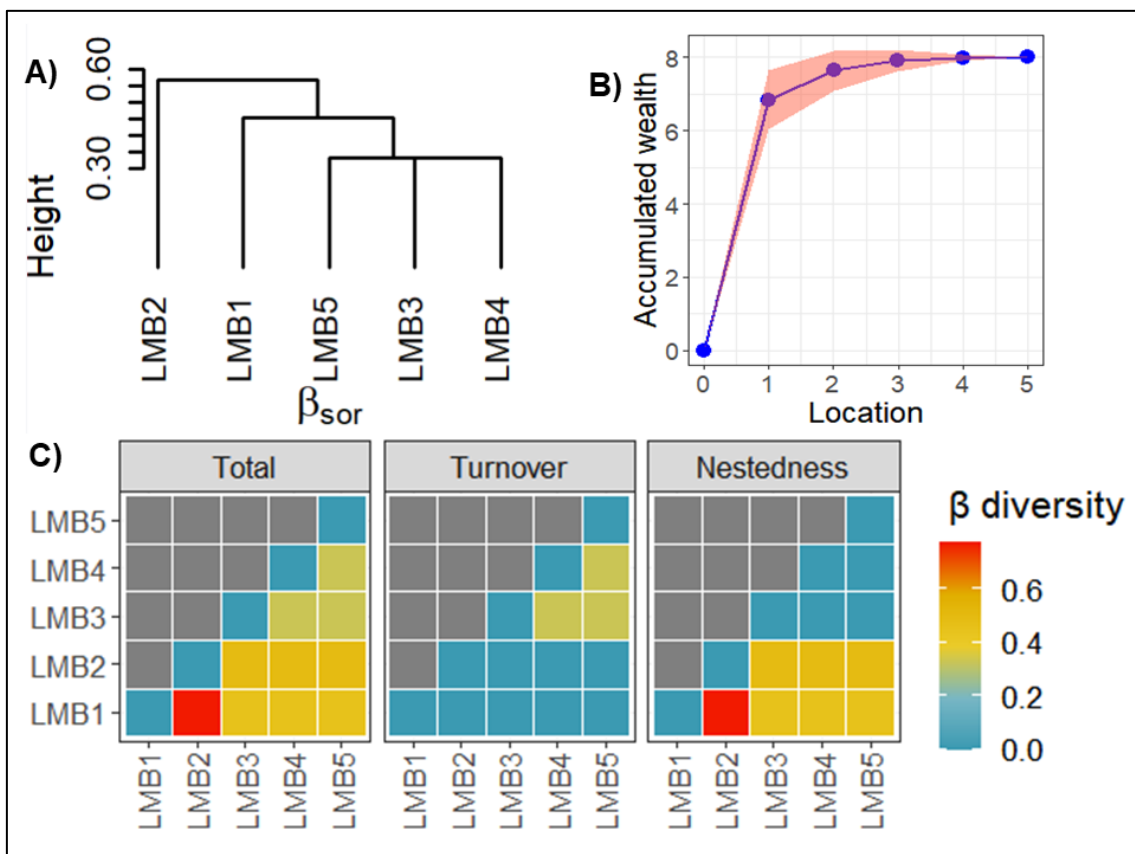
The diversity of the fluvial macrofauna revealed significant variations between the different sampling points. Notably, point 6 (upstream point) on the Iô-Grande River stood out as presenting the greatest dissimilarity in relation to the other points. On the other hand, no statistically significant differences were observed in the mean dissimilarity between points 1 (point closest to the mouth) and point 4, as well as between points 3 and 5 (as shown in Figure 9). The mean dissimilarity between the sampled points (IG1, IG2, IG3, IG4, IG5 and IG6) was

calculated. The values represent the measures of dissimilarity between the groups, and a higher value indicates a greater dissimilarity. The results reveal that the IG2 point presents a mean dissimilarity of (0.5) in relation to the other points, while the IG3 point has a mean dissimilarity of (0.333) with IG1 and (0.273) with IG2. The IG4 point has a mean dissimilarity of (0.2) with IG1, (0.42)9 with IG2 and (0.5) with IG3. The IG5 Group has a mean dissimilarity of (0.333) with IG1, (0.273) with IG2 and (0.167) with GI3. Finally, the IG6 Group presents mean dissimilarities of (0.6) with IG1, (0.714) with IG2, (0.5) with IG3, (0.5) with IG4 and (0.5) with IG5. The analysis of the accumulation curve revealed patterns of diversity of the fluvial macrofauna along the river. There was an inflection in the curve around point 1 and stabilization from the fourth point (Figure 9).



**Figure 9:** (A,) Grouping using mean binding of  $\beta$  components (Sørensen dissimilarity) (B) Species accumulation curve (C) Components of Beta diversity between the points of the Iô Grande River (IG1-IG6), with data from June 2022 on the island of São Tomé.3.2.2. Variation in species composition between different sampling points in the Lembá River

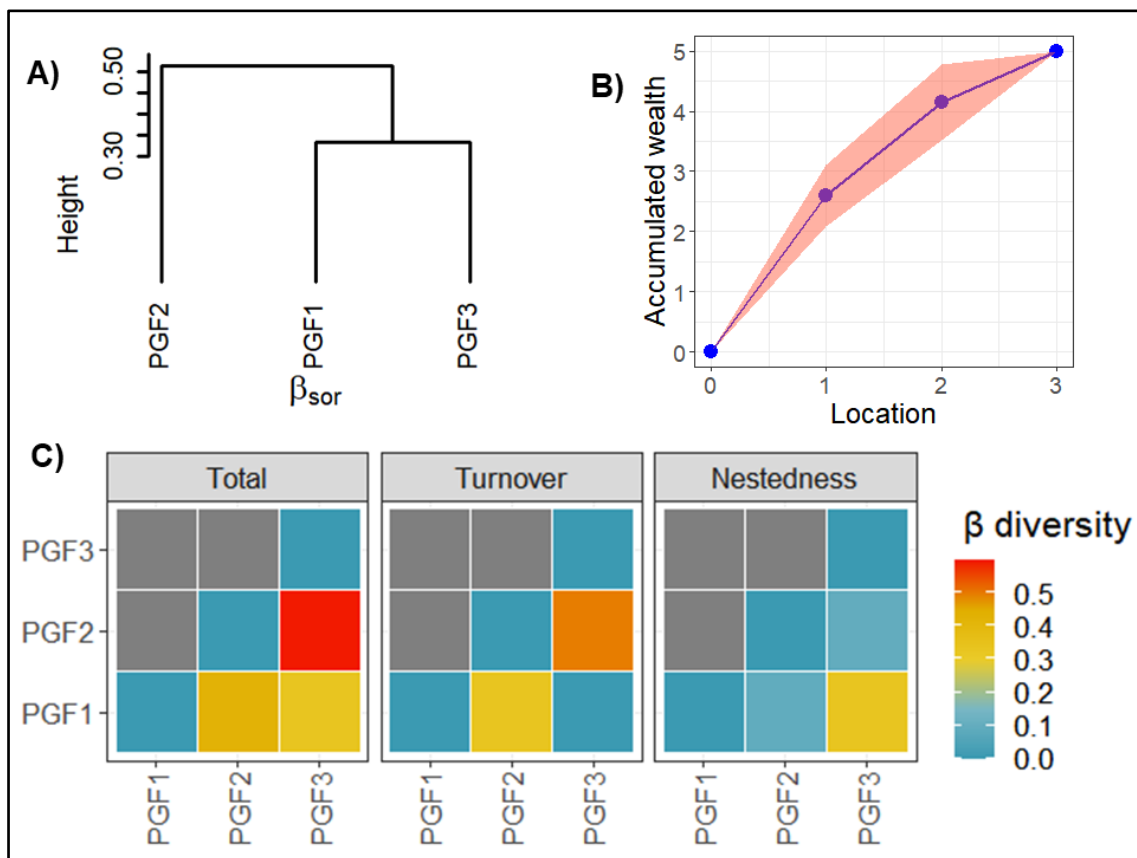
The analysis of the dissimilarity matrix between the sampling sites (LMB1, LMB2, LMB3, LMB4 and LMB5) showed variation in the diversity of the macrofauna along this river (Figure 10). For example, the value at the intersection between the LMB2 and LMB3 points is 0.5, indicating a 50% dissimilarity in macrofauna composition between these two sites. Similarly, the value between LMB3 and LMB4 is 0.333, suggesting a 33.3% dissimilarity in macrofauna composition between these two sites. In addition, an inflection in the species accumulation curve was observed around point 1 to 3, with stabilization from the fourth point. This suggests a transition in species diversity at the sampling sites of the Lembá River, indicating a change in the composition of the macrofaunal community along this environmental gradient. The analysis of the components of the Beta diversity between the points revealed the occurrence of the process of substitution of species, mainly explained by nesting (Figure 10).



**Figure 10:** (A,) Grouping using mean binding of  $\beta$  components (Sørensen dissimilarity) (B) Species accumulation curve (C) Components of Beta diversity between points of the Iô Lembá River (LMB1-LMB5), with data from June 2022 on the island of São Tomé.

### 3.2.3. Variation in species composition between different sampling points in Paga Fogo

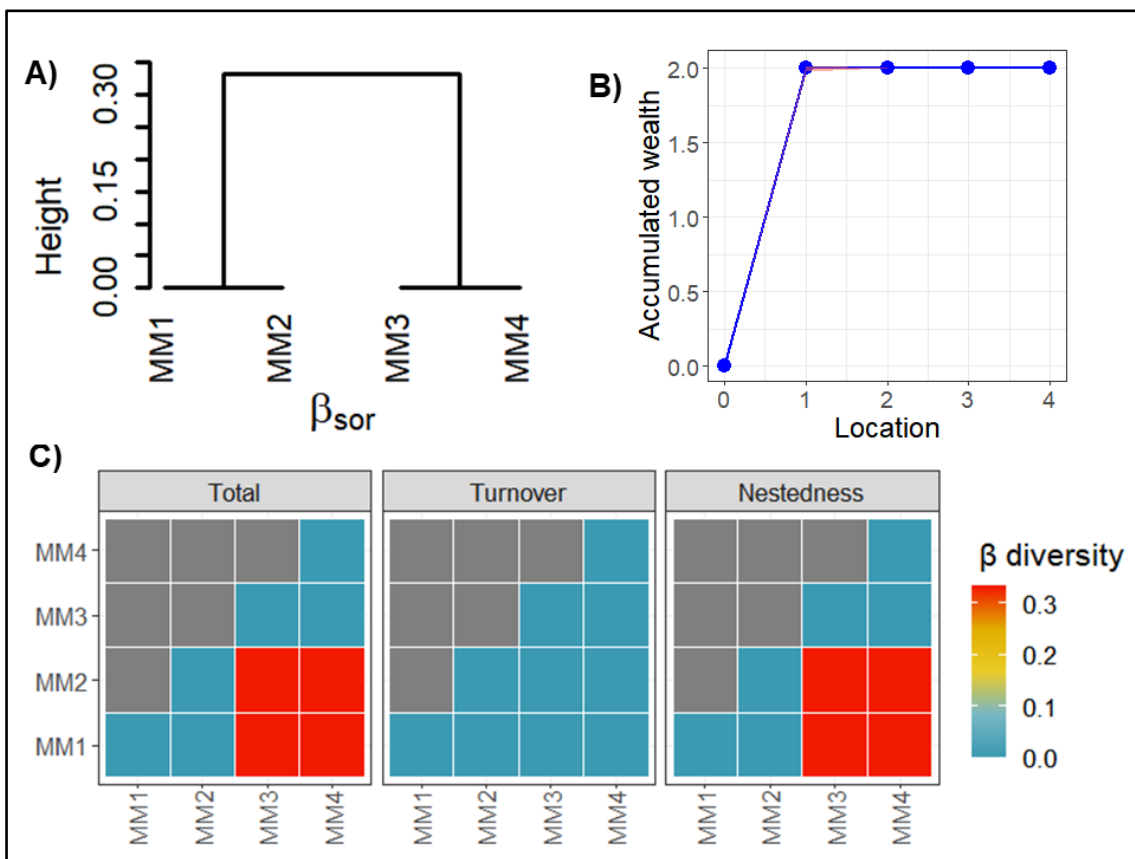
The analysis of the Sørensen dissimilarity matrix between the sites of the Paga Fogo river showed that the intersection between the points PGF2 and PGF3 is (0.333), which suggests a dissimilarity of 33.33% in the composition of the macrofauna between these two sites. Between PGF1 and PGF2, the dissimilarity is 42.86%. The species accumulation curve in the Paga Fogo River did not present a stabilization point, suggesting a potential greater diversity of macrofauna in this environment (Figure 11). In addition, the analysis of the components of beta diversity revealed a process of substitution of species between points 3 and 4, mainly due to the phenomenon of turnover (Figure 11).



**Figure 11:** (A,) Grouping using mean binding of  $\beta_{sor}$  components (Sørensen dissimilarity) (B) Species accumulation curve. (C) Components of the Beta diversity between the points of the Paga Fogo River (PGF1-PGF3), with data from June 2022 on the island of São Tomé.

### 3.2.4 Variation in species composition between different sampling points in Martim Mendes

The value at the intersection between the MM2 and MM3 points is (0.333), indicating a dissimilarity of 33.3% in the composition of the macrofauna between these two points. The same pattern occurs between the other sampling points (Figure 12). There was a low number of species recorded in Martim Mendes and the species accumulation curve did not reach a stabilization point. In addition, the analysis of beta diversity components showed the substitution of species between points 3 and 4, mainly due to the nesting process.



**Figure 12:** (A,) Grouping using mean binding of  $\beta$  components (Sørensen dissimilarity) (B) Species accumulation curve (C) Components of Beta diversity between points of the Martim Mendes River (MM1-MM4), with data from June 2022 on the island of São Tomé.



## **4. Discussion**

The main findings of this study were: 1) collection of 16 species, including unidentified species (*Dytiscus* sp., *Gerris* sp., Ephemeroptera unidentified species, Nemertini unidentified species); 2) the family Gobiidae is the most represented in the fluvial habitats studied; 3) the macrofaunal diversity varies between the rainy season and the dry season; 4) there was a slight variation in the diversity of macrofauna between rivers and along rivers; 5) temperature slightly influences macrofaunal diversity; and 6) the rivers presented little physical disturbance.

### **4.1 Variation of macro faunal diversity**

The reduced diversity of species observed can be associated with the insular nature of the region. As documented by Castelo (1994), the ichthyofauna of Bioko comprises only 17 species adapted to freshwater environments. The ichthyofauna in Bioko is less diverse due to the island's isolation from the mainland and the scarcity of different habitats (Thys van den Audenaerde, 1968). The dominance of the Gobiidae family may be due to the omnivorous feeding pattern observed in one of its representatives (Baptista et al., 2020; Liao et al., 2020; Blubaugh, 2023) which coincides with the pattern observed by (Castle, 1994) in Bioko. On the other hand, the family Gobiidae is among the most represented in the study on the coastal marine fish of São Tomé carried out by Wirtz et al. (2007).

The variation in macrofauna diversity between the rainy season and the dry season can be attributed to the substantial increase in river water flow during the rainy season compared to the dry season. This increase in flow results in an increased transport of nutrients and organic matter from river drainage areas to coastal ecosystems. This process enriches the availability of food and resources in these areas (Galegos et al., 1992; Macedo et al., 2023).

The results showed significant variations in alpha and beta diversity between rivers and along the river. This can be explained by the variation in the

watershed. Larger watersheds tend to house a greater variety of aquatic habitats and adjacent wetlands, providing a broad spectrum of ecological niches for different species to develop (Fonseca et al., 2021). In addition, the altitude of the region where the river is located is also a relevant element. Altitude variation can influence water temperature, nutrient availability, and atmospheric pressure. In higher altitude areas, conditions can be more extreme, selecting species adapted to these specific environments (Miranda et al., 2019). The rivers located in the northern region presented a higher richness of species compared to the rivers of the south. This may be related to differences in environmental characteristics and water flow patterns between the two regions. A noteworthy aspect was the low alpha diversity observed in the Martim Mendes River, where only two species predominated. This phenomenon can be explained by the quality of the water present in this river, which is subject to intense agricultural exploitation, especially monocultures. It is plausible that the pesticide residues used in these plantations are being carried into the river during the floods, resulting in adverse impacts on the macrofauna and, consequently, in the decrease of the observed diversity. There was a slight correlation between temperature and species diversity. There are several studies that address the influence of temperature on species (Baptista & Leitão, 2014; Floeter et al., 2007; Baptista et al., 2014).

#### **4.2. Effects of environmental parameters on macrofaunal diversity**

A crucial part of the analysis was related to environmental variables such as temperature, total dissolved solids (TDS), turbidity and salinity. The results indicated small variations in water temperatures, suggesting a certain environmental stability at the sampling points. In addition, the values of temperature and turbidity presented variable means, but in general, revealed acceptable levels along the rivers. This corroborates with the evaluation of habitat quality, which demonstrated, on average, low disturbance at the sampling sites. Turbidity can alter species community composition and biodiversity in estuarine ecosystems (Lunt & Smee, 2020). Increased turbidity in lakes may modify the community structure of benthic macroinvertebrates (Sosa-Aranda & Zambrano, 2019). Turbidity affects beta diversity of fish (Menezes et al., 2023). On the other

hand, according to the study conducted by Huxham (2008), no significant relationship was found between fish behavior and turbidity, suggesting that water transparency does not explain the low densities of fish in mangroves. Environmental changes, such as deforestation, habitat fragmentation, and urbanization, lead to a reduction in species diversity (Mulinge, 2023).

#### **4.3. Methodological limitations of the study**

It is essential to recognize the methodological limitations of this study, especially with regard to the sampling effort, which varied between stations and between rivers. In addition, the precise identification of species in river environments can be challenging, given the complexity of these ecosystems and the presence of species that present significant differences in terms of sexual characteristics and life cycles. Additionally, the sampling may not have covered all species present due to logistic limitations, which potentially influenced the results.

#### **4.4. Perspectives for Future Research**

For future research it would be important to carry out a long-term monitoring of the macro faunal diversity in the rivers occupied by the *peixinho* in São Tomé to better understand the seasonal and interannual variations to identify long-term trends in the macro faunal community. In addition, it would be valuable to incorporate additional variables, such as different types of substrates, for a deeper understanding of how these habitats may influence the distribution of species. It is also important to conduct more comprehensive analyses of water quality, especially in rivers subject to intense agricultural activities, in order to assess the effects of pesticides and other pollutants on aquatic macrofauna. Exploring the impact of climate change on the diversity of macrofauna in São Tomé, considering temperature and precipitation trends, is also fundamental. This information would be crucial for predicting how species would respond to climate change and human activities, thus contributing to the development of conservation plans, especially for endangered species.

## 5. Conclusion

The results indicated that there are variations in macro faunal diversity in the habitats occupied by the *peixinho* in São Tomé. This variation occurs between rivers and along rivers. The family Gobiidae is the most represented in the fluvial habitats studied. Macrofaunal diversity varies seasonally, with a significant increase during the rainy season. Environmental variables, such as temperature, total dissolved solids (TDS), turbidity and salinity, show small variations along the rivers studied. Temperature influences macro faunal diversity. The methodological limitations of this study include differences in sampling effort between seasons and rivers as well as the challenges in accurately identifying species during the dry season.

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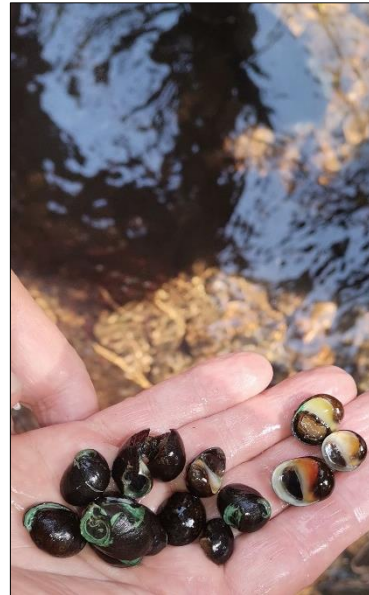
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**Appendix I - Samples collected in January 2023 from the Paga Fogo, Lembá, lô Grande, and Martim Mendes rivers on the island of São Tomé**



*Macrobrachium vollenhovenii* (Herklots, 1857)



*Nereina afra* (G. B. Sowerby I, 1836)



*Nesonautes saotome*(Cumberlidge & Daniels, 2018)



*unidentified*



*Peixinho* Rio Lembá, Junho 2023



*Atya scabra* (Leach, 1816)



*Nematogobius maindroni* (Sauvage, 1880)  
Photo: Peter Wirtz



*Eleotris annobonensis* (Blanc, 1968)  
Photo: Peter Wirtz



*Awaous lateristriga* (Duméril, 1861)  
Photo: Peter Wirtz



*Sicydium brevifile* Ogilvie-Grant, 1885  
Photo: Peter Wirtz



*Macrobrachium zariquieyi* Holthuis, 1949  
Photo: Peter Wirtz



*Sicydium bustamantei* Greeff, 1882  
Photo: Peter Wirtz

## Appendix II – Protocol used for habitat characterization during sampling in the dry season and wet season.

### Protocolo de Caracterização de Hábitats Físicos – Simplificado de Callisto et al, 2002

Utilizado em atividades de monitoramento participativo de qualidade ecológica de águas com estudantes de ensino básico.

Descrição do Ecossistema Aquático			
Nome (s) Pesquisador (es):			
Questões	Respostas		
1. O que existe em maior quantidade em torno do local?	(a) <b>Vegetação natural</b>	(b) Plantações, pastagens, monocultura	(c) Casas, lojas, indústrias
2. Existe assoreamento	(a) Não	(b) Pouco	(c) Muito
3. Existe lixo na (s) margem (s)?	(a) Não	(b) Pouco	(c) Muito
4. A água apresenta odor?	(a) Não	(b) Odor fraco	(c) Odor forte
5. Existe esgoto? (observar presença de canos de despejo de esgoto no local)	(a) Não	(b) Pouco	(c) Muito
6. Como é a transparência da água?	(a) A água é clara	(b) A água é um pouco escura	(c) A água é muito escura
7. Como é composto o leito do rio (maior parte)?	(a) Pedras e cascalhos	(b) Lama e areia	(c) Cimento
8. Como é a mata ciliar?	(a) Existem muitas árvores	(b) Existem poucas árvores	(c) Quase não existem árvores
9. Existe erosão nas margens?	(a) Não	(b) Pouco	(c) Muito
10. Qual a diversidade de habitats para organismos aquáticos	(a) Muito diverso	(b) Mais ou menos diverso	(c) Pouca diversidade (apenas 1 ou 2 tipos diferentes de habitats, ou seja, cascalho, areia, madeira)
Cálculo			
Letra marcada	Valor	Número de letras	Total de Pontos
(a)	10 pontos		
(b)	5 pontos		
(c)	0 pontos		
Pontuação Total			

#### Interpretação da Pontuação:

Maior que 68 pontos: **MÍNIMA PERTURBAÇÃO**

40 a 68 pontos: **MODERADA PERTURBAÇÃO**

Menor que 40 pontos: **ALTA PERTURBAÇÃO**