DOUGLAS DA SILVA FERREIRA

PRESERVED OR NOT PRESERVED? HOW MUCH THE QUALITY OF A STREAM CAN IMPROVE IN A CONSERVATION AREA, EVEN WHEN THE SPRING IS URBANIZED

Dissertation presented to the Universidade Federal de Viçosa, as part of the requirements of the Entomology Graduate Program, to obtain the title of *Magister Scientiae.*

Adviser: Frederico Falcão Salles

Co-adviser: Tathiana Guerra Sobrinho

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APPROVED: July 26, 2021.

Assent:

Douglas da Silva Ferreira
Author

Frederico Falcão Salles
Adviser

Aos meus pais amados Márcia e Claudinei

Aos meus irmãozinhos Davi e Lívia

Ao meu orientador Fred

À minha coorientadora Tathi

E a todos os meus amigos

OFEREÇO

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"It always seems impossible until it’s done."

- Nelson Mandela
1918-2013

BIOGRAPHY

Douglas da Silva Ferreira, son of Márcia da Silva dos Santos and Claudinei Ferreira de Souza. Graduated in Agronomy (2019) from the Federal Rural University of Rio de Janeiro (UFRRJ). He was monitor of General Entomology at UFRRJ (2018-2019), supervised by Professor André Luis Santos Resende. He was a FAPERJ Scientific Initiation Scholar at Embrapa Agrobiologia, from 2015 to 2018, being supervised by the researcher Alessandra de Carvalho Silva, where he worked with biological control, attractive plants and arthropod taxonomy. He was Administrative and Financial Director at the Junior Agronomy Company of UFRRJ - Rural Consultoria Júnior, where he also acted as coordinator of landscaping projects, between 2017 and 2018. In August 2019 he started the Master's Degree in Entomology at the Federal University of Viçosa, under the guidance of Professor Frederico Falcão Salles and co-supervision of Professor Tathiana Guerra Sobrinho from Federal University of Espírito Santo, whose dissertation is presented here.

BIOGRAFIA

Douglas da Silva Ferreira, filho de Márcia da Silva dos Santos e Claudinei Ferreira de Souza. Formado em Agronomia (2019) pela Universidade Federal Rural do Rio de Janeiro (UFRRJ). Foi monitor de Entomologia Geral da UFRRJ (2018-2019), orientado pelo Professor André Luis Santos Resende. Foi bolsista de iniciação científica FAPERJ na Embrapa Agrobiologia, no período de 2015 a 2018, sendo orientado pela Pesquisadora Alessandra de Carvalho Silva, onde trabalhou com controle biológico, plantas atrativas e taxonomia de artrópodes. Foi Diretor Administrativo e Financeiro na Empresa Júnior de Agronomia da UFRRJ - Rural Consultoria Júnior, onde também atuou como coordenador de projetos de paisagismo, entre 2017 e 2018. Em agosto de 2019 iniciou o Mestrado em Entomologia na Universidade Federal de Viçosa, sob orientação do Professor Frederico Falcão Salles e coorientação da Professora Tathiana Guerra Sobrinho da Universidade Federal do Espírito Santo, cuja dissertação é aqui apresentada.

ABSTRACT

FERREIRA, Douglas da Silva, M.Sc, Universidade Federal de Viçosa, July, 2021. **Preserved or not preserved? How much the quality of a stream can improve in a conservation area, even when the spring is urbanized.** Adviser: Frederico Falcão Salles. Co-adviser: Tathiana Guerra Sobrinho.

Rapid urbanization in Brazil has been dramatically deteriorating the water quality of streams and threatening aquatic ecosystem health. In this study, we aimed to address the question of how urbanization affects macroinvertebrate distribution patterns and the water quality of a strategic stream that runs from the city to an important conservation area. Environmental variables (spatial and temporal) and macroinvertebrate community (use of community deconstruction) data were collected on rainy and dry seasons of 2019 and 2020 at 10 sampling sites, of which four we categorized as impacted, three as altered and three as pristine, based on environment assessment protocols. We submitted the abundance and taxonomic richness data to a two-way ANOVA, then to a GLMMs. We also performed a nMDS to the macroinvertebrate community, followed by the ANOSIM and SIMPER test, respectively. The results of this study showed that impacted and altered streams had lower total richness and % Odonata, and higher % Chironomidae when compared to pristine streams. As expected Chironomidae had a positive relationship with total coliforms, and taxonomic richness and % EPT had a negative one, and % Odonata had a negative relationship with total dissolved nitrogen. Pristine stream community of benthic macroinvertebrates showed different composition between impacted and altered streams, however, communities of impacted and altered environments were generally similar. Our exploratory data showed that gathering collectors were representative in impacted and altered streams, and predators were representative in pristine areas. Moreover, our results suggest that urbanization resulted in less diverse and more tolerant stream macroinvertebrate assemblages.

Keywords: Aquatic insects. Conservation. Biomonitoring. Urban stream. Domestic sewage. Ecological indicator. Pollution.

RESUMO

FERREIRA, Douglas da Silva, M.Sc, Universidade Federal de Viçosa, julho de 2021. **Preservado ou não preservado? O quanto a qualidade de um riacho pode melhorar em uma área de conservação, mesmo quando a nascente é urbanizada.** Orientador: Frederico Falcão Salles. Coorientadora: Tathiana Guerra Sobrinho.

A rápida urbanização no Brasil tem deteriorado drasticamente a qualidade da água dos córregos e ameaçado a saúde do ecossistema aquático. Neste estudo, pretendemos abordar a questão de como a urbanização afeta os padrões de distribuição de macroinvertebrados e a qualidade da água de um córrego estratégico que vai da cidade à uma importante área de conservação. Variáveis ambientais (espaciais e temporais) e dados da comunidade de macroinvertebrados (uso de desconstrução de comunidade) foram coletados nas estações chuvosa e seca de 2019 e 2020 em 10 locais de amostragem, dos quais quatro foram categorizados como impactados, três como alterados e três como naturais, com base em protocolos de avaliação do ambiente. Submetemos os dados de abundância e riqueza taxonômica a uma ANOVA de duas vias e, em seguida, a um GLMMs. Também realizamos um nMDS para a comunidade de macroinvertebrados, seguido do teste ANOSIM e SIMPER, respectivamente. Os resultados deste estudo mostraram que córregos impactados e alterados apresentaram menor riqueza total e % Odonata, e maior % Chironomidae quando comparados com córregos naturais. Como esperado, Chironomidae teve uma relação positiva com coliformes totais, já a riqueza taxonômica e % EPT tiveram uma relação negativa, e % Odonata teve uma relação negativa com o nitrogênio total dissolvido. A comunidade de macroinvertebrados bentônicos em córregos naturais mostrou uma composição diferente dos córregos impactados e alterados, no entanto, as comunidades de ambientes impactados e alterados foram geralmente semelhantes. Nossos dados exploratórios mostraram que os coletores foram representativos em córregos impactados e alterados, e os predadores foram representativos em áreas naturais. Além disso, nossos resultados sugerem que a urbanização resultou em assembleias de macroinvertebrados de riachos menos diversificadas e mais tolerantes.

Palavras-chave: Insetos aquáticos. Conservação. Biomonitoramento. Córrego urbano. Esgoto doméstico. Indicador ecológico. Poluição.

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SUMMARY

1. INTRODUCTION

Freshwater ecosystems, despite occupying only 1% of the world's surface (Gleick, 1998), have a rich species diversity that contain about 12% of all known species (Garcia-Moreno *et al.*, 2014; Higgins *et al.*, 2021). These freshwater ecosystems directly or indirectly support most of life on Earth, as they provide goods and services that are essential to the economy and livelihood of billions of people (Russi *et al.*, 2012; Dodds, Perkin & Gerken, 2013; Higgins *et al.*, 2021). Thus, maintaining biodiversity is very important for the balance of aquatic ecosystems, ensuring the continuity of life across generations (Azevedo-Santos *et al.*, 2019; Padial *et al.*, 2021).

From this perspective, there are many debates about the conservation of freshwater biodiversity, which is extremely diverse, with high rates of endemism and also a high proportion of species with a lack of data (Azevedo-Santos *et al.*, 2019). However, any threats generated to these ecosystems (including their ecosystem services) cause changes in species biodiversity (Padial *et al.*, 2021) or even species extinction, changes in river flow, organic pollution (Dudgeon *et al.*, 2006; Callisto, Moreno & Rodrigues Macedo, 2019), biotic homogenization (Rahel, 2002), thermal changes, global climate change, increased ultraviolet radiation (Dodds *et al.*, 2013), and microplastic contamination (Zeng, 2018).

In Brazil, despite having one of the richest biodiversity in the world, threats to these freshwater ecosystems are even more problematic (Collen *et al.*, 2014) because they present intense anthropic interference (Agostinho, Thomaz & Gomes, 2005). All categories of water bodies (ponds, puddles, rivers, streams, canals and other wetlands) have been completely modified as a result of the disorderly increase in human activities (Callisto *et al.*, 2019). This panorama is easily found in cities where there has been a disorderly growth, with high population densities, mainly in urbanized areas, where water bodies directly receive sediment, garbage and domestic and industrial sewage without previous treatment (Martins *et al.*, 2017; Chen *et al.*, 2019; Roveri, Guimarães & Correia, 2020). Moreover, the high expansion of hydroelectric dams in the South and Southeast of Brazil during the last decades has caused great impacts on biodiversity (Agostinho *et al.*, 2005; Lima Junior *et al.*, 2018; Padial *et al.*, 2021). Recently, Brazil faced two major environmental disasters due to the collapse of dams caused by mining activities in Mariana (2015) and Brumadinho (2019), both in the state of Minas Gerais. In addition to the countless lives lost and many homeless, the tragedy affected the soil, making it infertile, polluting the waters and strongly damaging the aquatic ecosystem (Freitas *et al.*, 2019). These changes in the land use and the flow regime of freshwater ecosystems have been one of the causes of this loss of biological diversity and original identity (Moreno & Callisto, 2004; Cavalcante, Miranda & Medeiros, 2017).

Some of these Brazilian freshwater ecosystems are found in the Atlantic Forest (Agostinho *et al.*, 2005; Padial *et al.*, 2021). This South American forest extends along the Atlantic coast of Brazil from the state of Rio Grande do Norte in the northeast to the state of Rio Grande do Sul in the south (Padial *et al.*, 2021). Currently, about 90% of the Atlantic Forest original area has been lost (Myers *et al.*, 2000). Even so, this biome remains the fifth most important biodiversity hotspot due to its significant portion of Brazil's biological diversity, with high levels of endemism (Myers *et al.*, 2000; Ipema, 2005; Padial *et al.*, 2021). As far as it is concerned, almost all natural freshwater ecosystems in the Atlantic Forest are probably altered by human activities (Agostinho *et al.*, 2005), and coincidentally the greatest human occupation is found in these areas (Ribeiro *et al.*, 2011; Padial *et al.*, 2021). Thus, an effective strategy to conserve biodiversity would be the creation and maintenance of protected areas for aquatic systems (Azevedo-Santos *et al.*, 2019). However, there are few watersheds in the Atlantic Forest that are fully protected by conservation units (Azevedo-Santos *et al.*, 2019; Padial *et al.*, 2021).

Espírito Santo is a small Brazilian state, with 12 watersheds (Galter *et al.*, 2021), which still has a remnant of Atlantic Forest, also known as the Lowland Atlantic Forest (coastal plains) (Filho, Peixoto & Jesus, 2000a; Sarmento-Soares & Martins-Pinheiro, 2017). Currently, part of these remnants of Lowland Forests along the Atlantic Forest in Espírito Santo are found in conservation units (Ipema, 2005; Massariol, Soares & Salles, 2014; Sarmento-Soares & Martins-Pinheiro, 2017), represented by 14 federal and 17 state, which has a total area of 119,559.40 ha (Ipema, 2005). Regarding these units, four are Private Natural Heritage Reserves (RPPN), and there are also several private areas in the state, whose activities are aimed at conservation, such as the Vale Natural Reserve (RNV) in Linhares (Filho *et al.*, 2000a; Ipema, 2005).

Studies aiming to understand how human activities influence the quality of aquatic ecosystems, with the prospect of restoring degraded ecosystems, are urgent and essential (Jørgensen, 2015). In fact, the continuous loss of species indicates that the management of water resources in watersheds requires conservation strategies and sustainable solutions that address socio-cultural, economic, ecological and public policy measures, in order to ensure the recovery of the original identity of aquatic ecosystems (Allan & Flecker, 1993; Callisto *et al.*, 2019; Padial *et al.*, 2021).

Usually, the physical, chemical and biological conditions of water bodies are subject to geographical characteristics and changes in human occupation in watersheds and riparian meta-ecosystems (Vannote *et al.*, 1980; Allan, 2004). In the last decades, several studies have correlated land use with river conditions, adopting parameters, elaborating concepts of landscape ecology and health indicators of aquatic ecosystems (Moreno & Callisto, 2010; de Oliveira, Maillard & de Andrade Pinto, 2017; Linares, Callisto & Marques, 2018; Callisto *et al.*, 2019).

All of these studies list negative impacts, which are serious in southeastern Brazil, due to changes in natural vegetation in agricultural areas, pastures, eucalyptus monocultures, mining and urban areas (Pompeu, Alves & Callisto, 2005; Maillard & Pinheiro Santos, 2008; Dala-Corte *et al.*, 2020). Therefore, changes in land use and occupation have been the main factors causing pollution in water resources and, consequently, the main responsible for the reduction of aquatic biodiversity (Morley & Karr, 2002; Goulart & Callisto, 2003; Callisto *et al.*, 2019). In this sense, the forest massifs are responsible for the conservation around the streams, preserving their qualitative integrity and allowing the maintenance of this natural vegetation (Allan & Flecker, 1993; Couceiro *et al.*, 2007; Martins *et al.*, 2017).

Pollution caused by land use and occupation over water bodies can be assessed using monitoring strategies. There are several ways to assess water quality, such as the use of chemical parameters (level of dissolved oxygen, pH, total solids, heavy metals, organic matter and pesticides), physical parameters (temperature, color, turbidity and flavor) and biological parameters, related to the abundance and variety of flora and fauna in the aquatic environment (Hermes *et al.*, 2004; Martins *et al.*, 2017). Among these methods, the use of bioindicators has been recurrent in the assessment of these anthropogenic impacts (Callisto, Goulart & Moretti, 2001; Sumudumali & Jayawardana, 2021), since animals, plants, microorganisms and their complex interactions with the environment respond differently to land use (Piedras *et al.*, 2006). Through the information obtained with biomonitoring, it is possible to identify the presence of pollutants from occasional discharges of domestic sewage and industrial effluents, as well as to understand how these bioindicators interact with nature (Callisto *et al.*, 2001; Piedras *et al.*, 2006; Du *et al.*, 2021). For example, poor water quality can be identified through the abundant reduction of aquatic biodiversity, due to the ecological effects caused by multiple sources of pollution (Callisto *et al.*, 2001). Aquatic organisms, mainly invertebrates, are the ones that best respond to changes in environmental conditions (Piedras *et al.*, 2006). According to Arenas-Sánchez *et al.* (2021), biomonitoring should be performed frequently, once or twice a year (in summer and/or winter). In addition, sustainable programs and public strategies that combine scientific studies with socioeconomic conveniences must be implemented, with the objective of conserving freshwater ecosystems (Azevedo-Santos *et al.*, 2019).

Among all aquatic macroinvertebrates used in biomonitoring, insects represent the dominant group, with more than 60% of the total biodiversity (Balian *et al.*, 2008). The participation of these macroinvertebrates in the food chains of freshwater ecosystems is crucial, constituting a link between primary producers, detritic deposits and higher trophic levels, in addition of being vital for the functioning of water bodies, and contributing to the nutrient cycling and decomposition (Du *et al.*, 2021). Each taxonomic group has a behavior towards environmental impacts. Thus, previous studies are needed to identify the levels of pollution tolerance of these benthic systems that may be more tolerant or more sensitive to environmental impacts (Silveira, de Queiroz & Boeira, 2004; Moreno & Callisto, 2010).

The abundance and richness of these benthic macroinvertebrates have been used as parameters indicative of the conditions of aquatic ecosystems (Galdean, Callisto & Barbosa; Martins *et al.*, 2017; Roveri *et al.*, 2020). The numerical loss of sensitive species and the proliferation of tolerant species can be considered an indicator of human disturbances in ecosystems, mainly due to urbanization (Morley & Karr, 2002; Walters, Roy & Leigh, 2009; Callisto *et al.*, 2019). Ephemeroptera, Plecoptera and Trichoptera are good bioindicators of environmental quality, constituting the EPT index (Crisci-Bispo, Bispo & Froehlich, 2007). They are sensitive to chemical changes in the environment (excess waste) that can affect their feeding activity and limit their presence (Zequi *et al.*, 2019).

In the present study, we investigate the response of benthic macroinvertebrates to changes in water quality as the collected sites move away from the urbanized headwaters and enter protected areas, located in the state of Espírito Santo. In this sense, the change in the fauna of aquatic macroinvertebrates that respond directly and indirectly to water quality will be the focus of the research, whose community composition indicates the degree of human impact on streams.

2. OBJECTIVES

2.1. Main objective

i. In this study we aimed to evaluate the possible improvement in the quality of water in a stream as it moves away from the urbanized headwater (polluting area) and enters a conservation area. The research proposal has the following question: how is the stream's behavior when leaving an urbanized area upstream to an area of preserved forest downstream?

2.2. Specific objective

i. How do different diversity metrics respond to environmental indicators of water quality?

ii. How the composition of the aquatic macroinvertebrate community responds to the environmental quality of the water?

3. MATERIAL AND METHODS

3.1. Study area

We carried out this work on the D'Água stream and its tributaries (Fig. 1), which flow into the Pau Atravessado river and integrate the Barra Seca River inter watershed, located in the north of the state of Espírito Santo, in the municipalities of Sooretama and Linhares. The study area is located on the surface of coastal boards predominantly Yellow Argisol and transitions to Latosol and Spodosol (dos Santos *et al.*, 2004; Fontana, dos Anjos & Pereira, 2016), with altitudes ranging between 28 and 65m (Peixoto *et al.*, 2008). The region's climate is identified as humid tropical, with dry winter and concentrated rain in the summer, classified as Aw by Köeppen (Braz *et al.*, 2009). The average annual temperature is 24 ºC and the average annual precipitation is about 1200 mm (Peixoto *et al.*, 2008; Saiter, Rolim & Oliveira-Filho, 2016). About 80% of annual rainfall is distributed between October and March (Engel & Martins, 2005; Jesus & Rolim, 2005; Peixoto *et al.*, 2008). The water balance makes it possible to recognize a wet summer season (December to February) and a dry autumn-winter season (May to mid-September) separated from each other by transition seasons (Peixoto, Rosa & Joels, 1995; Engel & Martins, 2005; Saiter *et al.*, 2016).

In this study we performed the biomonitoring in landscape transition zones, upstream is the urban center of Sooretama and downstream is a conservation area of forest remnants of the Atlantic Forest of the Vale Natural Reserve (RNV) and between these two landscapes there is an area of Atlantic Forest deforested by cattle producers that gave rise to pasture (Fig. 1).

The headwater of the D'Água stream is fully urbanized, integrating two districts of Sooretama (Centro and Vale do Sol). Currently, Sooretama has an estimated population of 30,680 inhabitants (IBGE, 2020). This makes the region one of the most critical areas in this study, since the water courses are modified, receiving industrial and domestic sewage *in natura*, in addition to sediment and garbage, leaving the stream in the process of eutrophication.

At the other extreme, where RNV's protected forest is located, there are several wild animals that are protected and monitored (Ferreguetti *et al.*, 2016). When streams enter the protected forest, they play a fundamental role for these animals because they will serve as a water resource (Rocha *et al.*, 2016).

The forest block in which the RNV is inserted together with the Sooretama Biological Reserve and two other private reserves, constitute one of the few forest remnants of the Brazilian

Atlantic Forest, called the Linhares-Sooretama Block (SOS Mata Atlântica & INPE, 2014; Rocha *et al.*, 2016). This region has a large part of mammal species from all over the Atlantic Forest (Srbek-Araujo, Rocha & Peracchi, 2014) and is one of the last refuges in Espírito Santo for several species that are nationally threatened with extinction, such as the South American tapir (*Tapirus terrestris*), jaguar (*Panthera onca*) and the giant armadillo (*Priodontes maximus*) (Srbek-Araujo *et al.*, 2014; Ferreguetti *et al.*, 2016).

We established sampling sites along the D'Água stream (Table 1), which connects with the other affluent streams: Paciência, João Pedro and Alegre de Cima (Fig. 1). The D'Água stream is found in urbanized, agricultural and pasture areas (Fig. 1), the Alegre de Cima and Paciência streams are also found in these areas and in the protected forest of RNV. The João Pedro stream is found only in the RNV protected forest (Fig. 1), which presents a block of protected native forest in an area of 22,771 ha (Filho, Peixoto & de Jesus, 2000b).

In order to contemplate the studied territory, we distributed ten sampling points along the streams, which are interconnected, where the sampling of water and aquatic macroinvertebrates occurred. The collections were divided into two stages (campaigns): the first, in the rainy season (December 2019) with rainfall of 219.1 mm and the second in the dry season (September 2020) with approximately 38.5 mm (data collected from the meteorological station installed at RNV, in Linhares, ES).

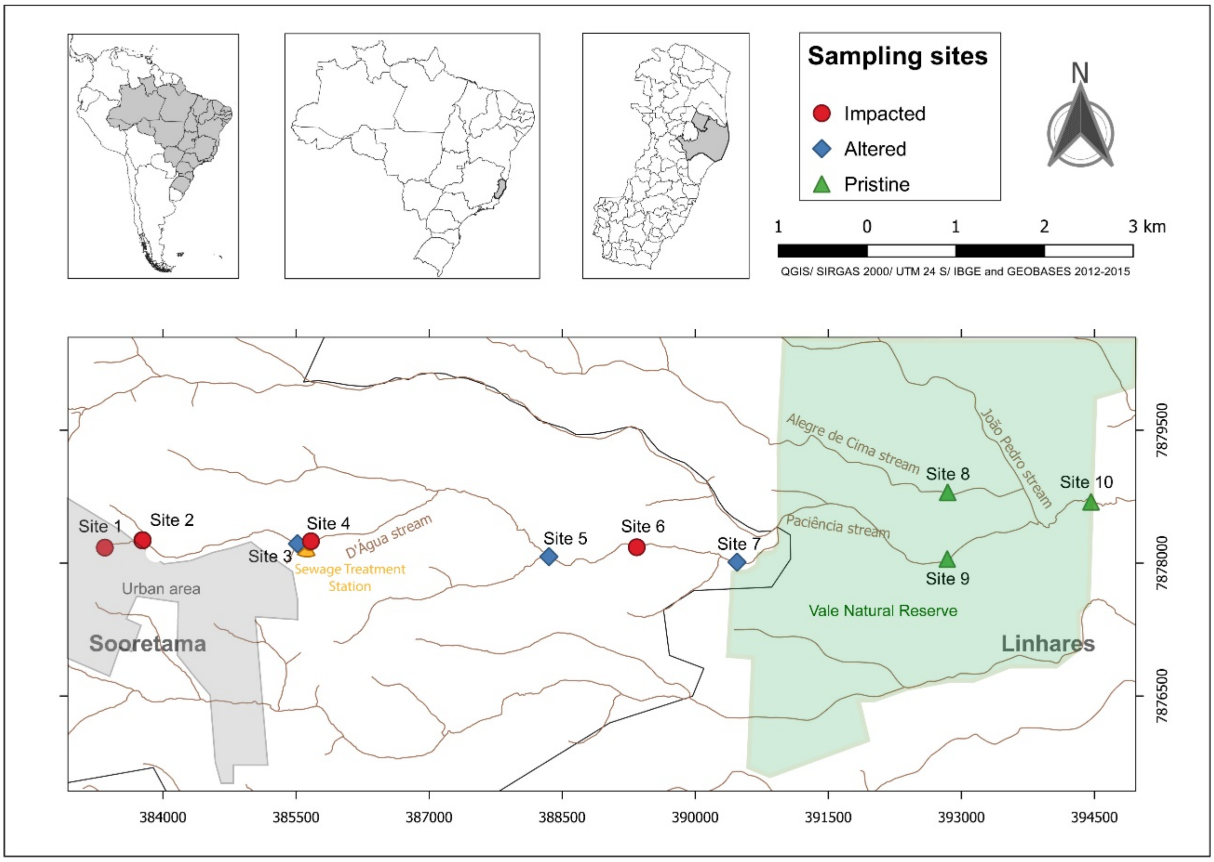

Fig. 1. Location of sampling sites and respective streams (D'Água, Paciência, Alegre de Cima and João Pedro) in the municipalities of Sooretama and Linhares, ES. Sampling sites represents the categorization of environmental integrity measured by the Rapid Assessment Protocol for Habitat Diversity of Callisto *et al.* (2002) (supplementary data).

Table 1. Coordinates of the monitoring sites related to the D'Água, Paciência, Alegre de Cima and João Pedro streams, in the municipalities of Sooretama and Linhares, ES.

Identification of collection sites	Coordinates	Description of the collection site
1	24K 383345 / 7878176 (55 m s.n.m.)	Headwater of the D'Água stream located in the urban area of Sooretama, ES, Brazil
2	24K 383772 / 7878258 (50 m s.n.m.)	Near the headwater of the D'Água stream in the urban area of Sooretama, ES, Brazil
3	24K 385516 / 7878220 (40 m s.n.m.)	Before the Sewage Treatment Station on the D'Água stream in the urban area of Sooretama, ES, Brazil
4	24K 385668 / 7878248 (40 m s.n.m.)	After the Sewage Treatment Station on the D'Água stream in the urban area of Sooretama, ES, Brazil
5	24K 388351 / 7878074 (30 m s.n.m.)	Pasture area in Sooretama, ES, Brazil
6	24K 389341 / 7878180 (25 m s.n.m.)	Dam in the pasture area in Sooretama, ES, Brazil

7	24K 390471 / 7878012 (25 m s.n.m.)	Pasture area bordering the Vale Natural Reserve in Sooretama, ES, Brazil
8	24K 392846 / 7878801 (20 m s.n.m.)	Vale Natural Reserve in Linhares, ES, Brazil
9	24K 392841 / 7878047 (15 m s.n.m.)	Vale Natural Reserve in Linhares, ES, Brazil
10	24K 394462 / 7878693 (15 m s.n.m.)	Vale Natural Reserve in Linhares, ES, Brazil

3.2. Biological assessment

In each of the ten sampling points, we collected the aquatic macroinvertebrates samples by the scanning method along the streams. We used an aquatic D-net (500 µm and 471 cm^2) at each point, and we performed six scans, with a sampling effort of 15 seconds each, totaling 60 scans in each campaign, and 120 at the end of the two campaigns. We adopted a new collection protocol, reducing the sampling effort. Many works reported the efficiency of the smallest sampling effort, facilitating its replicability (Saito, Fonseca-Gessner & Siqueira, 2015; Flotemersch *et al.*, 2017).

After finishing each sampling, we previously screened the biological material to remove impurities, later we preserved in 80º ethyl alcohol solution, and transferred to the laboratory of the Entomology Museum of the Federal University of Viçosa (UFV). We performed the invertebrate identification to the lowest taxonomic level possible using literature on the regional fauna (Calor, 2007; Domínguez & Fernández, 2009; Hamada, Thorp & Rogers, 2018) and compared with the collection of aquatic insects of the Entomology Museum.

3.3. Assessment of land use and coverage and urbanization

Together with the biological material sampling, at each site, we used two protocols for assessing land use and coverage and urbanization. The first, proposed by Callisto *et al.* (2002), which aims to conduct a rapid assessment of the types of habitats present at the collection site, entitled: Rapid Assessment Protocol for Habitat Diversity (PAR) in stretches of watersheds, based on the protocol of the Ohio Environmental Protection Agency (USA) (Ohio EPA, 1987) (supplementary data). With this protocol we classified the freshwater environments into three categories: pristine, altered and impacted (Fig. 1). The second, proposed by Nessimian *et al.* (2008), where the Habitats Integrity Index (HII), based on the Petersen protocol (1992), is presented. Index values close to zero indicate an impacted environment, while values close to one indicate a pristine environment (supplementary data).

3.4. Water analysis

The RNV team carried out the water collections, in December 2019 and September 2020, and transported to the Fullin Environmental Analysis laboratory where they were subjected to physical-chemical analysis of the following parameters: Heterotrophic Bacteria, Chloride, Total Coliforms, Electrical Conductivity , Apparent Color, Chemical Oxygen Demand, Total Hardness (calc.), *Escherichia coli*, Total Iron, Phosphorus, Nitrate, Nitrite, Ammoniacal Nitrogen, Organic Nitrogen, pH, Total Solids, Sulfate. The analyzes were performed according to the version of the Standard Methods for the Examination of Water & Wastewater 23nd 2017 (SMEWW) and NBR (when applicable) (supplementary data).

We used the analytical results to compose the indices and interpretations, which consider the presence and concentration of toxic chemical contaminants, their effect on aquatic organisms (toxicity) and two of the variables considered essential for biota (pH and chemical oxygen demand).

3.5. Statistical analysis

In order to assess the ecological quality of streams in December 2019 (rainy season) and September 2020 (dry season), we used the following diversity metrics of aquatic macroinvertebrates as our response variables: total abundance, total taxonomic richness and percentage of bioindicator groups (% EPT [Ephemeroptera, Plecoptera and Trichoptera], % Chironomidae and % Odonata). We calculated the total taxonomic richness in order to exclude abundance effects (Gotelli & Colwell, 2001). For the explanatory variables, we used in addition to the integrity indexes (PAR and HII), as well as metrics related to tolerance / intolerance (BMWP and EPT / Chironomidae). We used the biotic index BMWP (Biological Monitoring Working Party System) (Armitage *et al.*, 1983) for its wide use in tropical and subtropical regions (Domínguez & Fernández, 2009; Zequi *et al.*, 2019; Das & Maity, 2021). This index relates the number of total taxa with a tolerance/intolerance value using the family as the taxonomic level. The final value is obtained by the sum of the values of the scores of each family, ranging from 0 to 10. Families with values closer to zero are more tolerant of pollution, and those closer to ten are more intolerant (Domínguez & Fernández, 2009).

3.5.1. Abundance and richness analysis

Firstly, we tested if there were differences in each diversity metrics between seasons (rainy and dry seasons) and urbanization gradient levels (pristine, altered and impacted) using a two-way ANOVA. We assumed the normal distribution, tested by Shapiro-Wilk, was used for the total richness, Poisson distribution, corrected for over dispersion, for total abundance and Binomial distribution, suitable for percentage data, for the % EPT, % Chironomidae and % Odonata.

So, for each diversity metrics we proceed linear regressions through the construction of GLMMs (mixed generalized linear models), in which the response variables were total richness, total abundance, % EPT, % Chironomidae and % Odonata, and the explanatory variables in each model were measures used to assess environmental quality (HII, BMWP, EPT / Chironomidae, total coliforms, pH, oxygen demand and total nitrogen), using the same distribution errors listed above to each response variable.

From the complete model, we removed non-significant terms, using the simplification and model selection method, always keeping the model as simple as possible, applying the principle of parsimony. At the end of each test, we performed a residual analysis to verify the adequacy of the models, test the distribution of errors used, to correct over or under dispersion when necessary, as well as the verification and removal of outliers, when present. We performed all statistical analyses in R software (R CoreTeam, 2020) using the all necessary packages.

3.5.2. Composition analysis

To compare the species composition, we used non-metric multidimensional scaling (nMDS) to assess macroinvertebrate community similarities between habitat integrity, along the urbanization gradient, based on PAR (pristine, altered and impacted). We used presence-absence data in order to evaluate families of macroinvertebrates composition with 9999 permutations. To assess the variability of macroinvertebrate communities along the urbanization gradient, we calculated Bray-Curtis dissimilarities between each stream based on PAR. After the nMDS, we performed a Similarity Analysis test (ANOSIM), which corresponds to a non-parametric test, which seeks significant differences between two or more groups based on any measure of distance (Clarke, 1993), which in our study case was also the Bray-Curtis dissimilarity index. Then we carried out a SIMPER test to compare the three communities in terms of differences in the main taxa that can cause the separation of the groups. In the test, we used 50% of the most influential taxa in the separation of communities, in order to identify possible bioindicator groups. These analyses were performed in Past free software (Hammer, Harper & Ryan, 2001).

4. RESULTS

4.1. Biological assessment

4.1.1. General data

We identified a total of 8,806 specimens of benthic macroinvertebrates, from the two sampling campaigns, distributed in four Phyla: Annelida, Arthropoda, Mollusca and Platyhelminthes. Arthropoda stood out (Fig. 2A), since Insecta alone represented 99% of the collected material (Fig. 2B). Diptera represented 88% of the total number of invertebrates (Fig. 2C). Chironomidae stood out from the other families, encompassing 6,837 specimens, being responsible for 98% (Fig. 2D and Table 2).

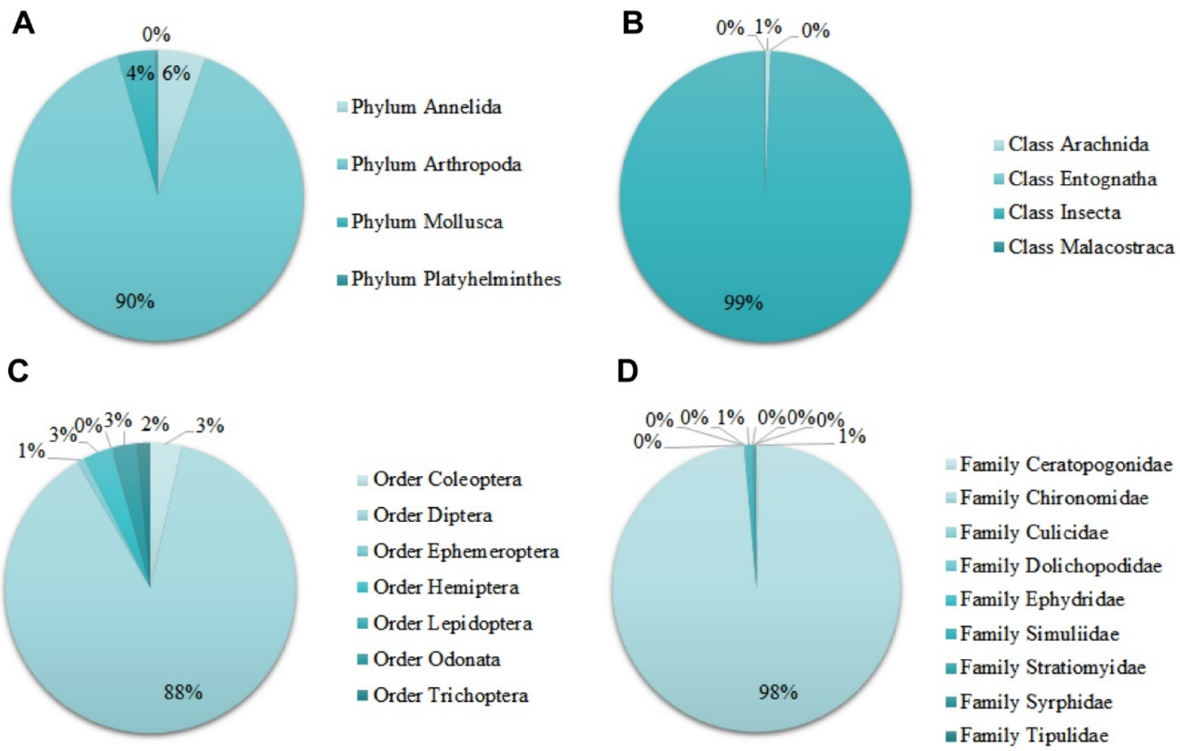

Fig. 2. Relative proportions of aquatic macroinvertebrates, distributed in Phylum (A), classes of the Phylum Arthropoda (B), orders of the Class Insecta (C) and families of the Order Diptera (D). Data referring to the two campaigns carried out in the streams of Sooretama and Linhares - ES, in the rainy season (December 2019) and in the dry season (September 2020).

Concerning insects, six orders were obtained (Coleoptera, Diptera, Ephemeroptera, Hemiptera, Odonata and Trichoptera) and 36 families (Aeshnidae, Baetidae, Belostomatidae, Caenidae, Ceratopogonidae, Chironomidae, Coenagrionidae, Corixidae, Culicidae, Curculionidae, Dolichopodidae, Dryopidae, Dytiscidae, Elmidae, Ephydridae, Gerridae, Hydrophilidae, Hydropsychidae, Lampyridae, Leptoceridae, Leptophlebiidae, Lestidae, Libellulidae, Meruidae, Naucoridae, Nepidae, Noteridae, Notonectidae, Pleidae, Scirtidae, Simuliidae, Staphylinidae, Stratiomyidae, Syrphidae, Tipulidae, and Veliidae) (Table 2).

In the first campaign, carried out in the rainy season (December 2019), we collected a total of 5,200 specimens. As shown above in the total data, Chironomidae had the largest number of individuals collected (4,106) (Table 2). During the second campaign, carried out in the dry season (September 2020), we collected a total of 3,606 specimens. Once again, Chironomidae stood out with 2,731 individuals (Table 2).

Table 2. Aquatic macroinvertebrates collected in the two campaigns, in the rainy season (December 2019) and in the dry season (September 2020), carried out in the streams of Sooretama and Linhares, ES.

Taxon	Number of specimens collected		
	Rainy season	Dry season	Total
Phylum Annelida	**329**	**140**	**469**
Class Clitellata	**329**	**140**	**469**
Subclass “Oligochaeta”	198	65	263
Subclass Hirudinea	131	75	206
Phylum Arthropoda	**4655**	**3288**	**7943**
Class Arachnida	**11**	**33**	**44**
Family Hydrachnidae	11	33	44
Class “Entognatha”		**3**	**3**
Subclass Collembola		3	3
Class Insecta	**4635**	**3252**	**7887**
Order Coleoptera	**93**	**184**	**277**
Family Curculionidae	2	5	7
Family Dryopidae		1	1
Family Dytiscidae	11	43	54
Family Elmidae	1	46	47
Family Hydrophilidae	70	52	122
Family Lampyridae		2	2
Family Meruidae		1	1
Family Noteridae	9	12	21
Family Scirtidae		18	18
Family Staphylinidae		4	4
Order Diptera	**4195**	**2761**	**6956**
Family Ceratopogonidae	11	10	21
Family Chironomidae	4106	2731	6837
Family Culicidae	3		3

Taxon	Number of specimens collected		
	Rainy season	Dry season	Total
Family Dolichopodidae	1		1
Family Ephydridae	16		16
Family Simuliidae	43		43
Family Stratiomyidae	9	12	21
Family Syrphidae	3	4	7
Family Tipulidae	3	4	7
Order Ephemeroptera	**44**	**22**	**66**
Family Baetidae	15	16	31
Family Caenidae	22	6	28
Family Leptophlebiidae	7		7
Order Hemiptera	**87**	**166**	**253**
Family Belostomatidae	17	58	75
Family Corixidae	30	34	64
Family Gerridae		1	1
Family Naucoridae	7	5	12
Family Nepidae	1	1	2
Family Notonectidae	29	45	74
Family Pleidae	2	21	23
Family Veliidae	1	1	2
Order Odonata	**122**	**93**	**215**
Family Aeshnidae	8	2	10
Family Coenagrionidae	55	61	116
Family Lestidae	1	2	3
Family Libellulidae	58	28	86
Order Trichoptera	**94**	**26**	**120**
Family Hydropsychidae	91	3	94
Family Leptoceridae	3	23	26
Class Malacostraca	**9**		**9**
Order Decapoda	**9**		**9**
Family Penaeidae	2		2
Family Trichodactylidae	7		7
Phylum Mollusca	**216**	**151**	**367**
Class Gastropoda	**216**	**151**	**277**
Order Architaenioglossa	**216**	**61**	**277**
Family Ampullariidae	216	61	277
Class Bivalvia		**90**	**90**
Order Veneroida		**90**	**90**
Family Sphaeriidae		90	90
Phylum Platyhelminthes		**27**	**27**
Class Rhabditophora		**27**	**27**
Order Tricladida		**27**	**27**

Taxon	Number of specimens collected		
	Rainy season	Dry season	Total
Superfamily Planarioidea		27	27

4.1.2. Population distribution of aquatic macroinvertebrates by sampling site

In the first year of the campaign, site one (headwater) had the largest number of individuals (3,680), due to the abundance of Chironomidae at this site (Fig. 3). The headwater was surrounded by houses and hovels, so that the stream showed high signs of an impacted environment, such as dumped garbage, an unpleasant smell and a darkened color. In addition to the population peaks of Chironomidae, the aquatic snails (Ampullariidae) showed a peak in the first campaign at the third site, where the Sewage Treatment Station is located (Fig. 3). Other highlights of the first campaign were water worms ("Oligochaeta") and leeches (Hirudinea) at site seven and nine, Corixidae only at site seven. Hydrophilidae, Simuliidae and Libellulidae at site eight (Fig. 3).

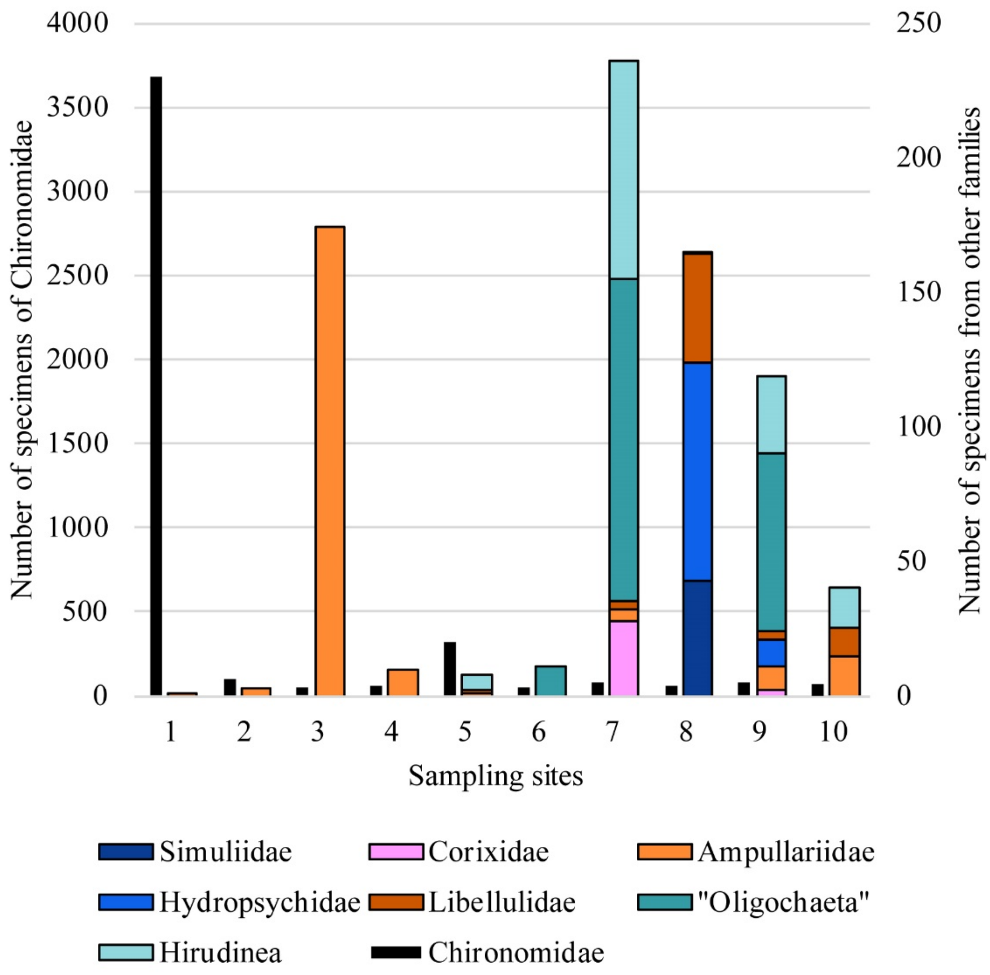

Fig. 3. Relative proportions of the main aquatic macroinvertebrates found in the first sampling campaign, carried out in the streams of Sooretama and Linhares - ES, in the rainy season December 2019).

On the other hand, in the second year, the first site was dry, which made it impossible to collect aquatic macroinvertebrates. Site seven was the most abundant of this campaign with 1,537 individuals, also due to the abundance of Chironomidae larvae (Fig. 4). Chironomidae showed high abundance at sites three and four, where there was a Sewage Treatment Station (Fig. 4). Still at the Sewage Station, Hydrophilidae showed a high abundance at site three. Ampullariidae, “Oligochaeta”, Dytiscidae and Hirudinea peaked at site five (Fig. 4), in the pasture area. At site six, due to the dam made in the middle of pasture, a lentic environment, the individuals: Notonectidae, Belostomatidae, Corixidae, Hydrophilidae and Hydrachnidae became the most abundant groups. At site eight, Elmidae, Leptoceridae and Planarioidea had high abundance. Finally, site ten showed a high number of freshwater mussels (Sphaeriidae) (Fig. 4).

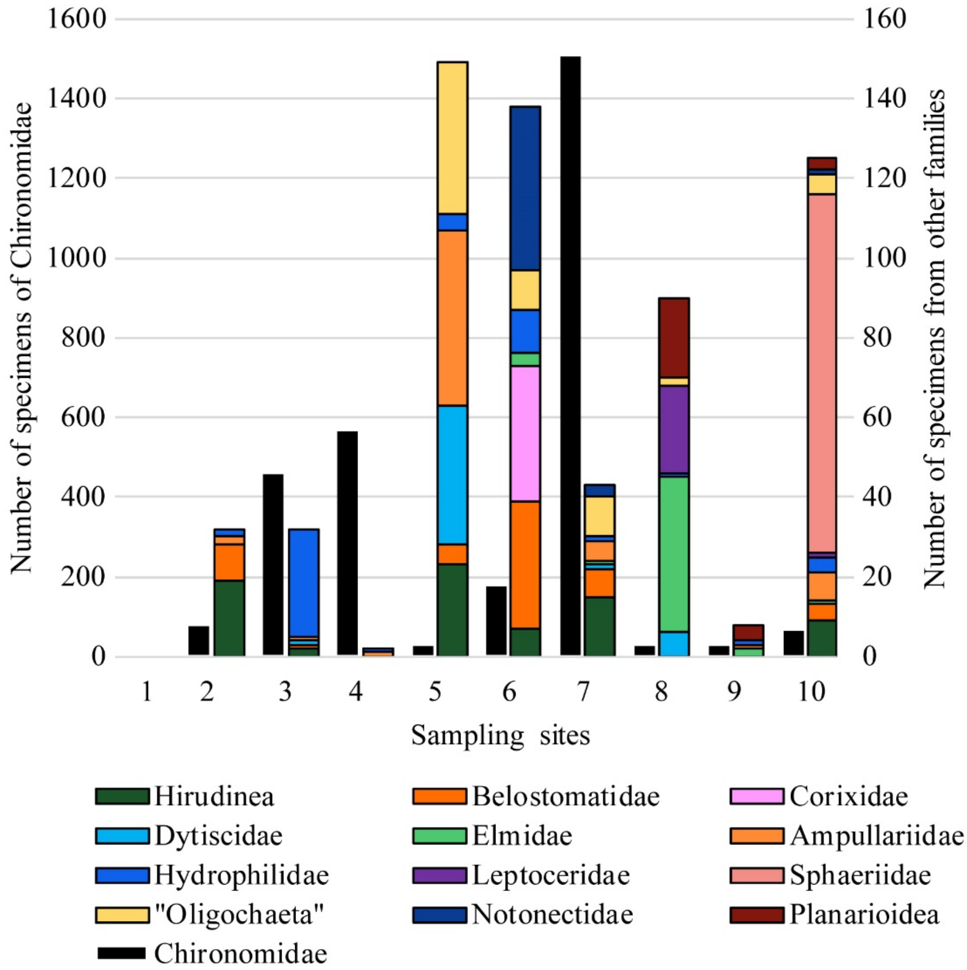

Fig. 4. Relative proportions of the main aquatic macroinvertebrates found in the second sampling campaign, carried out in the streams of Sooretama and Linhares - ES, in the dry season (September 2020).

4.2. Effects of urbanization gradient on invertebrate metrics

4.2.1. Total abundance and richness

Total abundance did not vary according to the type of environment ($F_{2.15} = 0.9324$; p = 0.4153) or between the dry and rainy seasons ($F_{1.17} = 0.1243$; p = 0.7293). Total taxonomic richness, on the other hand, varied according to the type of environment ($F_{2.15} = 3.9955$; p = 0.04065), being higher in natural environments and not differing between altered and impacted environments (p> 0.05, a result revealed by the analysis of contrasts *a posteriori*) (Fig. 5). There were no significant differences in taxonomic richness between the dry and rainy seasons ($F_{1.17}$ = 0.85593; p = 0.366861) (Fig. 5).

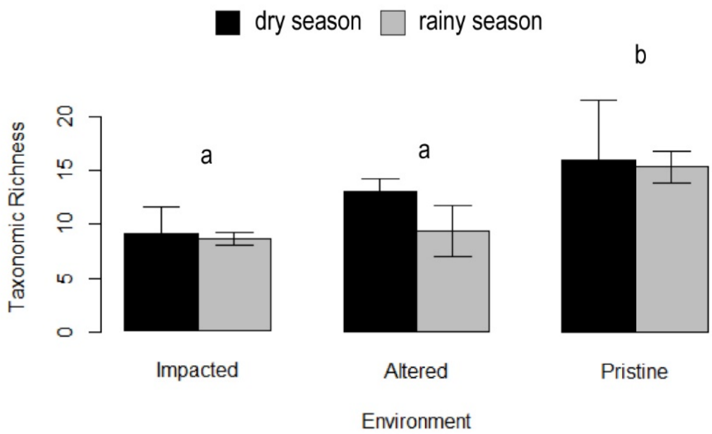

Fig. 5. Taxonomic richness according to the types of environments (altered, impacted and pristine) in the dry (black) and rainy (gray) seasons.

4.2.2. Bioindicator groups

All key groups (% EPT, % Chironomidae and % Odonata) had some kind of response to pristine, altered and impacted environments, but none of these groups bioindicators showed significant differences between the dry and rainy seasons (Table 3). The percentage of EPT ($F_{2.15} = 3.9406$; $p = 0.04213$) was higher and not differing between the pristine and impacted environments, and being statistically lower (null) in the altered (Fig. 6). The percentage of Chironomidae ($F_{2.15} = 4.6614$; $p = 0.02664$) was lower in the natural environment, and not differing in the impacted and altered environments (Fig. 6). And in the last group, the percentage of Odonata varied according to the three types of environment ($F_{2.15} = 6.4949$; $p = 0.00994$), being higher in the natural environment, followed by the impacted one and being lower in the altered one (Fig. 6). All differences were revealed by the analysis of contrasts *a posteriori*.

Table 3. F and p values of percentage of EPT, Chironomidae and Odonata according to the types of environments (altered, impacted and pristine). Significative values are in bold and marginally significative in italic.

Metrics	% EPT	% Chironomidae	% Odonata
Season	$F_{1.17} = 3.667$ $p = 0.07482$	$F_{1.17} = 0.712$ $p = 0.79316$	$F_{1.17} = 0.07060$ $p = 0.78657$
Environment	$F_{2.15} = 3.9406$ **$p = 0.04213$**	$F_{2.15} = 4.6614$ **$p = 0.02664$**	$F_{2.15} = 3.4079$ *$p = 0.060$*

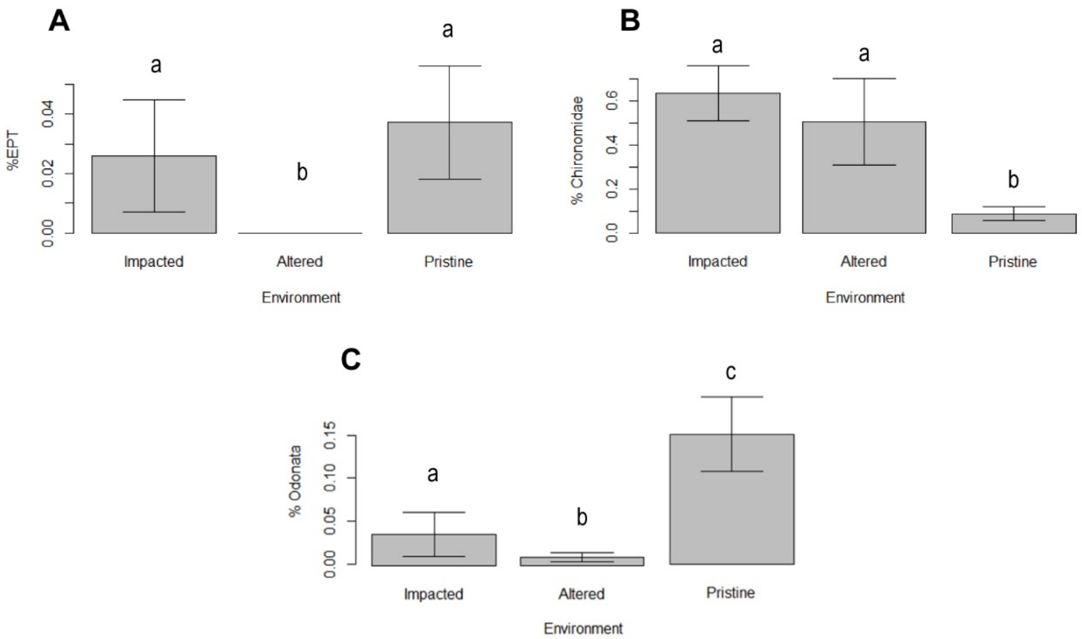

Fig. 6. Percentages of EPT, Chironomidae and Odonata according to the types of environments (altered, impacted and pristine).

4.3. Environmental quality metrics

4.3.1. Total abundance and richness

Although total abundance did not respond significantly to any environmental quality metrics (HII, BMWP, EPT / Chironomidae, fecal coliforms, pH, oxygen demand and total nitrogen), for total taxonomic richness we found significant responses for HII, BMWP and fecal coliforms (Fig. 7 and Table 4). We found a positive relationship between taxonomic richness for the metrics HII and BMWP, and a negative relation for the total coliforms (Fig. 7).

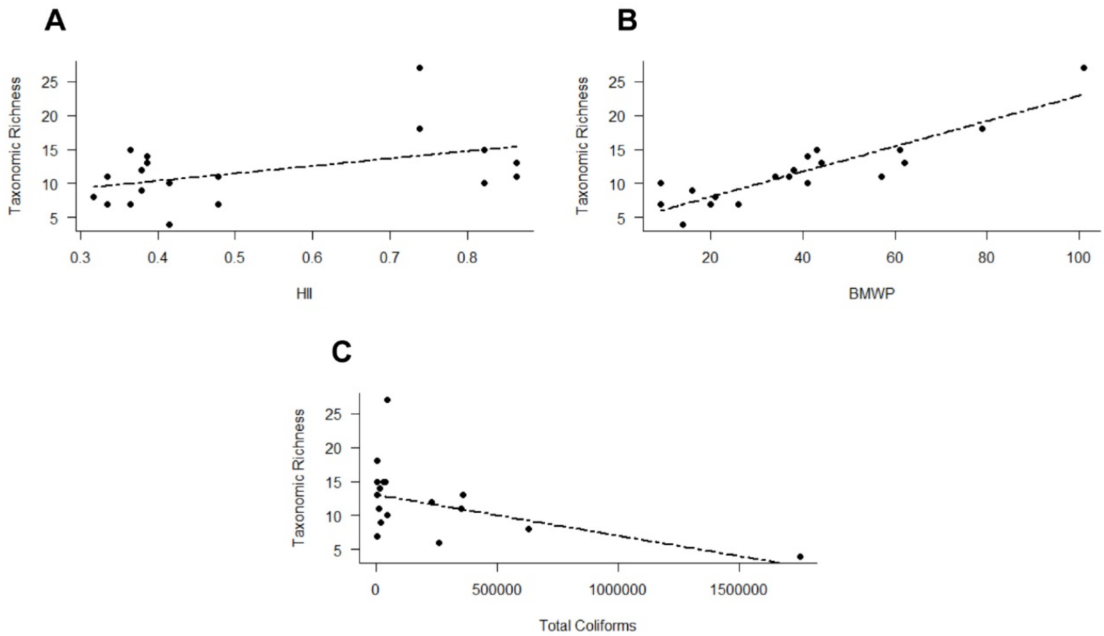

Fig. 7. Relationship between taxonomic richness and the metrics HII (A), BMWP (B) and fecal coliforms (C).

Table 4. F and p values of total richness and total abundance according to the types of environmental quality metrics. Significative values are in bold.

Metrics	Total Abundance	Total richness
HII	$F_{1.7}$= 0.0811; p = 0.7794	$F_{1.8}$ = 4.2856; **p = 0.055**
BWMP	$F_{1.8}$ = 0.5286; p = 0.4784	$F_{1.8}$ = 69.29; **p < 0.001**
EPT/Chironomidae	$F_{1.7}$ = 0.207; p = 0.6607	$F_{1.8}$ = 0.3535; p = 0.5738
Total coliforms	$F_{1.8}$ = 2.311; p = 0.1479	$F_{1.8}$ = 5.4784; **p = 0.0325**
pH	$F_{1.8}$ = 0.4774; p= 0.5001	$F_{1.8}$ = 0.5454; p = 0.4716
Oxygen demand	$F_{1.8}$ =1.2688; p = 0.2789	$F_{1.8}$ = 0.0080; p = 0.9262
Total nitrogen	$F_{1.7}$ = 2.8857; p = 0.1087	$F_{1.8}$ = 2.5736; p = 0.1283

4.3.2. Bioindicator groups

The results of linear regressions for each of the metrics used to assess the quality of the environment revealed a positive relationship between the % EPT ($F_{1.7}$ = 6.2369, p = 0.0238) and % Odonata ($F_{1.8}$ = 7.2855; p = 0.0152) and the HII (Fig. 8 and Table 5). On the other hand, a negative relation was found between % Chironomidae ($F_{1.8}$ = 9.0164, p = 0.00800) with the HII (Fig. 8 and Table 5). Regarding the percentages of EPT ($F_{1.7}$ = 5.0764, p = 0.03864) and Odonata ($F_{1.8}$ = 10.743; p = 0.004439) a positive relation for the BMWP was found (Fig. 9 and Table 5). Also, a positive relation was obtained between % EPT ($F_{1.8}$ = 31.64; p < 0.001) and EPT / Chironomidae ratio (Fig. 10 and Table 5). As expected, a negative relation was found between % EPT ($F_{1.7}$ = 6.7997; p < 0.01839) and total coliforms, and a positive relation between % Chironomidae ($F_{1.7}$ = 6.1319; p = 0.02402) for the total coliforms (Fig. 11 and Table 5). Finally, the percentage of Odonata presented a negative relation with total dissolved nitrogen (Fig. 12 and Table 5).

Table 5. F and p values of percentages of EPT, Chironomidae and Odonata according to the types of quality environmental metrics. Significative values are in bold.

Metrics	% EPT	% Chironomidae	% Odonata
HII	$F_{1.7}$=6.2369; **p=0.0238**	$F_{1.8}$=9.0164; **p=0.00800**	$F_{1.8}$=7.2855; **p=0.0152**
BWMP	$F_{1.7}$=5.0764; **p=0.03864**	$F_{1.8}$=3.6386; p=0.0735	$F_{1.8}$=10.743; **p=0.004439**
EPT/Chironomidae	$F_{1.8}$=31.64; **p<0.001**	$F_{1.8}$=3.1144; p=0.0955	$F_{1.7}$=2.1853; p=0.1588
Total coliforms	$F_{1.7}$=6.7997; **p=0.01839**	$F_{1.7}$=6.1319; **p=0.02402**	$F_{1.8}$=2.4725; p=0.1343
pH	$F_{1.8}$=2.3088; p=0.147	$F_{1.8}$=0.2024; p=0.6585	$F_{1.8}$=0.0274; p=0.8704
Oxygen demand	$F_{1.8}$=1.1377; p=0.3011	$F_{1.8}$=2.7634; p=0.1148	$F_{1.8}$=1.1413; p=0.3003
Total nitrogen	$F_{1.8}$=3.6811; p=0.07198	$F_{1.8}$=1.7043; p=0.2091	$F_{1.8}$=4.2543; **p=0.05477**

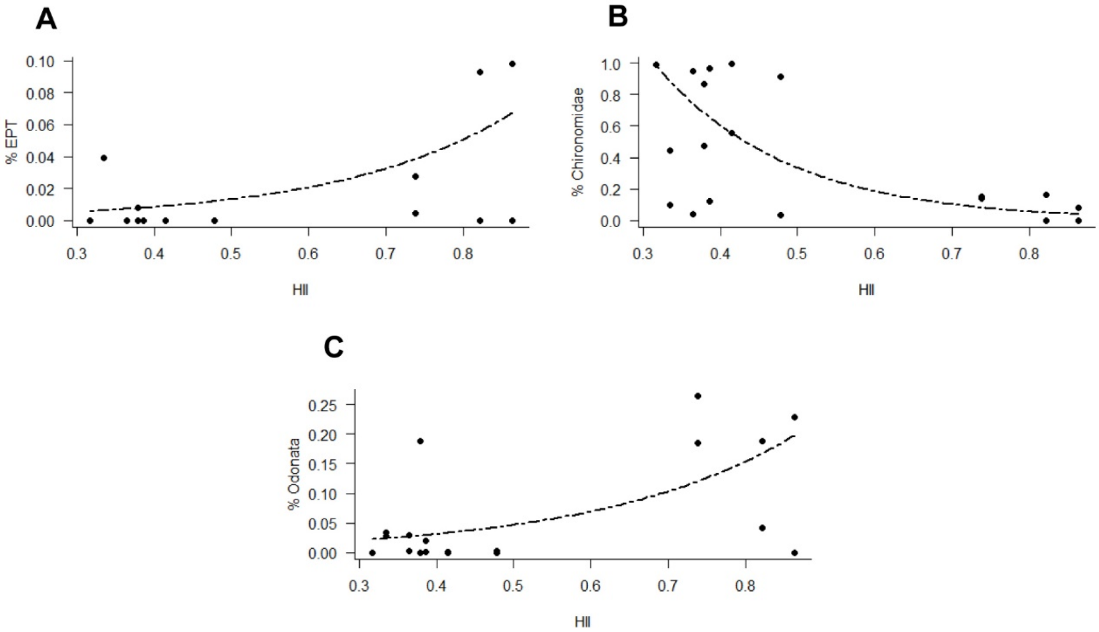

Fig. 8. Relationship between percentages of EPT (A), Chironomidae (B) and Odonata (C) with the metric HII.

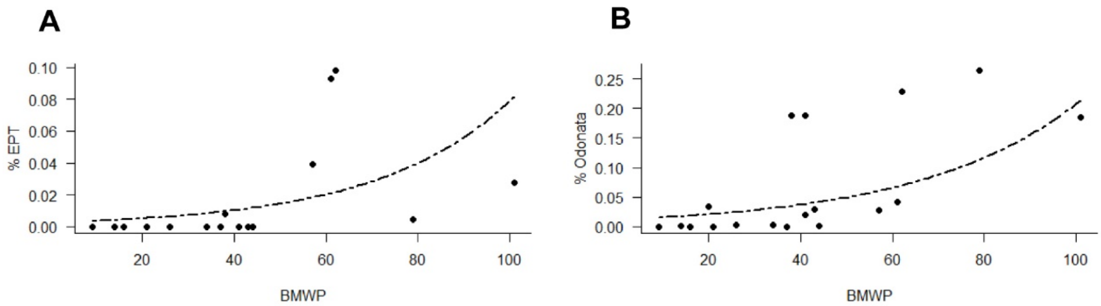

Fig. 9. Responses of EPT (A) and Odonata (B) to the metric BMWP.

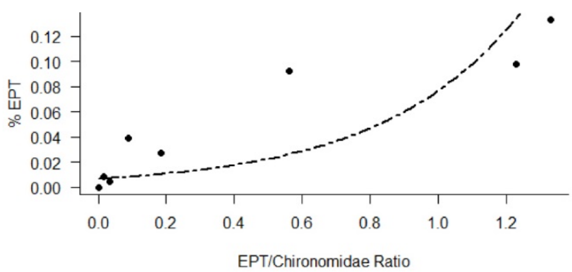

Fig. 10. Response of percentage of EPT to the metric EPT/Chironomidae.

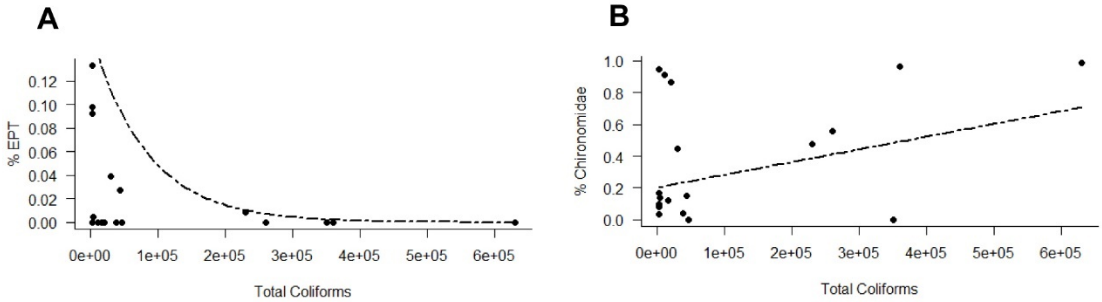

Fig. 11. Responses of percentages of EPT (A) and Chironomidae (B) to the total coliforms.

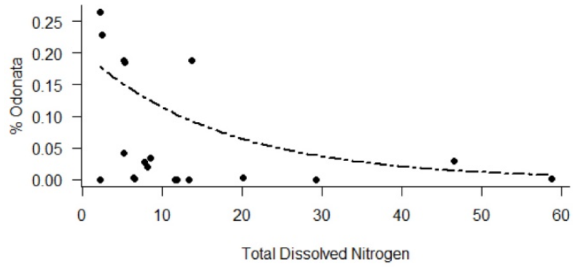

Fig. 12. Response of percentage of Odonata to the total dissolved nitrogen.

4.4. Community composition

Through the nMDS analysis we tested the compositions of the communities of aquatic organisms that showed variations between the pristine, altered and impacted environments. The stress value in our nMDS (0.2459) on two axes of ordination was at the upper end close to the acceptable range (0.0–0.2; Clarke, 1993), indicating moderate lack of fit. However, it is necessary to observe the other parameters, given by ANOSIM.

Species communities in pristine environments clustered separately from communities in altered environments, indicating that community compositions differed (Fig. 13). As for the pristine and impacted environments, they have a small overlapping part (Fig. 13). The the altered and impacted environments are also overlapping, indicating that there was no difference in the communities composition of the altered environments in the face of the impacted environments (Fig. 13). Points representing community composition in impacted environments were widely scattered in our nMDS plot, indicating inter-environment variability in community composition (Fig. 13).

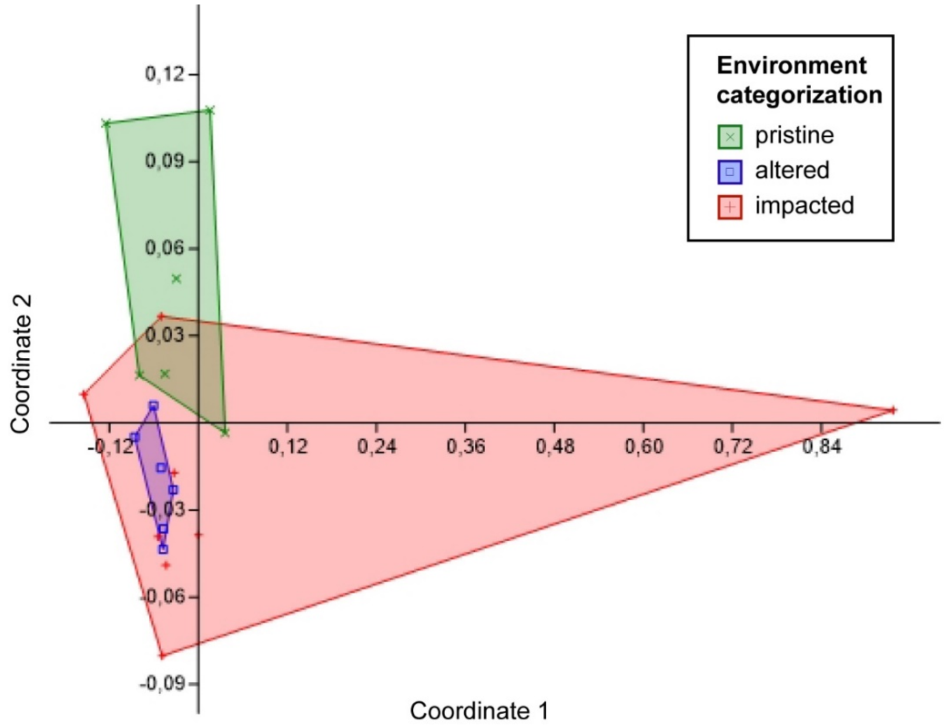

Fig. 13. Non-metric multidimensional (nMDS) ordination of macroinvertebrate community composition in pristine (green), altered (blue) and impacted (red) environment (n = 19; stress = 0.2459).

The results of ANOSIM showed that when we look at the three communities together (pristine, altered and impacted) there are no significant differences (R = 0.116 and p = 0.064, marginally significant) (Table 6). However, when we check the p values for the communities pair by pair, we observe that there are significant differences for the communities between the pristine and altered environments (p = 0.0016) and almost significant for pristine and impacted (p = 0.0705). There were no significant differences between the communities in the impacted and altered environments (Table 6).

Table 6. Pairwise comparisons of environments sampled. Results from ANOSIM based on Bray-Curtis distances between streams in pristine, altered and impacted. The omnibus test indicated significant difference among environments (number of permutations = 9999, R = 0.116, p = 0.0626). Significative values of ANOSIM are in bold.

Environment	Altered	Impacted	Pristine
Altered	-	0.6033	**0.0016**
Impacted	0.6033	-	0.0705

Pristine	**0.0016**	0.0705	-

Through the relative frequencies (up to 50% of the contribution of the most influential taxa in the separation of communities) obtained with the SIMPER analysis, we found that Tipulidae, Baetidae, Hydropsychidae, Planarioidea, Naucoridae and Caenidae characterized pristine environments, with an mean frequency = 0 in the altered environment, in order of cumulative percentage contribution (%) respectively (Table 7). No family was found to characterize an altered environment (Table 7).

Table 7. Similarity of percentage (SIMPER) analysis based on Bray-Curtis distances of macroinvertebrate community composition in Espírito Santo stream environments. Values represent the cumulative percentage contribution (%) and the mean frequency of each taxon to the similarities of the altered and pristine environments (only the most important taxon contributing globally up to 50% of the environment similarity are shown, overall average dissimilarity = 63.44).

Taxon	Cumulative %	Mean frequency Altered	Mean frequency Pristine
Tipulidae	4.119	0	0.667
Elmidae	8.213	0.167	0.667
Baetidae	12.11	0	0.667
Hydropsychidae	15.62	0	0.5
Coenagrionidae	19.11	0.333	0.667
Hirudinea	22.58	0.833	0.5
Belostomatidae	26.05	0.833	0.5
“Oligochaeta”	29.46	0.5	0.667
Planariodea	32.82	0	0.5
Stratiomyidae	36	0.167	0.5
Dytiscidae	39.14	0.5	0.333
Naucoridae	42.17	0	0.5
Caenidae	45.12	0	0.5
Libellulidae	47.94	0.667	0.833
Ceratopogonidae	50.7	0.333	0.333

When we ran the SIMPER analysis of the similarities between impacted and pristine environments, we found that Tipulidae, Hydropsychidae, Planarioidea, Naucoridae and Caenidae characterized pristine environment, presenting mean frequency = 0 in the impacted environment, in order of cumulative percentage contribution (%) respectively (Table 8). However, none of the families characterized impacted environments (Table 8).

Table 8. Similarity of percentage (SIMPER) analysis based on Bray-Curtis distances of macroinvertebrate community composition in Espírito Santo stream environments. Values represent the cumulative percentage contribution (%) and the mean frequency of each taxon to the similarities of the impacted and pristine environments (only the most important taxon contributing globally up to 50% of the environment similarity are shown, overall average dissimilarity = 71.8).

Taxon	Cumulative %	Mean frequency Impacted	Mean frequency Pristine
Elmidae	4.554	0.125	0.667
Tipulidae	8.907	0	0.667
“Oligochaeta”	12.98	0.25	0.667
Coenagrionidae	16.78	0.25	0.667
Hydropsychidae	20.55	0	0.5
Libellulidae	24.32	0.5	0.833
Baetidae	27.97	0.375	0.667
Planarioidea	31.6	0	0.5
Ampullariidae	35.08	0.5	0.667
Stratiomyidae	38.51	0.375	0.5
Naucoridae	41.71	0	0.5
Caenidae	44.8	0	0.5
Chironomidae	47.85	0.875	0.667
Belostomatidae	50.84	0.25	0.5

As for the similarities between altered and impacted environments, we did not find families that characterized any of them (Table 9). Both environments presented taxa with similar mean frequencies (Table 9) and there were no significant differences between them (Table 9). However, Ampullariidae and Hydrophilidae occurred in 100% of the samples from the altered environment, presenting mean frequency = 1 (Table 9).

Table 9. Similarity of percentage (SIMPER) analysis based on Bray-Curtis distances of macroinvertebrate community composition in Espírito Santo stream environments. Values represent the cumulative percentage contribution (%) and the mean frequency of each taxon to the similarities of the altered and impacted environments (only the most important taxon contributing globally up to 50% of the environment similarity are shown, overall average dissimilarity = 58.81).

Taxon	Cumulative %	Mean frequency Altered	Mean frequency Impacted
Belostomatidae	7.403	0.833	0.25
Hirudinea	14.81	0.833	0.25
Ampullariidae	21.07	1	0.5

Libellulidae	26.96	0.667	0.5
Dytiscidae	32.17	0.5	0.25
“Oligochaeta”	36.98	0.5	0.25
Noteridae	41.65	0.5	0.125
Syrphidae	45.98	0.167	0.375
Hydrophilidae	50.16	1	0.75

5. DISCUSSION

Our study showed that (1) impacted and altered streams had lower total richness and Odonata percentage, and higher Chironomidae percentage when compared to pristine streams; (2) as expected Chironomidae had a positive relationship with total coliforms, and total richness and EPT percentage had a negative one, and Odonata percentage had a negative relationship with total dissolved nitrogen; (3) pristine stream community of benthic macroinvertebrates showed different composition between impacted and altered streams, however, communities of impacted and altered environments were generally similar.

Through these results, we were able to understand that within the urbanized area (upstream), water bodies had the worst conditions in our study, with high concentrations of pollution indicator metrics, such as fecal coliforms, total nitrogen and total solids (supplementary data). Furthermore, these streams had high numbers of pollution-tolerant macroinvertebrates, such as dipterans and mainly chironomids, in contrast, they had low numbers of pollution-sensitive macroinvertebrates, such as the EPT group and Odonata.

We were able to verify that when the stream enters the preserved region, the water quality conditions start to improve. There is an increase in taxonomic richness and in aquatic macroinvertebrates sensitive to pollution. However, this does not mean that the conditions in the preserved area are ideal, in fact, the situation in the urbanized area is unbalanced, with a lot of pollution. This pollution is diluted and runs all the way to the preserved area. Despite mitigation of these pollutants from the urbanized region to the Vale reserve, we cannot guarantee that living beings dependent on water bodies are safe in the face of this situation. This panorama corroborates our hypothesis, where impacted and altered environments lacked pollution-sensitive macroinvertebrates and, antagonistically, pristine environments had a better abundance of these bioindicators.

According to Arenas-Sánchez *et al.* (2021), pollution results in more tolerant and less diverse communities, with polluted sites suffering from severe hydrological variation (urban and agricultural sites) showing a trend towards lower richness and functional richness, and significantly lower functional diversity. Our results were very similar, with impacted and altered streams showing lower richness and Odonata percentage, and higher Chironomidae percentage when compared to pristine streams.

One factor that can help mitigate this scenario is the allocation of areas for conservation, through which we can maintain the minimum acceptable standards for the existence of the species (Nessimian *et al.*, 2008; Hauer *et al.*, 2013; Pereira-Moura *et al.*, 2021). Many of the streams sampled in this study are located in the remnants of the Atlantic Forest, however, they already suffer the effects of population growth and land use, which occupy the soil with farms, residences and pastures. Many of these streams are now on private properties and have already lost characteristics that influence the local fauna, mainly due to the pollution of garbage and sewage deposited in the stream beds, as well as the alteration of marginal vegetation, which is the basis of habitat integrity assessments, and the home site of many aquatic macroinvertebrates (Nessimian *et al.*, 2008).

These streams suffer impacts on their environmental structure, since both environments had a higher proportion of sediments than the pristine ones. The high amount of sediment deposited in the bed of these streams is a direct effect of soil disturbance in these areas. Some studies described around the world have shown this same problem in different environments with human activities (Relyea, Minshall & Danehy, 2012; Correa *et al.*, 2015; Leitner *et al.*, 2015; Manoel & Uieda, 2021).

Our results were significantly correlated with most of our explanatory variables (HII, BMWP, EPT / Chironomidae, total coliforms and total nitrogen). Furthermore, our results showed significant correlation between HII values and faunal metrics (taxonomic richness, EPT, Odonata and Chironomidae). Similar results were presented in a work developed in Pará, Brazil (Pereira-Moura *et al.*, 2021). However, the work carried out by Nessimian *et al.* (2008), which we base ourselves on to carry out our study, found the opposite of ours. Despite, the environmental water quality metrics we used in this study were sufficient to understand spatial variations, however, we would need more studies to be able to list the best metrics to understand temporal variations.

Likewise, the aquatic macroinvertebrate community responds to variations in the environmental quality of water and we expected to find differences in the community in the impacted and altered environment, however, through this community we realize that the impacted and altered environments represented a single environment, that is, the altered environment is a variation of the impacted one. By the way, in the works by Callisto *et al.* (2002) and Nessimian *et al.* (2008) that categorize the environments, they point out the fluidity of the categorizations, because depending on the type of vegetation cover or land use (e.g., cattle raising, different agricultural practices, forestry), changes in vegetation cover can lead to few or discrete physical changes in streams. Thus, hindering our interpretation of the state of the quality of the environment. Furthermore, they explain the importance of considering the parameters of categorization of habitats individually, aiming at a better interpretation of ecological conditions. According to Nessimian *et al.* (2008), sediment inputs induce siltation, leading to habitat changes, disturbance of trophic resources and feeding mechanisms of streams fauna. In this way, it alerts us to the situation of altered environments that, quickly, can become the most accentuated variation of a polluted environment.

Other important data obtained in this work are the taxonomic richness and abundance, where only the first can explain the variations in water quality in this study, because the total abundance data showed some outliers that were removed from the statistical analysis, these data were referring, in mostly, to chironomid peaks in polluted environments, reaching more than a thousand individuals in a single site. Therefore, for these conditions in the studied region, abundance was not effective to detect variations in water quality, or contrary to the taxonomic richness that significantly correlated with our explanatory variables.

The higher values of total coliforms and total dissolved nitrogen found in non-pristine streams are probably due to the garbage and sewage that are deposited into the stream. According to Martins *et al.* (2017) the impact of urbanization streams is accentuated when there is an increase in deforestation and sewage input. These increased impacts result in a decrease in sensitive taxa (e.g., EPT and Odonata families) and an increase in tolerant organisms (e.g., Chironomidae and “Oligochaeta”) (Moreno & Callisto, 2010; Martins *et al.*, 2017; Callisto *et al.*, 2019; Mendoza-Penagos, Calvão & Juen, 2021), as well as found in our results. Although the pristine streams have been affected by the impacts of urbanization, they are located in conservation areas that generally mitigate the negative impact. Moreover, the water in pristine streams ingested by this fauna is responsible for the nutrition of the cellular tissue and for the maintenance of homeothermy, regulating the temperature of the body and internal organs. However, inadequate water supply decreases the animal's performance (Andersson, 1978). Therefore, efficient public policies need to be designed to prevent intensification of the effects of urbanization in Espírito Santo streams.

Our findings suggest that Tipulidae, Baetidae, Hydropsychidae, Planarioidea, Naucoridae and Caenidae tended to characterize pristine streams as compared to impacted and altered streams. According to Nessimian *et al.* (2008), *Macrostemum* (Hydropsychidae) was positively correlated with pristine areas. On the other hand, their results showed conversely, *Callibaetis* (Baetidae) and *Smicridea* (Hydropsychidae) were negatively correlated and occurred only in non-pristine areas. Moreover, Caenidae and several Diptera taxa have shown a relatively high tolerance to pollution and hydrological stress in other studies performed in the Mediterranean region (Sabater *et al.*, 2016; Kalogianni *et al.*, 2017). This suggests that our featured families may not really represent a pristine stream. We probably have more influences from urbanization in pristine areas that, unfortunately, we cannot detect.

This study shows that healthier streams had a higher richness of EPT and Odonata and a lower richness of Chironomidae. Moreover, the impact of anthropogenic pollution on the richness and community composition of aquatic macroinvertebrates is lower in impacted and altered streams (in both seasons). This was potentially due to higher coliforms and nitrogen dilution and physically altered habitats, but further studies should be performed to confirm the factors related with pollution affecting this response. In general, pollution resulted in more tolerant and less diverse communities, with polluted sites suffering from severe hydrological variation (urban and agricultural sites) showing a trend towards lower richness and functional diversity. However, our pristine streams probably present a high influence of urbanization that we cannot detect. In addition, our study shows that our variables can identify differences between polluted and non-polluted sites, we were able to find an improvement in biodiversity provided by the conservation area, but lacks sensitivity to assess macroinvertebrate responses to alterations of seasonal hydrological and/or chemical pollution. Moreover, for this region the use of the taxonomic richness, % EPT, % Odonata, and % Chironomidae as metrics are sufficient to measure the environmental water quality. Therefore, this study supports the evaluation of other taxonomic variables and approaches for the ecological status assessment of Espírito Santo streams.

The development of future monitoring campaigns and variables should consider the combined effects of multiple stressors. The out-comes of our study indicate that rainy and dry seasons seem to show an impact to functional biodiversity by the higher pollution conditions in the urbanized streams. Therefore, further studies and monitoring campaigns assessing the effects of pollution within these periods are recommended. We must also develop strategies so that the legislation (under the terms of articles 4, 5 and 6 of Law No. 12651/12 - New Forest Code) is complied with, maintaining an area of riparian vegetation (permanent preservation area - PPA) with 30 m of width from the stream bank (Valera *et al.*, 2019). Furthermore, public policies and partnership projects with private companies need to be taken to revitalize the streams studied, both non-pristine and pristine, so that the local aquatic macrofauna can be re-established, as well as all fauna that directly or indirectly use these streams will not be impacted. However, all types of elaborated projects must be thought out very carefully, because the streams in the urbanized region (water spring) are populated by needy people who built shacks inside the rivers. These people are not in this situation of inequality because they want to, but because they were neglected by society. So, it is up to us scientists, collaborators and the State, to take measures that consider the revitalization of the streams, but also measures of decent housing for these people.

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SUPPLEMENTARY DATA

Assessment of land use and coverage and urbanization

Both methods of analysis presented similar habitats integrity qualitative data, where the urbanized sites present qualitative data impacted by the PAR and the lowest scores by the HII, indicated by sites one, two and four, except the site three that received the altered classification by the PAR. The pasture sites (five, six and seven) received low scores by the HII, however the site six received the altered classification by the PAR. And all the last sites have received the pristine classification by the PAR and the highest scores by the HII (Table S1).

Table S1. Data of the Rapid Assessment Protocol for Habitat Diversity (PAR) and the Habitats Integrity Index (HII) to the different analyzed sites, in Sooretama and Linhares. ES, Brazil.

Sites	PAR	HII
1	impacted	0.317
2	impacted	0.379
3	altered	0.478
4	impacted	0.415
5	altered	0.365
6	impacted	0.335
7	altered	0.386
8	pristine	0.863
9	pristine	0.821
10	pristine	0.738

Water analysis

The assessment of physical-chemical quality reflects the current situation, being an efficient tool for the assessment of point contamination, as well as allowing the interpretation of water quality buoyancy in the environment. Thus, its interpretation must be accompanied by data from the zoobenthos, as they reflect a longer period due to their life cycle present in the stream.

We observed that sites four and seven showed high changes in some parameters. such as electrical conductivity, chemical oxygen demand and total solids, either in 2019. or in 2020 (Table S2 and Table S3). However, the pH did not show major changes along the sites. The altered results may be indicative of anthropic changes. For the initial points, changes in these data were already expected due to the proximity to the urban area of Sooretama (Table S2 and Table S3). However, these high values at site seven in September 2020 are worrisome, as this impacted panorama of the stream may be being transported to the preserved forest region of Vale Natural Reserve, located at sites eight, nine and ten of this stream (Table S3).

Table S2. Analytical results of the water analysis of the first campaign, in the rainy season (December 2019), carried out at the collection sites (1 to 10), in the streams of Sooretama and Linhares - ES.

Analyzed Parameter	Unit	LQ	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	Site 7	Site 8	Site 9	Site 10
Heterotrophic Bacteria	UFC/mL	1	> 5700	3500	1000	> 5700	1400	2500	770	730	990	1100
Chloride	mg/L	0.7	68.8	51.2	37.2	63.4	41.8	29.2	28.2	27.3	24.2	24.7
Total Coliforms	UFC/100mL	1	$6.30x10^5$	$2.0x10^4$	$2.50x10^3$	$2.60x10^5$	$2.66x10^3$	$2.08x10^3$	$1.62x10^4$	$1.90x10^3$	$2.30x10^3$	$4.30x10^3$
Electric conductivity	µS/cm	0.01	614	455	336	596	438	253	256	84.1	170.3	153.4
Apparent Color	mg Pt-Co/L	7	241	79	114	1742	198	294	162	318	178	206
Chemical Oxygen Demand	mg/L	100	32	25	25	86	30	45	25	55	26	36
Total Hardness (calc.)	mg/L	0.2	100.1	74.55	61.88	50.76	57.26	43.55	45.64	31.28	34.83	37.84
Escherichia coli	UFC/100mL	1	10000	Absence	Absence	Absence	Absence	Absence	Absence	Absence	Absence	Absence
Total Iron	mg/L	0.15	0.61	0.43	0.88	0.94	1.15	1.37	1.92	1.84	2.19	1.69
Phosphor	mg/L	0.15	0.398	0.128	0.106	1.419	1.006	0.632	0.685	0.033	0.398	0.272
Nitrate	mg/L (NO3- - N)	0.5	3.8	4.2	8.1	4.2	0.8	1.2	0.7	1.1	2	0.9
Nitrite	mg/L (NO2- - N)	0.01	0.227	0.43	0.486	0.328	< 0.01	0.529	0.028	< 0.01	0.038	< 0.01
Ammoniacal Nitrogen	mg/L (NH3 -N)	2	6.61	6.59	1.78	23.2	17.5	4.69	6.19	0.15	0.394	0.15
Organic Nitrogen	mg/L	1.2	< 1.2	2.1	< 1.2	1.6	1.8	2.1	< 1.2	< 1.2	2.8	< 1.2
pH	-	2 a 13	6.67	6.76	6.86	6.82	6.97	7.32	7.25	7.43	7.19	7.17
Total solids	mg/L	12	340	238	172	424	170	160	298	142	110	86
Sulfate	mg/L	5	50	29	12	16	10	6	6	5	5	5
Turbidity	UNT	0.5	41.9	8.36	13.6	108	14.8	43.8	14	6.09	9.68	6.42

Table S3. Analytical results of the water analysis of the second campaign, in the dry season (September 2020), carried out at the collection sites (2 to 10), in the streams of Sooretama and Linhares - ES.

Analyzed Parameter	Unit	LQ	Site 2	Site 3	Site 4	Site 5	Site 6	Site 7	Site 8	Site 9	Site 10
Heterotrophic Bacteria	UFC/mL	1	> 5700	28	> 5700	> 5700	150	> 5700	> 5700	> 5700	> 5700
Chloride	mg/L	0.7	31	32	117.1	47.1	31.5	39.6	11.6	23.1	24.4
Total Coliforms	UFC/100mL	1	2.30x10^{5}	1.10x10^{4}	1.75x10^{6}	3.80x10^{4}	3.00x10^{4}	3.60x10^{5}	3.50x10^{5}	4.70x10^{4}	4.40x10^{4}
Electric conductivity	µS/cm	0.01	304	306	973	450	245	155.7	74.2	188.7	174.2
Apparent Color	mg Pt-Co/L	7	833	80	1560	2906	440	19712	302	409	273
Chemical Oxygen Demand	mg/L	100	19	29	255	166	25	1140	38	60	76
Total Hardness (calc.)	mg/L	0.2	20.38	18.7	24.43	13.37	8.12	12.74	4.53	8.12	7.06
Escherichia coli	UFC/100mL	1	1.00x103	Absence	2.2x105	Absence	Absence	Absence	2.00x103	Absence	Absence
Total Iron	mg/L	0.15	2.72	0.43	0.39	20.5	1.44	41	1	2.7	2.19
Phosphor	mg/L	0.15	0.339	0.271	3.58	1.676	0.947	1.453	0.016	0.76	0.894
Nitrate	mg/L (NO3- - N)	0.5	10.9	1.4	0.5	< 0.5	< 0.5	0.5	0.8	1.5	1.4
Nitrite	mg/L (NO2- - N)	0.01	0.036	0.035	< 0.010	< 0.010	0.011	< 0.010	< 0.010	0.015	0.022
Ammoniacal Nitrogen	mg/L (NH3 -N)	2	0.544	3.77	50.8	19.9	5.07	0.463	< 0.150	2.13	0.321
Organic Nitrogen	mg/L	1.2	2.17	< 1.2	7.46	26.19	2.14	5.54	< 1.2	1.5	3.6
pH	-	2 a 13	6.09	6.75	7.4	6.76	8.99	6.01	6.32	6.75	6.42
Total solids	mg/L	12	726	198	514	1052	266	4192	74	248	144
Sulfate	mg/L	5	8	10	29	9	< 5	< 5	< 5	15	< 5
Turbidity	UNT	0.5	251	8.86	232	417	80.1	3650	18.4	22.7	15.2

Table S4. Composition data, in the rainy season (December 2019), carried out at the collection sites (1 to 10), in the streams of Sooretama and Linhares - ES.

Site	Stage	Family	Genus	Localization	Date	Number
1	larva	Chironomidae	-	ES, BR, Sooretama, Nascente de Sooretama	18.XII.2019	3624
1	adulto	Hydrophilidae	morfo 1	ES, BR, Sooretama, Nascente de Sooretama	18.XII.2019	2
1	adulto	Hydrophilidae	morfo 2	ES, BR, Sooretama, Nascente de Sooretama	18.XII.2019	3
1	larva	Hydrophilidae	-	ES, BR, Sooretama, Nascente de Sooretama	18.XII.2019	3
1	ninfa	Libellulidae	-	ES, BR, Sooretama, Nascente de Sooretama	18.XII.2019	1
1	larva	Syrphidae	morfo 1	ES, BR, Sooretama, Nascente de Sooretama	18.XII.2019	1
1	larva	Syrphidae	morfo 2	ES, BR, Sooretama, Nascente de Sooretama	18.XII.2019	1
1	larva	Ceratopogonidae	-	ES, BR, Sooretama, Nascente de Sooretama	18.XII.2019	4
1	larva	Stratiomyidae	Odontomyia	ES, BR, Sooretama, Nascente de Sooretama	18.XII.2019	6
1	larva	Ephydridae	morfo 1	ES, BR, Sooretama, Nascente de Sooretama	18.XII.2019	1
1	pupa	Chironomidae	-	ES, BR, Sooretama, Nascente de Sooretama	18.XII.2019	10
1	pupa	Ceratopogonidae	-	ES, BR, Sooretama, Nascente de Sooretama	18.XII.2019	4
1	pupa	Culicidae	-	ES, BR, Sooretama, Nascente de Sooretama	18.XII.2019	2
1	larva	Ephydridae	morfo 2	ES, BR, Sooretama, Nascente de Sooretama	18.XII.2019	15
1	larva	Ceratopogonidae	-	ES, BR, Sooretama, Nascente de Sooretama	18.XII.2019	3

2	larva	Chironomidae	-	ES, BR, Sooretama, Próximo à Nascente	17.XII.2019	58
2	adulto	Hydrophilidae	-	ES, BR, Sooretama, Próximo à Nascente	17.XII.2019	3
2	larva	Syrphidae	-	ES, BR, Sooretama, Próximo à Nascente	17.XII.2019	1
2	-	Ampullariidae (caramujo)	morfo 1	ES, BR, Sooretama, Próximo à Nascente	17.XII.2019	2
2	-	Ampullariidae (caramujo)	morfo 2	ES, BR, Sooretama, Próximo à Nascente	17.XII.2019	1
2	adulto	Dytiscidae	-	ES, BR, Sooretama, Próximo à Nascente	17.XII.2019	1
2	adulto	Hydrophilidae	-	ES, BR, Sooretama, Próximo à Nascente	17.XII.2019	1
3	-	Ampullariidae (caramujo)	morfo 1	ES, BR, Sooretama, ETE	19.XII.2019	172
3	-	Ampullariidae (caramujo)	morfo 2	ES, BR, Sooretama, ETE	19.XII.2019	1
3	-	Ampullariidae (caramujo)	morfo 3	ES, BR, Sooretama, ETE	19.XII.2019	1
3	larva	Chironomidae	-	ES, BR, Sooretama, ETE	19.XII.2019	6
3	larva	Hydrophilidae	-	ES, BR, Sooretama, ETE	19.XII.2019	3
4	larva	Chironomidae	-	ES, BR, Sooretama, ETE	19.XII.2019	15
4	adulto	Hydrophilidae	-	ES, BR, Sooretama, ETE	19.XII.2019	2
4	-	Ampullariidae (caramujo)	morfo 1	ES, BR, Sooretama, ETE	19.XII.2019	10
5	larva	Chironomidae	-	ES, BR, Sooretama, Pastagem	18.XII.2019	275
5	ninfa	Belostomatidae	-	ES, BR, Sooretama, Pastagem	18.XII.2019	5
5	ninfa	Libellulidae	-	ES, BR, Sooretama, Pastagem	18.XII.2019	1
5	-	Sanguessuga (Hirudinea)	-	ES, BR, Sooretama, Pastagem	18.XII.2019	6
5	adulto	Hydrophilidae	-	ES, BR, Sooretama, Pastagem	18.XII.2019	1
5	ninfa	Nepidae	-	ES, BR, Sooretama, Pastagem	18.XII.2019	1
5	-	Ampullariidae (caramujo)	morfo 1	ES, BR, Sooretama, Pastagem	18.XII.2019	1

6	adulto	Dytiscidae	-	ES, BR, Sooretama, Pastagem	18.XII.2019	1
6	pupa	Chironomidae	morfo 1	ES, BR, Sooretama, Pastagem	18.XII.2019	1
6	pupa	Chironomidae	morfo 2	ES, BR, Sooretama, Pastagem	18.XII.2019	2
6	ninfa	Baetidae	-	ES, BR, Sooretama, Pastagem	18.XII.2019	4
6	ninfa	Aeshnidae	-	ES, BR, Sooretama, Pastagem	18.XII.2019	1
6	adulto	Dytiscidae	-	ES, BR, Sooretama, Pastagem	18.XII.2019	2
6	adulto	Noteridae	-	ES, BR, Sooretama, Pastagem	18.XII.2019	7
6	larva	Noteridae		ES, BR, Sooretama, Pastagem	18.XII.2019	1
6	-	Minhoca d'água (Oligochaeta)	-	ES, BR, Sooretama, Pastagem	18.XII.2019	11
7	adulto	Hydrophilidae	-	ES, BR, Sooretama, Divisa com a Vale	17.XII.2019	7
7	larva	Leptoceridae	-	ES, BR, Sooretama, Divisa com a Vale	17.XII.2019	3
7	larva	Libellulidae	morfo 1 do ponto 7	ES, BR, Sooretama, Divisa com a Vale	17.XII.2019	1
7	larva	Libellulidae	morfo 2 do ponto 7	ES, BR, Sooretama, Divisa com a Vale	17.XII.2019	2
7	larva	Coenagrionidae	-	ES, BR, Sooretama, Divisa com a Vale	17.XII.2019	3
7	larva	Chironomidae	-	ES, BR, Sooretama, Divisa com a Vale	17.XII.2019	37
7	adulto	Corixidae	-	ES, BR, Sooretama, Divisa com a Vale	17.XII.2019	28
7	adulto	Pleidae	-	ES, BR, Sooretama, Divisa com a Vale	17.XII.2019	1
7	ninfa	Belostomatidae	-	ES, BR, Sooretama, Divisa com a Vale	17.XII.2019	2
7	-	Sanguessuga (Hirudinea)	-	ES, BR, Sooretama, Divisa com a Vale	17.XII.2019	81
7	-	Minhoca d'água (Oligochaeta)	-	ES, BR, Sooretama, Divisa com a Vale	17.XII.2019	120
7	adulto	Noteridae	-	ES, BR, Sooretama, Divisa com a Vale	17.XII.2019	1
7	-	Ácaro azul (Arachnida)	-	ES, BR, Sooretama, Divisa com a Vale	17.XII.2019	11
7	-	Penaeidae (camarão)	-	ES, BR, Sooretama, Divisa com a Vale	17.XII.2019	2
7	-	Ampullariidae (caramujo)	morfo 2	ES, BR, Sooretama, Divisa com a Vale	17.XII.2019	1
7	-	Ampullariidae (caramujo)	morfo 3	ES, BR, Sooretama, Divisa com a Vale	17.XII.2019	1
7	-	Ampullariidae (caramujo)	morfo 4	ES, BR, Sooretama, Divisa com a Vale	17.XII.2019	2

8	larva	Chironomidae	morfo 1	BR, ES, Linhares, Reserva Vale, PILOTO	16.XII.2019	6
8	larva	Chironomidae		BR, ES, Linhares, Reserva Vale, PILOTO	16.XII.2019	6
8	larva	Chironomidae	morfo 2	BR, ES, Linhares, Reserva Vale, PILOTO	16.XII.2019	5
8	larva	Simuliidae	morfo 1	BR, ES, Linhares, Reserva Vale, PILOTO	16.XII.2019	14
8	larva	Simuliidae	morfo 1	BR, ES, Linhares, Reserva Vale, PILOTO	16.XII.2019	28
8	larva	Tipulidae	-	BR, ES, Linhares, Reserva Vale, PILOTO	16.XII.2019	1
8	ninfa	Libellulidae	-	BR, ES, Linhares, Reserva Vale, PILOTO	16.XII.2019	7
8	ninfa	Libellulidae	-	BR, ES, Linhares, Reserva Vale, PILOTO	16.XII.2019	33
8	ninfa	Aeshnidae	-	BR, ES, Linhares, Reserva Vale, PILOTO	16.XII.2019	1
8	ninfa	Coenagrionidae	-	BR, ES, Linhares, Reserva Vale, PILOTO	16.XII.2019	4
8	ninfa	Coenagrionidae	-	BR, ES, Linhares, Reserva Vale, PILOTO	16.XII.2019	3
8	ninfa	Lestidae	-	BR, ES, Linhares, Reserva Vale, PILOTO	16.XII.2019	1
8	larva	Hydropsychidae	-	BR, ES, Linhares, Reserva Vale, PILOTO	16.XII.2019	54
8	larva	Hydropsychidae	-	BR, ES, Linhares, Reserva Vale, PILOTO	16.XII.2019	27
8	ninfa	Caenidae	-	BR, ES, Linhares, Reserva Vale, PILOTO	16.XII.2019	4
8	ninfa	Leptophlebiidae	-	BR, ES, Linhares, Reserva Vale, PILOTO	16.XII.2019	7
8	ninfa	Caenidae	-	BR, ES, Linhares, Reserva Vale, PILOTO	16.XII.2019	1

8	ninfa	Baetidae	morfo 1	BR, ES, Linhares, Reserva Vale, PILOTO	16.XII.2019	4
8	ninfa	Baetidae	morfo 2	BR, ES, Linhares, Reserva Vale, PILOTO	16.XII.2019	5
8	-	Minhoca d'água (Oligochaeta)	-	BR, ES, Linhares, Reserva Vale, PILOTO	16.XII.2019	1
8	larva	Simuliidae	morfo 2	BR, ES, Linhares, Reserva Vale, PILOTO	16.XII.2019	1
8	larva	Dolichopodidae	-	BR, ES, Linhares, Reserva Vale, PILOTO	16.XII.2019	1
9	ninfa	Coenagrionidae	-	BR, ES, Linhares, Reserva Vale	17.XII.2019	5
9	larva	Hydropsychidae	-	BR, ES, Linhares, Reserva Vale	17.XII.2019	10
9	larva	Stratiomyidae	-	BR, ES, Linhares, Reserva Vale	17.XII.2019	3
9	ninfa	Baetidae	-	BR, ES, Linhares, Reserva Vale	17.XII.2019	1
9	ninfa	Caenidae	-	BR, ES, Linhares, Reserva Vale	17.XII.2019	17
9	ninfa	Belostomatidae	-	BR, ES, Linhares, Reserva Vale	17.XII.2019	7
9	ninfa	Libellulidae	-	BR, ES, Linhares, Reserva Vale	17.XII.2019	3
9	adulto	Elmidae	-	BR, ES, Linhares, Reserva Vale	17.XII.2019	1
9	larva	Chironomidae	-	BR, ES, Linhares, Reserva Vale	17.XII.2019	30
9	adulto	Corixidae	-	BR, ES, Linhares, Reserva Vale	17.XII.2019	2
9	-	Ampullariidae (caramujo)	-	BR, ES, Linhares, Reserva Vale	17.XII.2019	9
9	-	Sanguessuga (Hirudinea)	-	BR, ES, Linhares, Reserva Vale	17.XII.2019	29
9	-	Trichodactylidae (caranguejo)	-	BR, ES, Linhares, Reserva Vale	17.XII.2019	6
9	-	Minhoca d'água (Oligochaeta)	-	BR, ES, Linhares, Reserva Vale	17.XII.2019	66
9	adulto	Hydrophilidae	-	BR, ES, Linhares, Reserva Vale	17.XII.2019	3
9	pupa	Chironomidae	-	BR, ES, Linhares, Reserva Vale	17.XII.2019	2
10	ninfa	Baetidae	-	BR, ES, Linhares, Reserva Vale	20.XII.2019	1
10	ninfa	Aeshnidae	-	BR, ES, Linhares, Reserva Vale	20.XII.2019	5
10	ninfa	Libellulidae	-	BR, ES, Linhares, Reserva Vale	20.XII.2019	10
10	ninfa	Coenagrionidae	-	BR, ES, Linhares, Reserva Vale	20.XII.2019	40

10	adulto	Curculionidae	-	BR, ES, Linhares, Reserva Vale	20.XII.2019	2
10	adulto	Hydrophilidae	-	BR, ES, Linhares, Reserva Vale	20.XII.2019	3
10	adulto	Pleidae	-	BR, ES, Linhares, Reserva Vale	20.XII.2019	1
10	larva	Dytiscidae	-	BR, ES, Linhares, Reserva Vale	20.XII.2019	2
10	larva	Hydrophilidae	-	BR, ES, Linhares, Reserva Vale	20.XII.2019	32
10	larva	Hydrophilidae	-	BR, ES, Linhares, Reserva Vale	20.XII.2019	2
10	adulto	Notonectidae	-	BR, ES, Linhares, Reserva Vale	20.XII.2019	29
10	ninfa	Naucoridae	-	BR, ES, Linhares, Reserva Vale	20.XII.2019	7
10	ninfa	Belostomatidae	-	BR, ES, Linhares, Reserva Vale	20.XII.2019	3
10	larva	Culicidae	-	BR, ES, Linhares, Reserva Vale	20.XII.2019	1
10	ninfa	Aeshnidae	-	BR, ES, Linhares, Reserva Vale	20.XII.2019	1
10	adulto	Veliidae	-	BR, ES, Linhares, Reserva Vale	20.XII.2019	1
10	-	Sanguessuga (Hirudinea)	-	BR, ES, Linhares, Reserva Vale	20.XII.2019	15
10	larva	Chironomidae	-	BR, ES, Linhares, Reserva Vale	20.XII.2019	29
10	-	Ampullariidae (caramujo)	-	BR, ES, Linhares, Reserva Vale	20.XII.2019	15
10	-	Trichodactylidae (caranguejo)	-	BR, ES, Linhares, Reserva Vale	20.XII.2019	1
10	larva	Tipulidae	-	BR, ES, Linhares, Reserva Vale	20.XII.2019	2
10	adulto	Hydrophilidae	-	BR, ES, Linhares, Reserva Vale	20.XII.2019	5
10	adulto	Dytiscidae	-	BR, ES, Linhares, Reserva Vale	20.XII.2019	5

Table S5. Composition data, in the dry season (September 2020), carried out at the collection sites (2 to 10), in the streams of Sooretama and Linhares - ES.

Ponto	**Estágio**	**Família**	**Gênero**	**Local**	**Data**	**Quantidade**
1	-	-	-	-	-	-
2	-	Ampullariidae (caramujo)	-	ES, BR, Sooretama, Próximo à Nascente	21.IX.2020	2
2	ninfa	Libellulidae	-	ES, BR, Sooretama, Próximo à Nascente	21.IX.2020	10
2	larva	Hydrophilidae	-	ES, BR, Sooretama, Próximo à Nascente	21.IX.2020	2
2	larva	Stratiomyidae	-	ES, BR, Sooretama, Próximo à Nascente	21.IX.2020	1
2	larva	Ceratopogonidae	-	ES, BR, Sooretama, Próximo à Nascente	21.IX.2020	4
2	pupa	Chironomidae	-	ES, BR, Sooretama, Próximo à Nascente	21.IX.2020	6
2	larva	Chironomidae	-	ES, BR, Sooretama, Próximo à Nascente	21.IX.2020	52
2	-	Sanguessuga (Hirudinea)	-	ES, BR, Sooretama, Próximo à Nascente	21.IX.2020	19
2	ninfa	Baetidae	-	ES, BR, Sooretama, Próximo à Nascente	21.IX.2020	1
2	ninfa	Coenagrionidae	-	ES, BR, Sooretama, Próximo à Nascente	21.IX.2020	13
2	adulto	Staphylinidae	-	ES, BR, Sooretama, Próximo à Nascente	21.IX.2020	1
2	ninfa	Belostomatidae	-	ES, BR, Sooretama, Próximo à Nascente	21.IX.2020	9
2	larva	Syrphidae	-	ES, BR, Sooretama, Próximo à Nascente	21.IX.2020	2
3	adulto	Hydrophilidae	-	ES, BR, Sooretama, ETE	21.IX.2020	13
3	larva	Stratiomyidae	-	ES, BR, Sooretama, ETE	21.IX.2020	4
3	larva	Syrphidae	-	ES, BR, Sooretama, ETE	21.IX.2020	1

3	ninfa	Coenagrionidae	-	ES, BR, Sooretama, ETE	21.IX.2020	1
3	-	Ampullariidae (caramujo)	-	ES, BR, Sooretama, ETE	21.IX.2020	1
3	larva	Chironomidae	-	ES, BR, Sooretama, ETE	21.IX.2020	392
3	pupa	Chironomidae	-	ES, BR, Sooretama, ETE	21.IX.2020	45
3	pupa	Ceratopogonidae	-	ES, BR, Sooretama, ETE	21.IX.2020	2
3	adulto	Hydrophilidae	-	ES, BR, Sooretama, ETE	21.IX.2020	8
3	-	Sanguessuga (Hirudinea)	-	ES, BR, Sooretama, ETE	21.IX.2020	2
3	adulto	Dytiscidae	-	ES, BR, Sooretama, ETE	21.IX.2020	1
3	larva	Hydrophilidae	-	ES, BR, Sooretama, ETE	21.IX.2020	6
3	larva	Coleophoridae	-	ES, BR, Sooretama, ETE	21.IX.2020	1
3	ninfa	Belostomatidae	-	ES, BR, Sooretama, ETE	21.IX.2020	1
4	ninfa	Libellulidae	-	ES, BR, Sooretama, ETE	22.IX.2020	1
4	pupa	Chironomidae	-	ES, BR, Sooretama, ETE	22.IX.2020	3
4	pupa	Chironomidae	-	ES, BR, Sooretama, ETE	22.IX.2020	543
4	larva	Hydrophilidae	-	ES, BR, Sooretama, ETE	22.IX.2020	1
4	-	Ampullariidae (caramujo)	-	ES, BR, Sooretama, ETE	22.IX.2020	1
5	ninfa	Libellulidae	-	ES, BR, Sooretama, Pastagem	22.IX.2020	4
5	-	Ampullariidae (caramujo)	morfo 2	ES, BR, Sooretama, Pastagem	22.IX.2020	24
5	-	Ampullariidae (caramujo)	morfo 1	ES, BR, Sooretama, Pastagem	22.IX.2020	20
5	adulto	Belostomatidae	-	ES, BR, Sooretama, Pastagem	22.IX.2020	2
5	ninfa	Belostomatidae	-	ES, BR, Sooretama, Pastagem	22.IX.2020	3
5	-	Sanguessuga (Hirudinea)	-	ES, BR, Sooretama, Pastagem	22.IX.2020	23
5	ninfa	Aeshnidae	-	ES, BR, Sooretama, Pastagem	22.IX.2020	2
5	larva	Hydrophilidae	-	ES, BR, Sooretama, Pastagem	22.IX.2020	4
5	adulto	Dytiscidae	-	ES, BR, Sooretama, Pastagem	22.IX.2020	35
5	adulto	Noteridae	-	ES, BR, Sooretama, Pastagem	22.IX.2020	9
5	adulto	Pleidae	-	ES, BR, Sooretama, Pastagem	22.IX.2020	3
5	larva	Coleophoridae	-	ES, BR, Sooretama, Pastagem	22.IX.2020	2
5	larva	Scirtidae	-	ES, BR, Sooretama, Pastagem	22.IX.2020	18
5	larva	Lampyridae	-	ES, BR, Sooretama, Pastagem	22.IX.2020	1
5	-	Minhoca d'água (Oligochaeta)	-	ES, BR, Sooretama, Pastagem	22.IX.2020	38
5	larva	Chironomidae	-	ES, BR, Sooretama, Pastagem	22.IX.2020	8

5	adulto	Curculionidae	-	ES, BR, Sooretama, Pastagem	22.IX.2020	4
6	ninfa	Belostomatidae	-	ES, BR, Sooretama, Pastagem	22.IX.2020	32
6	ninfa	Libellulidae	-	ES, BR, Sooretama, Pastagem	22.IX.2020	1
6	adulto	Notonectidae	-	ES, BR, Sooretama, Pastagem	22.IX.2020	1
6	adulto	Notonectidae	-	ES, BR, Sooretama, Pastagem	22.IX.2020	40
6	adulto	Pleidae	-	ES, BR, Sooretama, Pastagem	22.IX.2020	7
6	-	Sanguessuga (Hirudinea)	-	ES, BR, Sooretama, Pastagem	22.IX.2020	7
6	adulto	Elmidae	-	ES, BR, Sooretama, Pastagem	22.IX.2020	3
6	-	Ácaro azul (Arachnida)	-	ES, BR, Sooretama, Pastagem	22.IX.2020	25
6	ninfa	Coenagrionidae	-	ES, BR, Sooretama, Pastagem	22.IX.2020	9
6	ninfa	Baetidae	-	ES, BR, Sooretama, Pastagem	22.IX.2020	14
6	pupa	Chironomidae	-	ES, BR, Sooretama, Pastagem	22.IX.2020	23
6	larva	Chironomidae	-	ES, BR, Sooretama, Pastagem	22.IX.2020	135
6	adulto	Hydrophilidae	-	ES, BR, Sooretama, Pastagem	22.IX.2020	8
6	-	Minhoca d'água (Oligochaeta)	-	ES, BR, Sooretama, Pastagem	22.IX.2020	10
6	ninfa	Corixidae	-	ES, BR, Sooretama, Pastagem	22.IX.2020	34
6	larva	Hydrophilidae	-	ES, BR, Sooretama, Pastagem	22.IX.2020	3
6	adulto	Nepidae	-	ES, BR, Sooretama, Pastagem	22.IX.2020	1
6	adulto	Stratiomyidae	-	ES, BR, Sooretama, Pastagem	22.IX.2020	2
7	ninfa	Belostomatidae	-	ES, BR, Sooretama, Divisa com a Vale	22.IX.2020	7
7	ninfa	Libellulidae	-	ES, BR, Sooretama, Divisa com a Vale	22.IX.2020	2
7	-	Ampullariidae (caramujo)	-	ES, BR, Sooretama, Divisa com a Vale	22.IX.2020	5
7	-	Sanguessuga (Hirudinea)	-	ES, BR, Sooretama, Divisa com a Vale	22.IX.2020	15
7	adulto	Notonectidae	-	ES, BR, Sooretama, Divisa com a Vale	22.IX.2020	3
7	adulto	Noteridae	-	ES, BR, Sooretama, Divisa com a Vale	22.IX.2020	2
7	larva	Noteridae	-	ES, BR, Sooretama, Divisa com a	22.IX.2020	1

				Vale		
7	pupa	Chironomidae	-	ES, BR, Sooretama, Divisa com a Vale	22.IX.2020	1
7	larva	Chironomidae	-	ES, BR, Sooretama, Divisa com a Vale	22.IX.2020	1485
7	adulto	Dryopidae	-	ES, BR, Sooretama, Divisa com a Vale	22.IX.2020	1
7	larva	Ceratopogonidae	-	ES, BR, Sooretama, Divisa com a Vale	22.IX.2020	2
7	adulto	Hydrophilidae	-	ES, BR, Sooretama, Divisa com a Vale	22.IX.2020	1
7	adulto	Elmidae	-	ES, BR, Sooretama, Divisa com a Vale	22.IX.2020	1
7	-	Minhoca d'água (Oligochaeta)	-	ES, BR, Sooretama, Divisa com a Vale	22.IX.2020	10
7	adulto	Dytiscidae	-	ES, BR, Sooretama, Divisa com a Vale	22.IX.2020	1
8	larva	Hydropsychidae	-	BR, ES, Linhares, Reserva Vale, PILOTO	23.IX.2020	2
8	larva	Leptoceridae	morfo 1	BR, ES, Linhares, Reserva Vale, PILOTO	23.IX.2020	5
8	larva	Leptoceridae	morfo 2	BR, ES, Linhares, Reserva Vale, PILOTO	23.IX.2020	6
8	adulto	Elmidae	morfo 1	BR, ES, Linhares, Reserva Vale, PILOTO	23.IX.2020	17
8	-	Planária (Tricladida)	-	BR, ES, Linhares, Reserva Vale, PILOTO	23.IX.2020	18
8	larva	Tipulidae	-	BR, ES, Linhares, Reserva Vale, PILOTO	23.IX.2020	1
8	-	Minhoca d'água (Oligochaeta)	-	BR, ES, Linhares, Reserva Vale, PILOTO	23.IX.2020	2
8	larva	Dytiscidae	-	BR, ES, Linhares, Reserva Vale, PILOTO	23.IX.2020	5
8	larva	Ceratopogonidae	-	BR, ES, Linhares, Reserva Vale,	23.IX.2020	1

				PILOTO		
8	adulto	Elmidae	morfo 1	BR, ES, Linhares, Reserva Vale, PILOTO	23.IX.2020	17
8	adulto	Elmidae	morfo 2	BR, ES, Linhares, Reserva Vale, PILOTO	23.IX.2020	3
8	adulto	Elmidae	morfo 3	BR, ES, Linhares, Reserva Vale, PILOTO	23.IX.2020	2
8	-	Planária (Tricladida)	-	BR, ES, Linhares, Reserva Vale, PILOTO	23.IX.2020	2
8	adulto	Staphylinidae	-	BR, ES, Linhares, Reserva Vale, PILOTO	23.IX.2020	3
8	-	Collembola	-	BR, ES, Linhares, Reserva Vale, PILOTO	23.IX.2020	3
8	larva	Hydropsychidae	-	BR, ES, Linhares, Reserva Vale, PILOTO	23.IX.2020	1
8	larva	Leptoceridae	morfo 1	BR, ES, Linhares, Reserva Vale, PILOTO	23.IX.2020	9
8	larva	Leptoceridae	morfo 2	BR, ES, Linhares, Reserva Vale, PILOTO	23.IX.2020	2
8	adulto	Dytiscidae	-	BR, ES, Linhares, Reserva Vale, PILOTO	23.IX.2020	1
8	adulto	Hydrophilidae	-	BR, ES, Linhares, Reserva Vale, PILOTO	23.IX.2020	1
9	ninfa	Lestidae	-	BR, ES, Linhares, Reserva Vale	23.IX.2020	1
9	ninfa	Libellulidae	-	BR, ES, Linhares, Reserva Vale	23.IX.2020	1
9	larva	Stratiomyidae	-	BR, ES, Linhares, Reserva Vale	23.IX.2020	2
9	larva	Hydrophilidae	-	BR, ES, Linhares, Reserva Vale	23.IX.2020	1
9	larva	Meruidae	-	BR, ES, Linhares, Reserva Vale	23.IX.2020	1
9	-	Planária (Tricladida)	-	BR, ES, Linhares, Reserva Vale	23.IX.2020	2
9	adulto	Elmidae	-	BR, ES, Linhares, Reserva Vale	23.IX.2020	2
9	adulto	Gerridae	-	BR, ES, Linhares, Reserva Vale	23.IX.2020	1
9	-	Ampullariidae (caramujo)	-	BR, ES, Linhares, Reserva Vale	23.IX.2020	1
9	ninfa	Lestidae	-	BR, ES, Linhares, Reserva Vale	23.IX.2020	1
9	-	Planária (Tricladida)	-	BR, ES, Linhares, Reserva Vale	23.IX.2020	2

9	ninfa	Naucoridae	-	BR, ES, Linhares, Reserva Vale	23.IX.2020	1
10	ninfa	Belostomatidae	-	BR, ES, Linhares, Reserva Vale	23.IX.2020	4
10	ninfa	Libellulidae	-	BR, ES, Linhares, Reserva Vale	23.IX.2020	9
10	ninfa	Naucoridae	-	BR, ES, Linhares, Reserva Vale	23.IX.2020	1
10	adulto	Naucoridae	-	BR, ES, Linhares, Reserva Vale	23.IX.2020	3
10	ninfa	Notonectidae	-	BR, ES, Linhares, Reserva Vale	23.IX.2020	1
10	adulto	Hydrophilidae	-	BR, ES, Linhares, Reserva Vale	23.IX.2020	1
10	ninfa	Coenagrionidae	-	BR, ES, Linhares, Reserva Vale	23.IX.2020	38
10	ninfa	Pleidae	-	BR, ES, Linhares, Reserva Vale	23.IX.2020	3
10	adulto	Pleidae	-	BR, ES, Linhares, Reserva Vale	23.IX.2020	8
10	-	Ácaro vermelho (Arachnida)	-	BR, ES, Linhares, Reserva Vale	23.IX.2020	2
10	larva	Stratiomyidae	-	BR, ES, Linhares, Reserva Vale	23.IX.2020	3
10	-	Sanguessuga (Hirudinea)	-	BR, ES, Linhares, Reserva Vale	23.IX.2020	9
10	-	Planária (Tricladida)	-	BR, ES, Linhares, Reserva Vale	23.IX.2020	3
10	-	Ampullariidae (caramujo)	-	BR, ES, Linhares, Reserva Vale	23.IX.2020	7
10	-	Sphaeriidae (mexilhão)	-	BR, ES, Linhares, Reserva Vale	23.IX.2020	90
10	larva	Chironomidae	-	BR, ES, Linhares, Reserva Vale	23.IX.2020	36
10	pupa	Chironomidae	-	BR, ES, Linhares, Reserva Vale	23.IX.2020	2
10	ninfa	Baetidae	-	BR, ES, Linhares, Reserva Vale	23.IX.2020	1
10	ninfa	Caenidae	-	BR, ES, Linhares, Reserva Vale	23.IX.2020	6
10	larva	Leptoceridae	-	BR, ES, Linhares, Reserva Vale	23.IX.2020	1
10	larva	Coleophoridae	-	BR, ES, Linhares, Reserva Vale	23.IX.2020	4
10	larva	Hydrophilidae	-	BR, ES, Linhares, Reserva Vale	23.IX.2020	2
10	-	Minhoca d'água (Oligochaeta)	-	BR, ES, Linhares, Reserva Vale	23.IX.2020	5
10	larva	Tipulidae	-	BR, ES, Linhares, Reserva Vale	23.IX.2020	3
10	larva	Lampyridae	-	BR, ES, Linhares, Reserva Vale	23.IX.2020	1
10	larva	Syrphidae	-	BR, ES, Linhares, Reserva Vale	23.IX.2020	1
10	adulto	Curculionidae	-	BR, ES, Linhares, Reserva Vale	23.IX.2020	1
10	ninfa	Veliidae	-	BR, ES, Linhares, Reserva Vale	23.IX.2020	1
10	-	não identificado	-	BR, ES, Linhares, Reserva Vale	23.IX.2020	2
10	adulto	Hydrophilidae	-	BR, ES, Linhares, Reserva Vale	23.IX.2020	1

10	adulto	Elmidae	-	BR, ES, Linhares, Reserva Vale	23.IX.2020	1
10	larva	Ceratopogonidae	-	BR, ES, Linhares, Reserva Vale	23.IX.2020	1
10	-	Ácaro azul (Arachnida)	-	BR, ES, Linhares, Reserva Vale	23.IX.2020	6