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IMPORTANCE OF A PROTECTED AREA FOR THE CONSERVATION OF ODONATA BIODIVERSITY

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Sumário

Abstract

The changes of the land uses and the loss of native vegetation are the mainly cause of the biodiversity loss. These modifications reduce availability habitat and foods, increases competition and isolates populations, causing local extinction of species in most cases. Thus, the preservation and maintenance of areas with native vegetation as the protect areas (PA) are of extreme importance for the conservation of biodiversity. In this perspective, the objective of this study was evaluated the role of the Private Reserve of Natural Heritage (RPPN) in the conservation of Odonata biodiversity when compared to unprotected areas around it. This dissertation is divided into three chapters. In the first, we assess the importance of a PA in the structuring of Odonata assemblages in streams within and around the RPPN Estação Veracel. In the second chapter, we expand the distribution of the *Leptagrion acutum,* critically endangered species to the southern region of the state of Bahia. And in the third chapter, our aim went to evaluate whether the Zygoptera/Anisoptera ratio proposed by Oliveira-Junior & Juen (2019) as a biological index to assess changes in aquatic ecosystems in the Amazon can be used as an indicator of changes in streams of the Atlantic Forest. 40 points were sampled, being 22 sites within the Reserva Particular de Patrimônio Natural (RPPN) Veracel Station and 18 sites in the areas around it, in the South region of Bahia, between the municipalities of Santa Cruz Cabrália and Porto Seguro. Were made two collections, one in September 2018, and another in February 2019, in periods of rain and drought to capture Odonata individuals in different seasons and increase the sampling effort of the assemblages. The mainly results were that the loss of native vegetation changes the composition of the Odonata assemblages found within and around the RPPN. Changes in the aquatic environments of the areas surrounding the RPPN may alter certain habitat characteristics and consequently affect the feeding, mating and reproduction sites of more specialist species, leading them to local extinction. Sites located around the RPPN favor the colonization of more generalist species, modifying the composition of the assemblages between the areas. The PA as the RPPN Veracel Station plays a very important role in preserving of the endangered species as *L. acutum*. Reaffirming the importance of preservation areas as places of extreme importance to maintain and conserve the biodiversity of threatened species. And that the Odonata suborders can to be considered as bioindicators to assess changes to the surroundings of the aquatic ecosystems. In short, the three chapters highlight that the creation and maintenance of protected areas such as the RPPN Estação Veracel are of fundamental importance for biodiversity conservation, especially in areas of Atlantic Forest.

Resumo

As mudanças de usos do solo e a perda de vegetação nativas são as principais causas da perda de biodiversidade. Essas modificações reduz a disponibilidade de habitat e alimentos, aumenta a competição e isola as populações, causando a extinção local de espécies. Assim, a preservação e manutenção de áreas com vegetação nativa como as áreas protegidas (PA) são de extrema importância para a conservação da biodiversidade. Nessa perspectiva, o objetivo deste trabalho foi avaliar o papel da Reserva Particular do Patrimônio Natural (RPPN) na conservação da biodiversidade de Odonata quando comparada às áreas não protegidas em seu entorno. Esta dissertação está dividida em três capítulos. No primeiro nós avaliamos a importância de uma AP na estruturação das assembléias de Odonata em riachos dentro e no entorno da RPPN Estação Veracel. No segundo capítulo nós ampliamos a distribuição do *Leptagrion acutum*, espécie criticamente ameaçada de extinção para a região sul do estado da Bahia. E no terceiro capítulo, nosso objetivo foi avaliar se a razão Zygoptera / Anisoptera proposta por Oliveira-Junior & Juen (2019) como um índice biológico para avaliar mudanças em ecossistemas aquáticos na Amazônia pode ser usado como um indicador de mudanças em riachos da Mata Atlântica. Foram amostrados 40 pontos, sendo 22 pontos dentro da Reserva Particular de Patrimônio Natural (RPPN) da Estação Veracel e 18 pontos nas áreas em seu entorno, na região Sul da Bahia, entre os municípios de Santa Cruz Cabrália e Porto Seguro. Foram feitas duas coletas, uma em setembro de 2018, e outra em fevereiro de 2019, nos períodos de chuva e estiagem para capturar indivíduos de Odonata em diferentes épocas do ano e aumentar o esforço amostral. Os resultados ressaltam que a perda de vegetação nativa altera a composição das assembléias de Odonata encontradas dentro e ao redor da RPPN. As mudanças nos ambientes aquáticos ao entorno da RPPN podem alterar certas características do habitat e, consequentemente, afetar os locais de alimentação, acasalamento e reprodução de espécies mais especialistas, levando-as à extinção local. Os pontos localizados no entorno da RPPN favorecem a colonização de espécies mais generalistas, modificando a composição das assembleias entre as áreas. As AP como a RPPN Estação Veracel pode estar desempenha um papel muito importante na preservação de espécies améacadas como *L. acutum*. Reafirmando a importância das áreas de preservação como locais de extrema importância para a manutenção e conservação da biodiversidade de espécies ameaçadas. E que as subordens de Odonata podem ser considerados como bioindicadores para avaliar as mudanças nos ecossistemas aquáticos. Em suma, os três capítulos destacam que a criação e manutenção de áreas protegidas como a RPPN Estação Veracel é de fundamental importância para a conservação da biodiversidade, principalmente em áreas de Mata Atlântica.

1. Introduction

The changes of the land uses and the loss of native vegetation for other land uses as livestock, agriculture and urbanization are seen as one of the mains problems for biodiversity conservation worldwide (Mittermeier et al., 2011; Pereira et al., 2018). These changes modify natural habitats, reduce food availability, increase competition, and isolate population. And most of the time causing local extinction of these species. (Hobbs et al., 2009; Tilman et al., 2011; Vanwalleghem et al., 2017). In Brazil, changes in natural landscapes resulting from the economic development of agriculture and ranching, coupled with the great growth of urban and industrial areas have created strongly fragmented landscapes. With a drastic reduction in the areas with native vegetation, especially in areas of Atlantic Forest domains (Vanwalleghem et al., 2017).

The Atlantic Forest domain is considered one of the priority areas for the conservation of biological diversity. It presents a great biodiversity, with many endemic species and an advanced state of degradation (Myers et al., 2000; Santos et al., 2018; SOS Mata Atlântica, 2018). This domain is distributed along over a large part of the Brazilian Atlantic coast from South to Northeast and the interior of some states in the South, Southeast and Center-West regions of the country, including parts of Argentina and Paraguay. The Atlantic Forest presents great variations in relief, pluviometric regimes and are formed by mosaics of phytogeographic units, which contribute to the great biodiversity found in these areas (Santos et al., 2018; SOS Mata Atlântica, 2018). Thus, the maintenance of areas with native vegetation within the Atlantic Forest are of extreme importance for the conservation of this biodiversity and the maintenance of ecosystem services (Myers et al., 2000; Mittermeier et al., 2011; Santos et al., 2018; SOS Mata Atlântica, 2018).

One way to conserve and maintain these areas with native vegetation is to create protected areas (PAs). The PAs, found in Brazilian territory are part of the National System of Nature Conservation Units (SNUC), which is divided into two different groups: Integral Protection UCs and Sustainable Use UCs (Brazil, Law No. 9.985/00). The PAs are usually created to encompass the uniqueness of the area/biome, the presence of endangered species, the formation of endangered species, the formation of corridors, the safeguarding of ecosystem integrity, biodiversity and associated environmental services such as soil conservation and watershed protection, pollination, nutrient recycling and climate changes (Leverington et al., 2010).

However, few studies have emphasized the role of the PAs in the conservation and maintenance of biodiversity, mainly when it comes to groups of aquatic invertebrates as the insects (Leverington et al., 2010). In general, the aquatic insects have a great ecological and economic importance, helping in the transformation of matter and the flow of energy of ecosystems and ecosystem services (Ballinger & Lake, 2006; Hoekman et al., 2011). They are increasingly used as indicators of changes in terrestrial and aquatic ecosystems (Couceiro et al., 2007; Dolny et al., 2012; Calvão et al., 2018). Among them, the insects of the order Odonata are organisms associated with the aquatic and terrestrial environments. The immatures ones develop in aquatic environments, and the adults use the terrestrial environment for feeding and reproduction (Valente-Neto et al., 2016).

The dragonflies are divided into two suborders Anisoptera and Zygoptera, and feature morphological, ecological and behavioral characteristics that allow the group to be used as indicators of changes in the environments (Simaika et al., 2013, Oliveira-Junior et al., 2015; Rodrigues et al., 2016; Valente-Neto et al., 2018; Rodrigues et al., 2018). The Anisoptera, in general the adults are more active, present a bigger body size and a high capacity of thermoregulation. These characteristics allow that the species of the group to have a greater capacity of dispersion and greater independence from environmental temperature (De Marco et al., 2015; Rodrigues et al., 2018; Calvão et al., 2018). They are found in more open areas with the exception of some groups such as the Gomphidae family, that adults are generally found in areas with a greater amount of vegetation around the aquatic environments. And the larvae are generally associated with streams or rivers more conserved, with greater flow in the channel and sandy sediments (Garisson et al., 2010).

The Zygoptera in general are less active, have smaller body size and are more dependent of environmental temperature for body temperature regulation. In general, they are found in more preserved environments and have a low dispersion capacity. The habitat loss or change can lead the most sensitive species to local extinction, due to their greater habitat specificities and low dispersion capacity when compared to Anisoptera (Corbet & May, 2008; De Marco et al., 2015). According to De Marco & Vianna (2005, 2012), the knowledge about the diversity and distribution of Odonata in Brazil is still little known. Only 29% of the Brazilian territory presents data about the distribution of the group in the country, constituting an obstacle for the conservation of the dragonflies. According with the authors, these information are concentrated in the states of the Southeast region. Studies with the group in other regions, mainly in the Brazilian Northeast are still extremely important.

Studies with the Odonata have shown that the preservation and maintenance of aquatic ecosystems and the areas around them as the riparian vegetation can affect Odonata assemblages (Dalny et al., 2014; Oliveira-Junior et al., 2015; Rodrigues et al., 2016). And that the changes in the physical characteristics of aquatic and terrestrial environments may alter the composition of Odonata species, which are highly dependent on environmental conditions (Vilela et al., 2016; Rodrigues et al., 2018; Calvão et al., 2018). Therefore, the use of the group as indicator may be a way to evaluate the role of protected areas for the conservation of Odonata biodiversity when compared to unprotected areas. Using the group as a "surrogate" in PA for other groups of invertebrates, mainly the aquatic insects.

In this sense, the objective of this study was to evaluate the role of a Private National Heritage Reserve (RPPN) in the conservation of Odonata's biodiversity when compared to unprotected areas around it. This dissertation is divided in three chapters* that will be presented as articles. In which, we investigate the importance of protected areas for the Odonata assemblages. The first chapter was intitle " Dragonflies in a protected area and across its boundary reveals the role of core sites to conserve forest specialists in the Atlantic Forest". In this study, we evaluated the role of a protected area and its surrounding landscapes on the Odonata assemblages in the Atlantic forest. The structure (composition and richness) of the Odonata assemblages in streams within and surrounding the PA was assessed as well as if any species could be considered as bioindicators of forest condition. We also assessed dragonfly patterns of local contribution to beta diversity (LCBD) and species contribution to beta diversity (SCDB) in streams within and surrounding of the PA.

In the second chapter intitle "New records of the critically endangered *Leptagrion acutum* Santos, 1961 (Odonata, Coenagrionidae) from southern Bahia, Brazil". We recorded *L. acutum* for the first time to the state of Bahia expanding its range of occurrence to northern areas of the Atlantic Forest. These results reaffirm the importance of areas as the RPPN Veracel Station for preserving the biodiversity of the Atlantic Forest. And the new records of *L. acutum* can help in future assessments about its current threat status and extend the knowledge of distribution of this species to a much larger area than previously known. The third chapter intitled "The Zygoptera/Anisoptera proportion to evaluate alterations in Atlantic Forest streams" have as objective to evaluate whether the Zygoptera/Anisoptera ratio proposed by Oliveira-Junior and Juen (2019) can be used to assess changes in aquatic ecosystems in streams from an Atlantic Forest region.

*The citations and references of each chapter are following the rules of the journals where the articles were and will be sent for publication. The journals are marked on the initial pages of each chapter.

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Chapter 1

Dragonflies in a protected area and across its boundary reveals the role of core sites to conserve forest specialists in the Atlantic Forest*

Orthemis attenuate (Erichson in Schomburgk, 1848) Foto: Ruy Penalva

*I wrote this article in collaboration with Marciel Elio Rodrigues e Fabio de Oliveira Roque. The manuscript will be submitted for Animal Conservation Journal

Dragonflies in a protected area and across its boundary reveals the role of core sites to conserve forest specialists in the Atlantic Forest

Abstract

Understanding the interactions between protected areas (PAs) and surrounding landscapes has become a central issue to conservation of biodiversity. The important role of protected areas to conserve biodiversity in tropical hotpots is widely recognized, but the role of landscapes surrounding them is poorly addressed, particularly for insects. In this study, we evaluated the composition, richness, and beta diversity of Odonata assemblages inside and in surrounding areas of a protected area in the Atlantic Forest hotspot. Sample collections were carried out in the Private Reserve of Natural Heritage Veracel Station and its surroundings in the southern region of Bahia. Forty sites were sampled, 22 sites within the reserve and 18 sites in the surrounding areas. We found higher total richness and of the suborder Anisoptera in surrounding areas. The species composition between the areas was also different, being more homogeneous with each other when compared to the surrounding areas. Some of the species found in protected areas contribute to a greater species contribution to beta diversity (SCDB). Our study suggests that although surrounding landscapes can contribute to the maintenance of regional diversity of dragonflies, the core areas inside protected areas play a vital role in supporting populations of forest specialists, such as phytotelmata species.

Keywords: Aquatic insects, bioindicators, Conservation Unit, damselfly, dragonfly, native vegetation.

1. Introduction

Protected areas (PAs) are at the core of efforts towards conserve local to global biodiversity, particularly threatened species, habitats and ecosystems (Mittermeier *et al*. 2005; Ferreira *et al*. 2014; Azevedo-Santos *et al*. 2017). The land-use changes and the expansion of PAs have amplified the interaction between protected and unprotected areas worldwide, multiplying contact zones and interactions between PAs and unprotected areas (Watson *et al*. 2014). In this context, cross-boundary interactions inside and around PAs have become central to issues around the conservation of biodiversity and ecosystem services (Blanco *et al*. 2020). In Brazil, protected areas cover more than approximately 150 million hectares, about 17% of its territory (MMA 2014). They are unevenly distributed across biomes and are still poorly studied in relation to their biodiversity (Oliveira *et al*. 2017; Fonseca and Venticinque 2018; Leal *et al*. 2020). This lack of information and the small number of areas in some regions create uncertainties about the effectiveness of PAs and its relations with the surrounding areas to conserve the country's biodiversity (Fonseca and Venticinque 2018).

Among the Brazilian forest domains, the Atlantic Forest considered a biodiversity hotspot (Mittermeier *et al*. 2011). The region still suffers from successive impacts resulting from different exploitation cycles, and the great concentration of the human population forming the largest urban and industrial centers from the country has led to a drastic reduction of native areas (Mello *et al*. 2020). The resulted is a heavily fragmented and man-dominated landscapes, with about of 12% original vegetation (S.O.S Mata Atlântica 2018), being mostly kept inside the PAs. Another issue is that the creation of PAs in Brazil has been strongly based on terrestrial biota and threats to these environments and its limits still do not consider the conservation of species and aquatic ecosystems, mainly the invertebrates (Fagundes *et al*. 2016; Samways 2017; Frederico *et al*. 2018; Reid *et al*. 2018; Basset and Lamarre 2019; Leal *et al*. 2020).

The invertebrates are among the most neglected groups when it comes to conservation (Mammola *et al*. 2020), even though they are the most diverse in number of species and they are among the most threatened groups due to anthropogenic actions (Cardoso *et al*. 2020; Sundar *et al*. 2020). The knowledge about the importance of these organisms for the ecosystems and the human population is still limited, leading to the exclusion of this large group on conservation programs (Cardoso *et al*. 2020; Samways *et al*. 2020, Mammola *et al*. 2020). With rare exceptions, such as the State Wildlife Refuge Dragonflies of Serra de São José created to protect especially species of dragonflies in the Atlantic forest (Bede *et al*. 2015), there is no protected areas designed to conserve aquatic insects in hotpots around the world (Sundar *et al*. 2020). Current studies have shown that the PAs have limited effectiveness in protecting freshwater invertebrates' biodiversity (Fagundes *et al*. 2016; Frederico *et al*. 2018; Leal *et al*. 2020). This causes a concern, as we still do not know the importance of PAs for local and regional biodiversity of invertebrates and the relationship with the surrounding areas.

Among freshwater aquatic invertebrates, dragonflies (Odonata) has been widely used as indicators of changes in natural ecosystems (Oliveira-Junior and Juen 2019; Voster *et al*. 2020). They are extremely dependent on aquatic and terrestrial ecosystems and the impacts on these environments may influence on the permanence and reproduction of adults and the development of immatures (Oliveira Junior *et al*. 2015; Valente-Neto *et al*. 2016; Rodrigues *et al*. 2018). Habitat changes may affect the capacity of thermoregulation, choice of partners, availability of sites for oviposition sites and substrates for adults and the availability of resources and habitats for the development of immatures (De Marco *et al*. 2015; Oliveira Junior *et al*. 2015; Valente-Neto *et al*. 2016; Rodrigues *et al*. 2018). These characteristics separate the species mostly into two large groups based on suborder Zygoptera and Anisoptera (De Marco *et al*. 2015). Anisoptera, are generally considered more generalist species, adapted to naturally more open environments or that have undergone environmental changes, mainly related to loss of native vegetation around the aquatic ecosystems. And the Zygoptera in general are considered more sensitive species and therefore always associated with more preserved environments (De Marco *et al*. 2015; Carvalho *et al*. 2018).

The changes or loss of the habitats leave odonates more specialist of forest to local extinction and facilitate the colonization of generalist species (Rodrigues *et al*. 2016; Carvalho *et* *al*. 2018; Rodrigues *et al*. 2018; Oliveira-Junior and Juen 2019), with drastic changes in the local diversity. In Atlantic Forest the majority areas around PAs is human-dominated landscapes, it is expected the core areas inside the PAs are the only refuges available for some forest specialist species.

Under this general expectation, in this study we evaluated the role of a protected area and its surrounding landscapes on the Odonata assemblages in the Atlantic forest. The structure (composition and richness) of the Odonata assemblages in streams within and surrounding the PA was assessed as well as if any species could be considered as bioindicators of forest condition. We also assessed dragonfly patterns of local contribution to beta diversity (LCBD) and species contribution to beta diversity (SCDB) in streams within and surrounding of the PA. Our prediction was that the composition and richness of species within protected areas would be significantly different from unprotected areas surrounding them. We also expected that the protected area would have an important role for the regional beta diversity by keeping exclusive species. Considering that PAs are known to be more suitable habitats for conservation and maintenance of more sensitive and exclusive species (Carvalho *et al*. 2018; Rodrigues *et al*. 2018) and based on the ecomorphological and behavioral characteristics of the suborders, we also expected that a greater richness of species of the suborder Zygoptera would be found in sites sampled within the PA, whereas in the surrounding areas there would be a greater richness of Anisoptera (De Marco *et al*. 2015; Rodrigues *et al*. 2016; Rodrigues *et al*. 2018). These eco-physiological characteristics are also expected to select groups of species that can be considered bioindicators of preserved and non-preserved areas (Rodrigues *et al*. 2019).

2. Materials and Method

2.1. Study Area

The study was carried out in the Private Reserve of Natural Heritage (RPPN) Veracel Station and its surrounding areas, located in the south of Bahia, between the municipalities of Santa Cruz Cabrália and Porto Seguro. The RPPN Veracel Station has an area of 6,069 ha. It is considered the largest private reserve in northeastern Brazil and the second largest in the Atlantic

Forest Biome. The area maintains characteristics of primary vegetation with high biological diversity (Veracel 2016). The region's climate is Af according to the Koppen classification, with a temperature above 18 °C and annual rainfall greater than 70mm.

The sampling stations inside the RPPN present a more closed and well-preserved canopy, formed by primary vegetation and without evidence of pollution, erosion and anthropogenic physical changes along the stream channel. The bottom of the sampled stream channels had a large amount of sediment with leaves, gravel, sand, wood logs, wood branches and particulate organic matter.

The areas surrounding the reserve are composed of pasture and agriculture matrix with settlement areas and large rural properties. The streams generally did not have uniform riparian vegetation and had a lower concentration of sediments such as leaves, wood logs and branches and particulate organic matter. The riparian vegetation presented, with greater frequency, locations that were more open and exposed to light. At some sampling sites, the stream channel was interrupted for the construction of dams for watering animals and/or uses in the property. It was also possible to observe a small amount of domestic waste and plastic bags in some sampling sites.

2.2. Sampling sites

Collections were carried out at 40 sampling sites, 22 of which within the Veracel Station Conservation Unit and 18 sites surrounding the RPPN. The streams were small and narrow (estimated from first to fourth order) in both areas. Two collections were performed at each sampling site (September 2018 and February 2019), in the rainiest and driest period of the season for a better characterization of the assemblages in the region (Fig. 1).

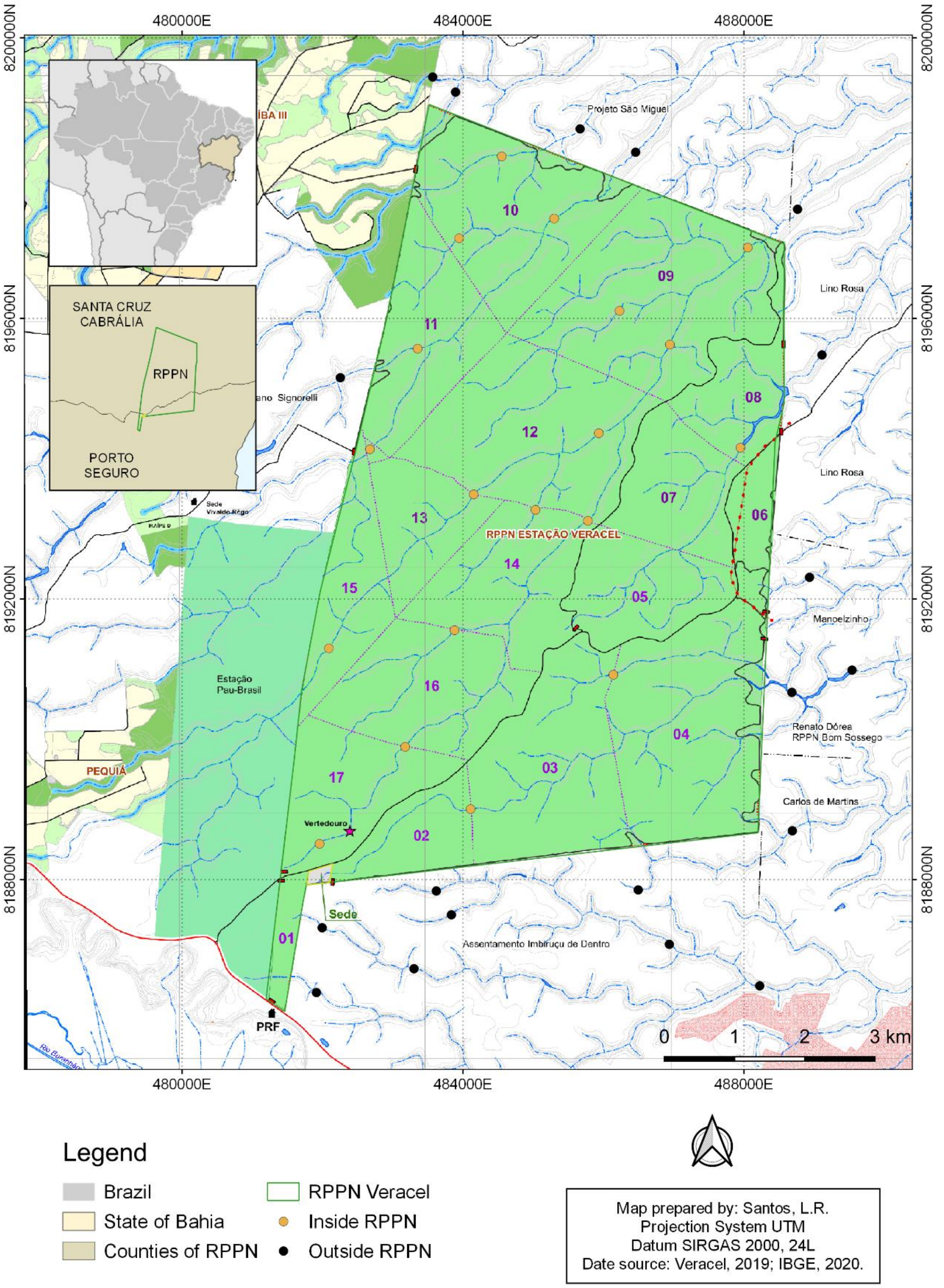

Fig.1: Map showing the area of RPPN Veracel Station, located in the municipality of Porto Seguro, BA. Red dots show the sampled locations inside the RPPN and black dots show the

sampled locations outside the RPPN. The numbers and lines in purple refer to the division of the RPPN area into blocks.

2.3. Specimen collection, curation and identification

The adults were captured through active collection with entomological nets. For each point, a 100-meter stretch of water was sampled on both banks for 90 minutes. The collections were carried out on non-rainy days at the time of the group's greatest activity, from 9 am to 4 pm (Oliveira-Junior and Juen 2019; Calvão *et al*. 2018). The adults were taken to the Laboratory of Aquatic Organisms (LOA) at the State University of Santa Cruz for further identification at the lowest possible taxonomic level with the aid of identification keys (Garrisson *et al*. 2010; Lencione 2005, 2006, 2017).

2.4. Statistical analyses

Species richness was calculated for the total community and for the suborders Zygoptera and Anisoptera. The data were compared using a Student t-test (Harris 2001), among the sites sampled within and surrounding the RPPN. The assumptions of homogeneity and normal distribution were tested *a priori* for each data set. In order to assess the composition of the assemblages within the RPPN and its surroundings. The principal coordinates analysis (PCOA) was used to summarize the species composition inside and in surrounding areas of protected area, using the Bray-Curtis dissimilarity index (Legendre & Legendre 1998). This grouping was tested through a permutational analysis of variance (PERMANOVA) (Anderson 2001), that was performed with 9999 replications (Anderson and Walsh 2013), to test for significant differences in species composition among conservation categories.

To evaluate the beta diversity within and surrounding the RPPN, we calculated total beta diversity (BDtotal), the local contribution to beta diversity (LCBD), and species contribution to beta diversity (SCBD) (Legendre and De Cáceres 2013). We first applied Hellinger transformation to community composition and, then, estimated BDtotal as the unbiased total sum of square of the species composition data (Legendre and De Cáceres 2013). The relative contribution of each sampling unit to beta diversity (LCDB), was estimated by the total variation in the species composition data (Legendre and De Cáceres 2013). To determine which taxa mostly contributed to beta diversity patterns in streams within and surrounding of the RPPN were retained those taxa with SCBD values greater than the mean of all taxa (i.e., species that has a disproportional contribution to beta diversity patterns) (Legendre and De Cáceres 2013). SCBD represents the degree of variation of individual species in the study area (Legendre and De Cáceres 2013). The association of data with beta diversity can indicate sites that disproportionally contribute to regional species pool relative to species richness and may be particularly useful to identify keystone sites (Legendre and De Cáceres 2013; Ruhí *et al*. 2017).

The indicator species analysis (IndVal), was calculated as proposed by De Cáceres *et al*. (2010). This analysis creates combinations for each species according to the selected groups and emits association values that are subsequently tested statistically. This analysis also shows two new components for species considered to be indicators. Component A, also called specificity, is the probability that an indicator species belongs to one of the selected target groups. Component B, also called fidelity, determines how much each species has been registered within the total samples of each selected category (De Cáceres *et al*. 2010). The analyses were performed using the statistical program R (R Core Team 2020) and the packages indicspecies (De Caceres & Legendre 2009).

3. Results

Forty-nine adult species were collected, belonging to 31 genera and eight families. Among the total species collected, six species were only sampled within the PA, 29 species in the surrounding areas and 14 species common to the two sampling areas (Appendix 1). The species of *Aceratobasis cornicauda, Leptagrion macrurum, Leptagrion acutum, Forcepsioneura sancta, Heliocharis amazona, Phyllogomphoides* sp., *Erythemis vesiculosa* and *Erythrodiplax funerea* were found only at sampling sites within the protected area. It is worth mentioning that the species of *Leptagrion macrurum and Leptagrion acutum* are species phytotelmata. *Leptagrion acutum* is considered critically endangered by the red list of endangered species in Brazil (IUCN 2014). The species of Zygoptera that showed a greater number of individuals were *Heteragrion aurantiacum*, *Hetaerina rosea*, *Argia hasemani* with 202, 79 and 71 specimens, respectively. The most abundant species of Anisoptera were *Erythrodiplax fusca* and *Erythrodiplax paraguayensis* with 30 and 12 individuals, respectively (Appendix 1).

The total adult Odonata richness showed a significant difference between the sites collected inside and outside the RPPN (T= -3.53; *p*= 0.001), with an average of 4.57 species inside and 7.63 species outside the RPPN. The suborder Anisoptera also showed significant differences (t= -3.75 and p= 0.001) between the sampled areas, with an average of 1.00 species inside and 4.00 species outside the RPPN. The richness of Zygoptera did not show significant differences between areas (t= -1.25, p= 0.91), with an average of 3.5 species for the sites inside the RPPN and 4.1 species in the surrounding sampling sites (Fig.2).

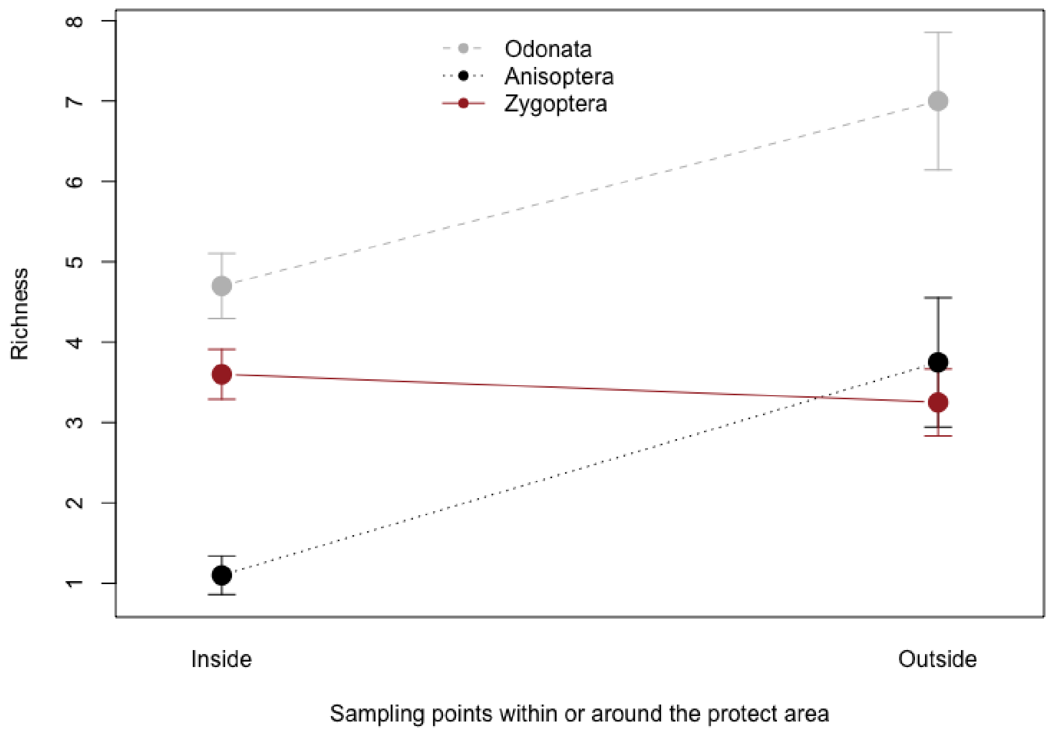

Fig.2: Graph of average richness of total Odonata (in gray) (T= -3.53; *p*= 0.001), of Anisoptera (in black) (t= -3.75 and *p*= 0.001) and of Zygoptera (in red) (t= -1.25, *p*= 0.91), in streams inside and outside the protected area.

In regard to the composition of species, the ordering analysis showed that the assemblages found in streams within the PA are different from the assemblages that were collected surrounding the protected area (PERMANOVA test p=0.001 and R= 0.5018) (Fig. 3). The assemblages found within the protected area are more similar to each other, suggesting less variation in their composition. The composition of the surrounding assemblages is much more varied, presenting a great variation of species between the areas sampled around the RPPN (Fig. 3). Some sites from outside the protected area had a very similar composition of species when compared with the sites within the protected area. This can be related to the greater integrity of some sampling sites of the surrounding area that were near the preserved area.

In relation to LCDB, eight sites had a significant contribution to the local beta diversity (p <0.05; LCBD> = 0.04). They are all outside the protected area (Appendix 2). However, when we estimate the SCDB, 24 species presented higher contribution values individual for beta diversity in the studies areas. Of these six species are associated with streams located within the RPPN. With three species that were found exclusively within the preserved area (*H. amazona*, *L. macrurum*, *L. acutum*) and three that were found most of the time within the protected area (*H. aurantiacum*, *H. longipes*, *E. cannacrioides*) (Appendix 1).

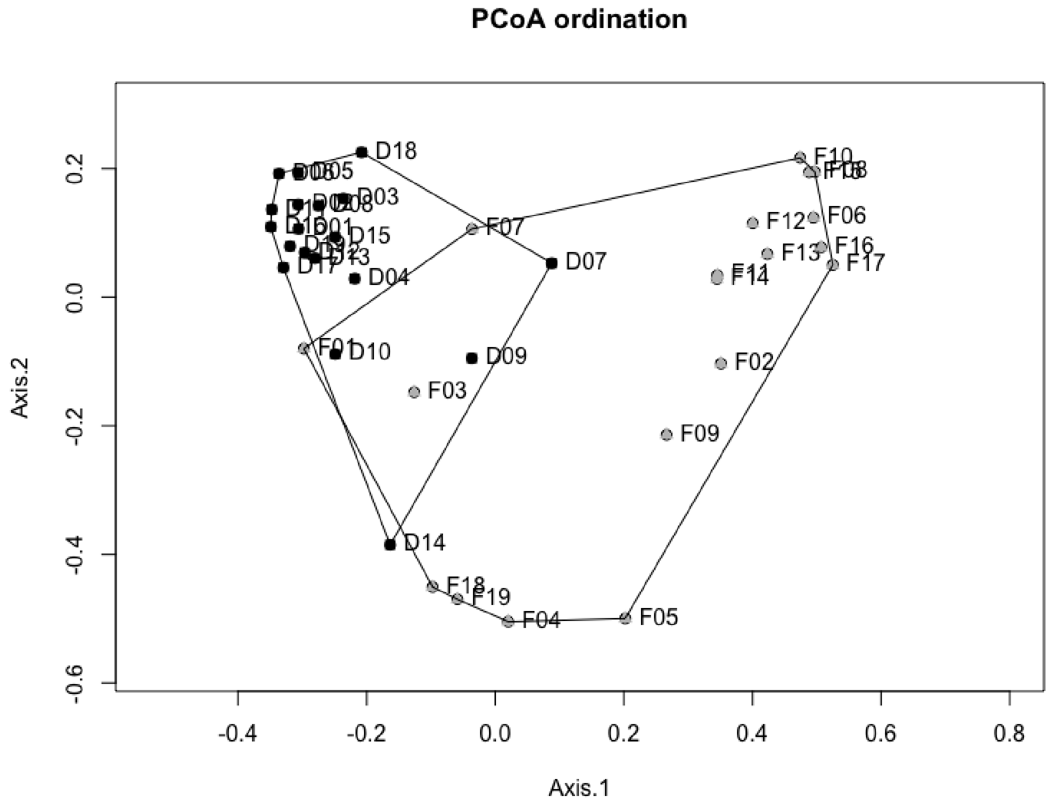

Fig.3: Graph of the PCOa analysis showing the similarity of the composition of the adult Odonata assemblages, between the areas inside and outside the RPPN. Black points or with the letter D show areas within the protected area and in gray points or with the letter F the surrounding areas.

Of the forty-nine adult species collected, seven responded as indicators of protected and non-protected areas. Three species was considered an indicator of streams located within the PA and four were considered to be indicative of the areas surrounding the PA (Table 1). Specificity (A), showed a variation from 0.71 to 1.0, and the selected species presented a high specificity to the sampling sites (inside and outside the PA). It means that all of them were only collected in the great majority of times in sampling sites within the PA in the case of *H. aurantiacum, Hetaerina longipes* and *Helicharis amazona* and in sites surrounding the PA, in the case of *Ischnura capreolus*, *Telebasis corallina*, *Erythrodiplax fusca* and *Erythrodiplax paraguayensis*. Regarding the fidelity (B), the values ranged from 0.26 to 0.84, emphasizing that *H. aurantiacum* (B = 0.84) was present in most of the sites sampled within the PA. The other species had low fidelity, meaning a low number of records within the total sites that were sampled inside of RPPN and in the surrounding areas (Table 1).

Table 1: Result of INDVAL test showing the environment indicator species in and around Veracel RPPN.

Species	Inside protected area	Outside protected area	Stat	p value	Specificity(A)	Fidelity(B)
Heteragrion aurantiacum Selys, 1862	X		0.90	0.001***	0.85	0.94
Hetaerina longipes Hagen in Selys, 1853	X		0.64	0.04*	0.71	0.58
Heliocharis amazona Selys, 1853	X		0.51	0.04*	1.00	0.26
Ischnura capreolus Hagen, 1861		X	0.71	0.003**	0.98	0.52
Telebasis corallina Selys, 1876		X	0.56	0.01*	1.00	0.31
Erythrodiplax fusca (Rambur, 1842)		X	0.54	0.003**	1.00	0.30
Erythrodiplax paraguayensis (Foster, 1905)		X	0.50	0.03*	1.00	0.26

4. Discussion

Our results corroborate with part of the predictions and emphasize that, in general, the assemblages collected within and in the surroudings of the PAs are different in terms of species richness and composition. Our results add evidences to the role of protected areas for the conservation of Odonata species that are considered forest specialists (e.g. phytothelmatous species) or sensitive to change in the natural environments, such as species dependent on conserved aquatic environments (Carvalho *et al*. 2018; Calvão *et al*. 2018; Rodrigues *et al*. 2018). Moreover, our study shows that core areas inside the PA is critical to conserve populations of threatened species, such as *L. acutum* listed as critically threatened by Brazil's red list of threatened species (ICMBio 2018, Ribeiro *et al*. 2021).

The surrounding areas showed greater total species richness and also greater species richness of the suborder Anisoptera when compared to the values in the protected area. This has also been observed in other studies on Odonata in areas under urbanization impacts (Monteiro Junior *et al*. 2013; Rodrigues *et al*. 2019), as well as in areas of intensive livestock and agriculture (Carvalho *et al*. 2018; Calvão *et al*. 2018). It may be related to different degrees of changes in the aquatic environments and surrounding habitats such as loss of riparian vegetation, siltations that changing the flow of channels from lotic to lentic, among others, that facilitate the colonization of mainly Anisoptera species, that are usually more generalist species and/or species adapted to lentic and altered environments (Juen and De Marco 2012; Monteiro-Junior *et al*. 2013; Calvão *et al*. 2018; Rodrigues *et al*. 2018; Carvalho *et al*. 2018; Rodrigues *et al*. 2019).

The richness of adult specimens of the suborder Zygoptera was similar among the sites sampled within the RPPN, not corroborating with our predictions. Among the streams sampled surrounding the protected area, almost all of them presented physical modifications of the channels and their surrounding areas as artificial barriers upstream or downstream the channels caused by erosion or modifications of anthropogenic origin for animal desedentation, among other uses. Changes in the flow of aquatic ecosystems from lotic to lentic can attract species that have adaptations to these types of environments. For example, *Acanthagrion cuyabae, Acanthagrion gracille, Telebasis longum, Telebasis corallina, Neoneura sylvatica, Neoneura minuta, Lestes forficula* and *Lestes tricolor* are species that were collected in the current study and are commonly found in lentic environments or open areas (Monteiro Junior *et al*. 2013; Rodrigues *et al*. 2016; Vilela and Ferreira 2016).

In regard to the composition of species, there was significant differences in the Odonata assemblages collected within and surrounding the PA. The assemblages within the PA did not suffer as much variation in the composition as that observed with the assemblages found in the surrounding areas, emphasizing that protected areas maintain a more stable community when compared to unprotected areas. Unprotected areas are subject to modifications of varying magnitude, allowing species with different eco-physiological requirements to be able to colonize and remain in certain locations (De Marco *et al*. 2015; Rodrigues *et al*. 2016, 2018). Studies with the group have already shown that species composition changes according to changes in natural environments, possibly due to the presence of more generalist species that may cause local extinction of specialist species. As we found in our study, this pattern seems to be more evident in Zygoptera (Juen and De Marco 2012; Monteiro-Junior *et al*. 2013; Rodrigues *et al*. 2016, 2018; Carvalho *et al*. 2018; Rodrigues *et al*. 2019).

Our results showed that the sites within the RPPN have low LCDB values when compared to the sites around the RPPN. This result is closely associated with the greater species richness found in the areas surrounding the RPPN. However, the SCDB analysis highlighted that among the species with the highest SCDB values, some of them have most of the time been found in streams within the RPPN as *E. cannacrioides, H. amazona*, *L. acutum*, *L. macrurum*. These species in their entirety are considered specialists in forested areas and specialists in relation to habitat (2 phytotelmata) (Carvalho *et al*. 2018; Rodrigues *et al*. 2019, Furieri *et al*. 2020, Ribeiro *et al*. 2021). Among species with high SCDB values and that were only collected in streams around the RPPN, some are species considered specialists in open areas or generalist habitat, such as *Diastatops obscura, E. fusca, E. paraguayensis*, *Erythrodiplax umbrata, I. capreolus, T. corallina* (Rodrigues *et al*. 2018; Carvalho *et al*. 2018, Rodrigues *et al*. 2019). Another relevant finding is that even though the Zygoptera richness has not had a significant difference between the protected area and its surroundings, six species that contribute the most to SCDB and were registered within the RPPN, five of them are from the suborder Zygoptera.

Among the species selected as bioindicators, three species (*H. aurantiacum, H. longipes* and *H. amazona)* showed preference for forested areas. The adults of these species prefer more shaded places and with greater environmental integrity of the surroundings and the stream channel

(Garisson *et al*. 2010, Ferreira-Puriquetti and De Marco 2002; Borges *et al.* 2019). Some studies have shown that their larvae are found in more conserved streams with running water in the channel (Garrison *et al*. 2010). Among the species that have been suggested as indicators of the areas surrounding the PA, *T. corallina* and *I. capreolus* are species generally associated with more open and lentic environments (Rodrigues *et al*. 2016; Rodrigues *et al*. 2019).

In summary, our results showed that the composition of the assemblages was a good surrogate to assess the importance of protected areas for Odonata communities. Streams located within the RPPN have species with high SCDB and are good candidates to be keystone communities because they have a disproportional contribution to regional species pool. Our study highlights the importance of PAs to the maintenance of the regional species pool, forest specialist species and threatened species. Management strategies are needed to maintain the disproportional contribution of sites located within protected areas to the regional species pool, particularly we call attention to the conservation of core areas inside the protected areas to support ecological conditions for forest specialists, such as big trees, high humid conditions, less variation in ambient temperature, greater integrity of habitats and availability of sites for reproduction and development of larvae. In fact, the conservation of these core areas inside protected areas not only play a vital role in supporting populations of forest specialist dragonflies but also they are fundamental for many other taxonomic groups (Fernández *et al*. 2004, 2012; Gray *et al*. 2016) and for ecological services, such as carbon storage (DeFries *et al.* 2010; Palomo *et al.* 2014; Cumming 2016; Maestre-Andrés *et al*. 2016). Therefore, the conservation strategies of dragonflies in the Atlantic Forest could be linked with more widely and inclusive movements of forest conservation and restoration with co-benefits at the landscape scale.

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6. Appendix

Appendix 1: Families and species (abundance) of the suborders Anisoptera and Zygoptera found in the streams inside and around the RPPN Veracel Station. And the values of species contribution to beta diversity (SCDB). In bold, the species with the highest contribution values.

Subordem	Family/Species	Places of sampled		SCDB
Anisoptera	**Gomphidae**	**Inside protected area**	**Outside protected area**	**Values**
	Epigomphus paludosos Hagen in Selys, 1854	-	2	0.005
	Phyllogomphoides sp Belle, 1970	1	-	0.003
	Progomphus montanos Belle, 1973	-	2	0.002
	Progomphus sp1 Selys, 1854	1	1	0.004
	Zonophora calippus spectabilis Campion, 1920	-	2	0.003
	Libellulidae			
	Anatya guttata Erichson in Schomburgk, 1884	-	2	0.002
	Diastatops obscura (Fabricius, 1775)	-	7	**0.015**
	Elasmothemis cannacrioides (Calvert, 1906)	7	1	**0.019**
	Erythemis credula (Hagen, 1861)	-	4	**0.011**
	Erythemis vesiculosa (Fabricius, 1775)	1	-	0.003
	Erythrodiplax avitata Borror, 1942	-	2	0.005
	Erythrodiplax funera (Hagen, 1861)	3	-	0.004
	Erythrodiplax fusca (Rambur, 1842)	-	30	**0.043**
	Erythrodiplax leticia Machado, 1996	-	4	**0.010**

	Erythrodiplax lygae Ris, 1911	-	1	0.001
	Erythrodiplax paraguayensis (Foster, 1905)	1	11	**0.036**
	Erythrodiplax umbrata (Linnaeus, 1758)	-	3	**0.012**
	Micrathyria artemis Ris, 1911	-	1	0.003
	Micrathyria atra (Martin, 1997)	1	1	0.005
	Micrathyria catenata Calvert, 1909	-	2	0.005
	Micrathyria menegeri menegeri Ris, 1919	-	1	0.001
	Micrathyria ungulata Forster, 1907	-	2	0.003
	Orthemis attenuata (Erichson in Schomburgk, 1848)	4	2	0.009
	Oligoclada umbricola Borror, 1931	-	2	0.004
	Perithemis lais (Perty, 1833)	-	5	**0.012**
	Perithemis thais Kirby, 1889	-	2	0.001
	Planiplax phoenicura Ris, 1912	-	9	**0.021**
	Zenithoptera viola Ris, 1910	-	5	0.009
Zygoptera	**Coenagrionidae**			
	Argia hasemani Calvert, 1909	48	23	**0.064**
	Acanthagrion cuyabae Calvert, 1909	-	2	0.003
	Acanthagrion gracile Rambur, 1842	-	5	**0.010**

	Aceratobasis cornicauda (Calvert 1909)	1	-	0.001
	Epipleoneura machadoi Rácenis, 1960	10	14	**0.040**
	Leptagrion macrurum Burmeister, 1839	10	-	**0.018**
	Leptagrion acutum Santos, 1961	3	-	**0.011**
	Telagrion longum Selys, 1876	-	1	0.001
	Telebasis corallina Selys, 1876	-	43	**0.051**
	Ischnura capreolus Hagen, 1861	1	44	**0.080**
	Neoneura sylvatica Hagen in Selys, 1886	-	5	**0.026**
	Nehalennia minuta Selys in Sagra, 1857	-	4	0.009
	Idioneura ancilla Selys, 1860	1	4	**0.010**
	Forcepsioneura sancta Hagen in Selys, 1860	4	3	**0.012**
	Megapodagrionidae			
	Heteragrion aurantiacum Selys, 1862	185	17	**0.187**
	Perilestidae			
	Perilestes fragilis Hagen in Selys, 1862	1	2	0.004
	Calopterygidae			
	Hetaerina rosea Selys, 1853	31	48	**0.097**
	Hetaerina longipes Hagen *in* Selys, 1853	27	12	**0.065**
	Dicteriadidae			
	Heliocharis amazona Selys, 1853	8	-	**0.015**
	Lestidae			

	Lestes forficula Rambur, 1842	-	8	**0.017**
	Lestes tricolor Erichson in Schomburgk, 1848	-	1	0.001

Appendix 2: Table with the values of the local contribution to beta diversity (LCDB) and their respective p values. In bold, the sites considered with greater contribution to beta diversity.

Sítios	Valor(p)	LCBD	Sampling location
D08	0.730	0.019	Inside RPPN
D07	0.250	0.032	Inside RPPN
D06	0.847	0.018	Inside RPPN
D09	0.512	0.023	Inside RPPN
D04	0.877	0.017	Inside RPPN
D11	0.942	0.015	Inside RPPN
D10	0.682	0.020	Inside RPPN
D12	0.831	0.017	Inside RPPN
D14	0.849	0.017	Inside RPPN
D02	0.932	0.016	Inside RPPN
D05	0.751	0.019	Inside RPPN
F04	0.574	0.022	Outside RPPN
F19	0.568	0.022	Outside RPPN
D13	0.990	0.013	Inside RPPN
F01	0.998	0.012	Outside RPPN
F02	**0.022**	**0.044**	**Outside RPPN**
D03	0.937	0.015	Inside RPPN
F03	0.863	0.017	Outside RPPN
D01	0.783	0.018	Inside RPPN
D18	0.932	0.016	Inside RPPN
D17	0.854	0.017	Inside RPPN
F18	0.445	0.025	Outside RPPN
F07	0.531	0.023	Outside RPPN
D19	0.707	0.020	Inside RPPN
F05	0.351	0.030	Outside RPPN
F06	0.113	0.038	Outside RPPN
F15	**0.035**	**0.042**	**Outside RPPN**
F13	0.149	0.036	Outside RPPN
F14	**0.062**	**0.041**	**Outside RPPN**
D16	0.992	0.013	Inside RPPN

F16	**0.064**	**0.040**	**Outside RPPN**
F12	0.093	0.038	Outside RPPN
D15	0.560	0.022	Inside RPPN
F11	**0.015**	**0.045**	**Outside RPPN**
F10	0.120	0.037	Outside RPPN
F09	**0.046**	**0.042**	**Outside RPPN**
F17	**0.074**	**0.039**	**Outside RPPN**
F08	**0.059**	**0.040**	**Outside RPPN**

Chapter 2

New records of the critically endangered *Leptagrion acutum* Santos, 1961 (Odonata, Coenagrionidae) from southern Bahia, Brazil**

Leptagrion acutum Santos, 1961 Foto: Karina Furieri

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New records of the critically endangered *Leptagrion acutum* Santos, 1961 (Odonata, Coenagrionidae) from southern Bahia, Brazil

Abstract

Leptagrion acutum Santos, 1961 is endemic to Brazil and since 2003, listed as critically endangered in the Red Book of Threatened Brazilian Fauna. In this study *L. acutum* is recorded for the first time to the state of Bahia expanding its range of occurrence to northern areas of the Atlantic Forest. Three males were collected in the RPPN Veracel. Information concerning distributional records of rare or endangered species is essential, because it expands the species' occurrence records and assist in future assessments about their threat status of conservation.

Key words

Atlantic Forest, Damselfly, distribution records, endangered species, phytotelmata, Zygoptera.

1. Introduction

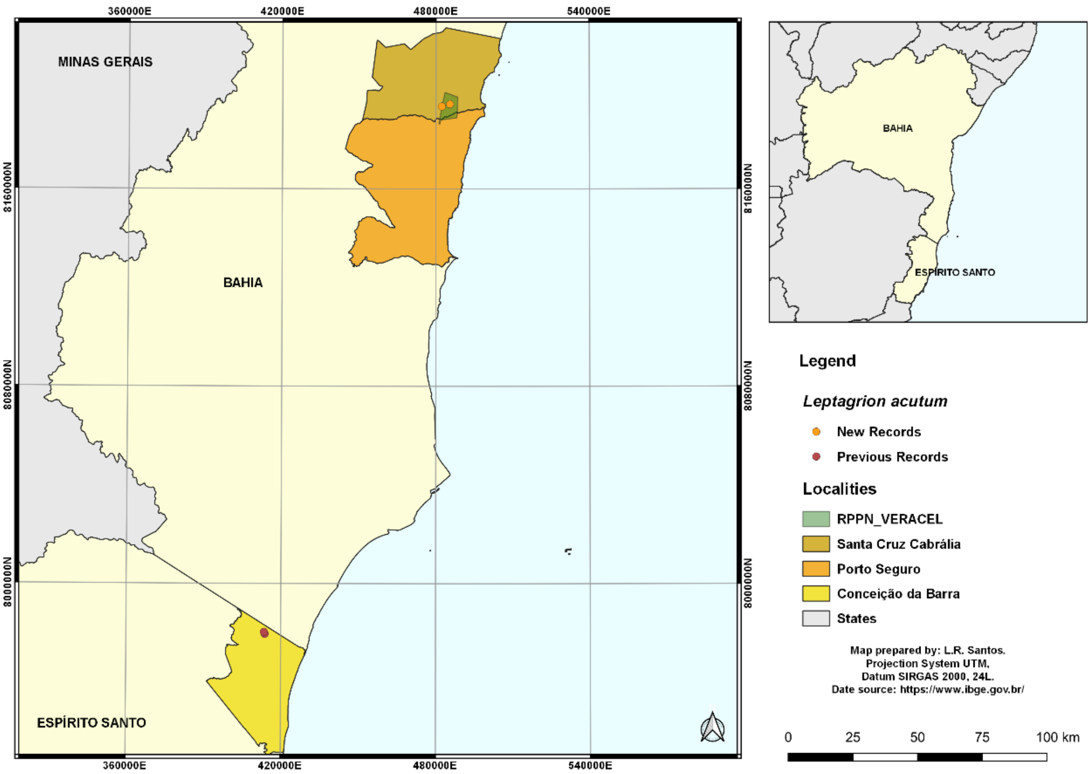
The genus *Leptagrion* Selys, 1876 (Coenagrionidae) has 17 described species and has been recorded in coastal areas of South America, from Venezuela south to Brazil, being highly diverse in the Atlantic Forest (Garrison et al. 2010, Lencioni 2017a, Furieri et al. 2020). The reproduction and development of these species depend on phytotelmata environments, breeding mainly in bromeliads (De Marco and Furieri 2000). However, the destruction and degradation of native habitats and the illegal extraction of these plants may lead to local extinctions, because most species are endemic and its populations are small (Galindo-Leal and Câmera 2003, Furieri 2008, Lencioni 2017a, ICMBio 2018).

Leptagrion acutum Santos, 1961 is an endemic species to Brazil with few records and very small populations (Costa and Garrison 2001, Furieri 2008, Lencioni 2017a). This species was described with no additional data further than Brazil as type locality, based on a holotype that was deposited in the entomological collection of the Museu Nacional, UFRJ, in Rio de Janeiro, Brazil (Santos 1961). In 1969 and 1971, two males were collected in Conceição da Barra in the Espírito Santo state (Costa and Garrison 2001). Since 2003, *L. acutum* has been in the list of endangered species and it is categorized as critically endangered in the Red Book of Threatened Brazilian Fauna (ICMBio 2018). These results indicate that populations of this species are severely fragmented due to the continuous decline of vegetation for agriculture and urbanization and the loss of habitat quality in its probable areas of occurrence (ICMBio 2018).

In 2005, the *Leptagrion* Project was launched in partnership with the Atlantic Forest Research Institute and approved by the National Environmental Fund in order to obtain more information on the distribution and populations of *Leptagrion* species. In this project, after 34 years of its last record, *L. acutum* was rediscovered in Conceição da Barra, in REBIO de Córrego Grande, a Federal Conservation unit, in the state of Espírito Santo (Furieri 2008, Furieri et al. 2020). The *Leptagrion* Project also extended its activities to the south of Bahia but it was unsuccessful in finding new records in that region (Furieri 2008). Therefore, this work aims to expand the distribution of *L. acutum* to the southern region of the state of Bahia and to describe the area of occurrence of the collected specimens.

2. Methods

The specimens were collected at the Veracel Station Private Reserve of Natural Heritage (Portuguese acronym RPPN), located between the municipalities of Santa Cruz Cabrália and



Porto Seguro in southern Bahia, Brazil (Figure 1). The RPPN Veracel Station is considered the largest private reserve in the Northeast region of Brazil and the second largest in the Atlantic Forest biome (Veracel 2016). The RPPN covers an area of 6,069 ha and consists of dense tropical rainforests, which, despite being homogeneous, exhibits tableland forest with gallery forests (Veracel 2016).

Figure 1. Map of southeastern Brazil between the states of Espírito Santos and Bahia showing the region of the occurrence of *L. acutum* Santos, 1961. Previous records in red, new records in orange.

The specimens were collected in September 2018 and February 2019, using an entomological net. They were packed in entomological envelopes and taken to the laboratory for identification, which followed the specifications of Lencioni (2006, 2017b). The specimens are deposited in the zoological collection of the State University of Santa Cruz in the Laboratory of Aquatic Organisms – LOA. Sampling was authorized by SISBIO with license number 57179-1.

3. Results

Leptagrion acutum Santos, 1961

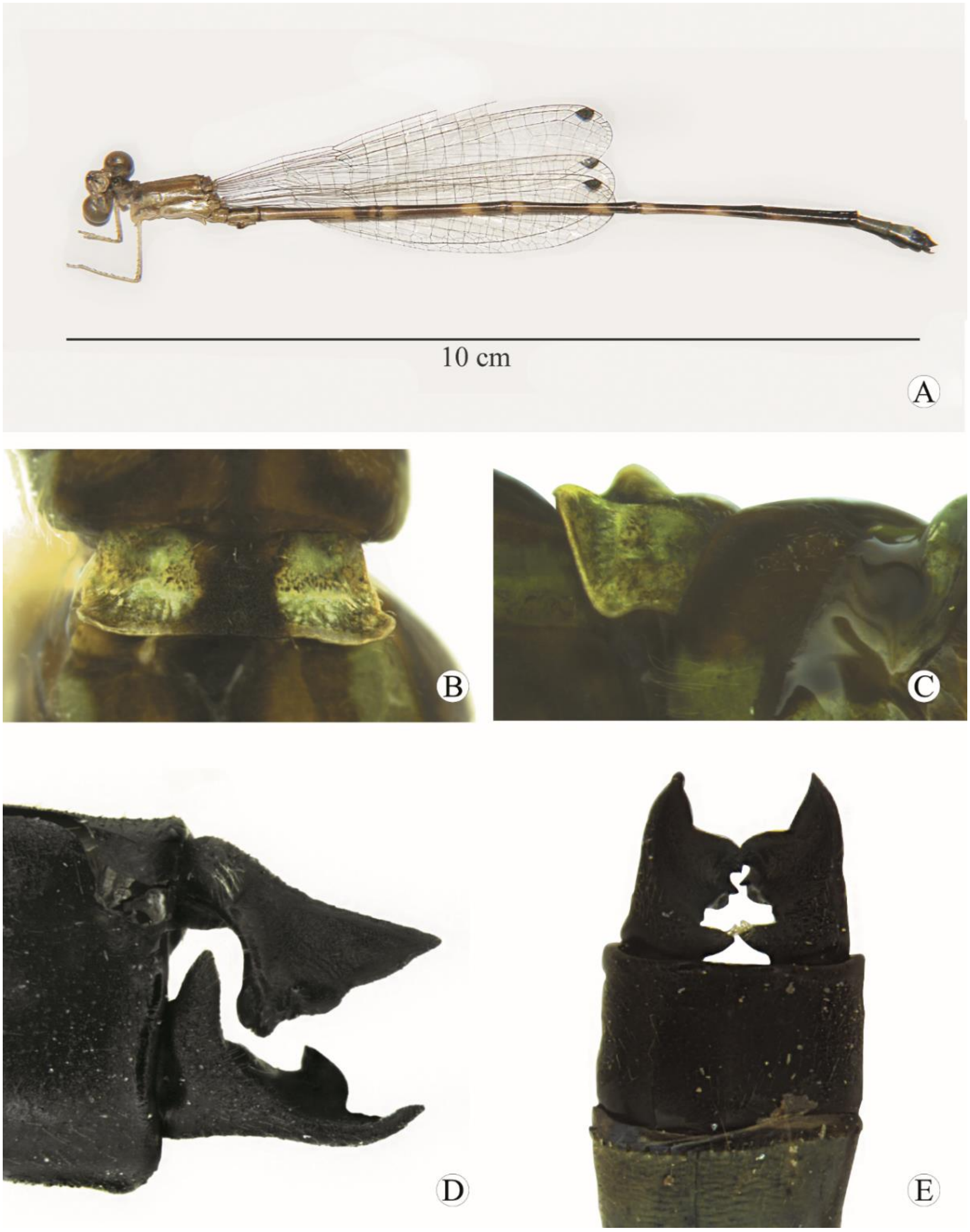


Figure 2. *Leptagrion acutum*. **A.** Photo of one of the collected specimens. **B.** Prothorax, dorsal view **C.** Prothorax, lateral view **D.** Superior appendages, mediodorsal view. **E.** Superior appendages, dorsal view.

New records. BRAZIL • 2 adults ♂; Bahia state, Porto Seguro, RPPN Veracel Station; -16.340556, -39.165556 (DD), datum WGS 84; 95 m elev. ; 09 Sep. 2018; M.E. Rodrigues leg.; Specimens were collected using an entomological net in a trail that is used for monitoring the RPPN Veracel Station; CZUESC 079BA, CZUESC 080BA. BRAZIL • 1 adult ♂; Bahia state, Porto Seguro, RPPN Veracel Station; -16.332222, -39.136106 (DD), datum WGS 84; 75 m elev.; 02 Feb. 2019; M.E. Rodrigues leg.; Specimen was collected using an entomological net in trail that is used for monitoring the RPPN Veracel Station; CZUESC 586BA.

Identification. The diagnosis of the species is based on the superior appendages and in the posterior region of the prothorax. The posterior lobe of prothorax in dorsal view features the edges slightly rounded and the posterior region almost straight (Figure 2b). In lateral view the posterior lobe is slightly tilted upwards (Figure 2c). Male cercus has one bifurcating at 2/3 of its length into a large dorsal branch and a thinner tapering ventral one (Figure 2d). In dorsal view the dorsal branch appears as a large acute tubercle covered with a small tuft of hairs directed distally. From the medial border of the ventral branch stems two stout subterminal tooth directed medially (Figure 2e).

4. Discussion

The specimens were close to two of the trails that are used for monitoring the RPPN Veracel Station, in areas of clearings in the forest, always resting on the vegetation. *L. acutum* apparently occurs in low population densities inside the RPPN. Only three specimens were observed and collected in more than 30 days of sampling within the area in different seasons of the year (September 2018, February 2019 and August 2019). The same was noted in the studies of Costa and Garrison (2001) and Furieri (2008), which only recorded two specimens in each study, all males. Until the present study, *L. acutum* had been recorded in 1969 and 1971 in Conceição da Barra, Espírito Santo (Costa and Garrison 2001) and in 2005 found again in Conceição da Barra, in Córrego Grande Biological Reserve (REBIO), also in the Espírito Santo state (Furieri 2008, Furieri et al. 2020) and now in the RPPN Veracel Station in the Bahia state.

The RPPN Veracel Station plays a very important role in preserving the biodiversity of the Atlantic Forest in southern Bahia because it is located in a strategic area forming corridors with others RPPNs and with the Pau Brasil National Park. Therefore, it increases the size and effectiveness of protected areas for the maintenance and preservation of the region's biodiversity, especially the endangered species (Veracel 2016). According to the Red Book of Threatened Brazilian Fauna published in 2018, *L. acutum* had an estimated distribution of 40km² (ICMBio 2018). With the new records herein provided, its occurrence expands to 345km in straight line from the place where the species was last recorded.

Protected areas are places of extreme importance to conserve biodiversity especially for threatened species. The Atlantic Forest Biome has suffered for decades with the loss of native areas and with the current neglect of politicians in the creation of new protected areas and maintenance of the existing ones. These results reaffirm the importance of areas as the RPPN Veracel Station for preserving the biodiversity of the Atlantic Forest. And the new records of *L. acutum* can help in future assessments about its current threat status and extend the knowledge of distribution of this species to a much larger area than previously known.

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6. Authors' Contributions

CRS and MER - Field collection, writing and manuscript revision. LRS - Field collection, manuscript review and map editing.

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Chapter 3

The Zygoptera/Anisoptera ratio as a tool to assess anthropogenic changes in Atlantic Forest streams***

Heteragrion aurantiacum Foto: Marciel Rodrigues

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The Zygoptera/Anisoptera ratio as a tool to assess anthropogenic changes in Atlantic Forest streams

Abstract

The changes in land use caused by human activities have directly impacted aquatic ecosystems, making these environments some of the most threatened places on the planet. It is increasingly necessary and urgent to develop tools to identify and assess the effects of human impacts on ecosystems and biodiversity. This study aimed to evaluate whether the Zygoptera/Anisoptera ratio can be an effective tool to measure ecological changes in Atlantic Forest streams. Adult Odonata were collected in 42 streams. An environmental integrity index was used as a measure of environmental change. The Zygoptera/Anisoptera ratio was efficient in classifying the state of preservation of streams; therefore, habitats with a proportion equal to or greater than 67 and 52% of richness and abundance of the suborder Anisoptera can be considered altered. Meanwhile, streams representing a proportion of 54 and 67% of richness and abundance of the suborder Zygoptera can be considered little altered or preserved. The proportions of responses were close to the proposals for streams in the Amazon rainforest region. The ease of identifying the specimens in the different suborders of Odonata is practical, enabling the implementation of participatory monitoring with quick responses for monitoring in the aquatic ecosystems assessed in the region. It is important to test the Zygoptera/Anisoptera ratio for a broader validation in biomes where this evaluation has not yet been carried out. In the future, this will enable the implementation of networks for monitoring the integrity of aquatic environments quickly, effectively and at a low cost.

Keywords: Lotic environments, bioindicators, Odonata, Aquatic ecosystems, Biological indices.

1. Introduction

Aquatic ecosystems are among the most threatened environments on the planet. Land use changes in natural environments for human purposes have a direct influence on aquatic ecosystems since any transformation that occurs around water bodies or in river basins can have direct and indirect consequences on these environments (Sala et al. 2000; Abell 2002; Dudgeon et al. 2006; Reid et al. 2018, Mello et al. 2020). Changes in land use for the development of activities such as agriculture, livestock, dam construction and mining, as well as the disorderly increase in urban centers are among the main impacts affecting aquatic ecosystems and their biodiversity (Sala et al. 2000; Dudgeon et al. 2006; Cabette et al. 2017; Reid et al. 2018; Barlow et al. 2018, Mello et al. 2020).

These changes over native environments, which are almost always executed without the proper management, cause drastic changes in the physical structure and water quality of aquatic ecosystems (Alan 2004; Dudgeon et al. 2006; Reid et al. 2018; Mello et al. 2020). It may also lead to an increase in the opening of canopies, a change in the structure of channels, a decrease in the flow of water and, consequently, a decrease in oxygenation due to an increase in sediment entry, causing silting along the channels, instability of the margins, and a decrease in water chemistry and homogenization of habitats (Alan 2004; Mello et al. 2020). These changes cause a decrease in abundance, local extinction and a change in biodiversity, especially for species that are more sensitive to environmental changes (Carvalho et al. 2013; Juen et al. 2014; Monteiro-Junior et al. 2015; Oliveira-Junior et al. 2015; Rodrigues et al. 2016; Rodrigues et al. 2018) since many species have specific needs conditioned by environmental conditions. Therefore, changes in environments with different magnitudes can alter the entire structure of the assemblages and the diversity of biological attributes, locally affecting the taxonomic and functional diversity of these groups in the altered environments (Hutchinson 1957; Southwood 1977).

Tropical regions, despite having the greatest biodiversity on the planet, also suffer the most from anthropogenic changes, mainly with deforestation for wood extraction and land use for agriculture and pasture (Barlow et al. 2016; Barlow et al. 2018; Mello et al. 2020). Among the Brazilian forest domains, the Atlantic Forest is one of the ecosystems most affected by the loss of its native vegetation and the exploitation of its natural resources (MMA 2010; SOS Mata Atlântica 2014). It is currently extremely fragmented, and the remaining cover corresponds to only 12.4% of its original vegetation with areas greater than 3 ha (Santos et al. 2018; SOS Mata Atlântica 2018). Moreover, it is considered a “hotspot” for biodiversity conservation (Myers et al. 2000; Mittermeier et al. 2011; Santos et al. 2018; SOS Mata Atlântica 2018), with a great appeal for the preservation, restoration of habitats and the conservation of its biodiversity.

The speed with which environmental changes are taking place is far greater than the ability of science to produce knowledge. Therefore, the increasingly rapid assessment and identification of these impacts and their effects on biodiversity are essential, considering that rapid identification contributes to decision-making in the conservation and monitoring of these ecosystems (Vorster et al. 2020). The search for effective indicators to assess the quality of aquatic ecosystems has been intense (Simaika and Samways 2012; Oliveira-Junior and Juen 2019; Vorster et al. 2020), mainly because these tools must follow some assumptions such as i) be an easy to understand method, as well as practical so that it can also be performed by non-specialists; ii) be applicable on a wide geographic scale; and iii) provide efficient and low-cost responses (Bonada et al. 2006; Resh 2008; Heink et al. 2010).

In this perspective, biological indices have gained prominence in assessments and monitoring of natural environments in the face of different types of anthropogenic changes

(Bonada et al. 2006; McGeoch 2007; Resh 2008; Simaika and Samways 2012; Oliveira-Junior and Juen 2019; Vorster 2020) mainly as indicators of physical and/or structural changes along the channels, in their surroundings and/or water contamination in aquatic ecosystems (Resh 2008; Samways and Simaika 2016). The variation in abundance, or the presence and absence of taxa or functional groups, reflects the state of the environment, whose values are indicative of magnitudes of different impacts and/or environmental changes (McGeoch 2007; Resh 2008), which makes them efficient tools to assist in the identification and monitoring of anthropogenic changes in natural ecosystems.

Aquatic insects are widely used as biological indicators, mainly due to some characteristics and peculiarities (Whittaker et al. 2005; Resh 2008), namely i) the wide distribution in the different continental aquatic systems as well as along them; ii) the composition of different taxonomic and functional groups, which are closely linked to different ecological functions and ecosystem services; iii) the wide range of tolerance that includes species that are sensitive and tolerant to several different types of anthropic changes; and iv) they are abundant organisms and easy to collect and/or observe in the field, reducing the problem that rarity could cause. These characteristics meet most of the requirements established as fundamental to be considered good indicators (Cairns and Dickson 1971; Resh 2008; Samways et al. 2010).

Among the aquatic insects, dragonflies (Odonata) have stood out as a target group in assessing and monitoring anthropogenic changes in aquatic and terrestrial ecosystems in various regions of the world (Chovanec and Waringer 2001; Simaika and Samways 2009; Chovanec et al. 2015; Valente-Neto et al. 2016; Miguel et al. 2017; Oliveira-Junior and Juen 2019; Sigutová et al. 2019; Vorster et al. 2020). Due to their sensitivity, there is a wide variation of ecophysiological requirements within the order, thus enabling predictions and testing different hypotheses. They are geographically well-distributed and charismatic organisms that are particularly easy to sample and are, therefore, susceptible to sympathy and popular support (species flags) (Carle 1979). All of these characteristics favored the development of biotic indices in various regions of the planet, such as the Odonata Habitat Index (OHI) (Chovanec and Waringer 2001) and the Dragonfly Association Index (DAI) (Chovanec et al. 2015), created in Australia to assess ecological conditions and the quality of rivers. Other indices include the Dragonfly Biotic Index (DBI) (Simaika and Samways, 2009), the Habitat Condition Scale (HSC) (Simaika and Samways 2012) and the African Dragonfly Biotic Index (ADBI) (Vorster et al. 2020), which were developed to assess the health of aquatic ecosystems in Africa.

Recently, in Brazil, Oliveira-Junior and Juen (2019) proposed an index that uses Odonata adults as indicators of physical changes in aquatic ecosystems for streams in the Amazon, with the ratio of abundance or species of the Zygoptera/Anisoptera suborders as a parameter. Because of the distinct ecophysiological requirements between the two suborders, it was expected that the frequency of Zygoptera would be higher in more preserved streams and with a more closed canopy. At the same time, Anisoptera would benefit from greater canopy opening, increasing the frequency of open areas (De Marco et al. 2015). According to the study, streams with a proportion of 54% or more of the richness or abundance of the suborder Zygoptera in relation to the suborder Anisoptera can be considered preserved or slightly altered. In contrast, streams where the proportion is equal to or more than 59% of the richness or abundance of the suborder Anisoptera in relation to the suborder Zygoptera can be considered altered and/or degraded (Oliveira-Junior and Juen 2019).

Therefore, the objective of this research was to evaluate whether the Zygoptera/Anisoptera ratio proposed by Oliveira-Junior and Juen (2019) can be used to assess changes in aquatic ecosystems in streams from an Atlantic Forest region. We predict that the Zygoptera/Anisoptera ratio is also efficient to indicate changes in streams in the Atlantic Forest. The study takes into account the ecophysiological and behavioral characteristics of the suborders (De Marco et al. 2015) and was created for a domain structurally very similar to the Atlantic Forest domain, with protected streamlets with very dense vegetation and a very closed canopy. In their original configuration, both have a phytophysionomy formed by forests surrounding the bodies of water. Thus, it is expected that the Odonata assemblies found in these two domains have very similar ecophysiological and behavioral responses.

2. Materials and Methods

2.1. Study area

The collections were performed in the municipalities of Santa Cruz Cabrália and Porto Seguro, in the southern region of the state of Bahia, Brazil. The sampled areas are located in the Atlantic Forest domain. According to the Koppen classification, the region's climate is type Af, with a temperature greater than 18°C and annual rainfall greater than 70 mm (Pell et al. 2007). Forty-two streams were sampled at 24 points within the Private Reserve of Natural Heritage (RPPN) Estação Veracel and 18 points around the RPPN (Figure 1). In both areas, the sampled streams are from first to third order (Strahler 1957), with an average width and depth of 2,5m and 0,40m, respectively. Furthermore, bottom sediment consisting of leaves, gravel, sand, pieces of logs and particulate organic matter.

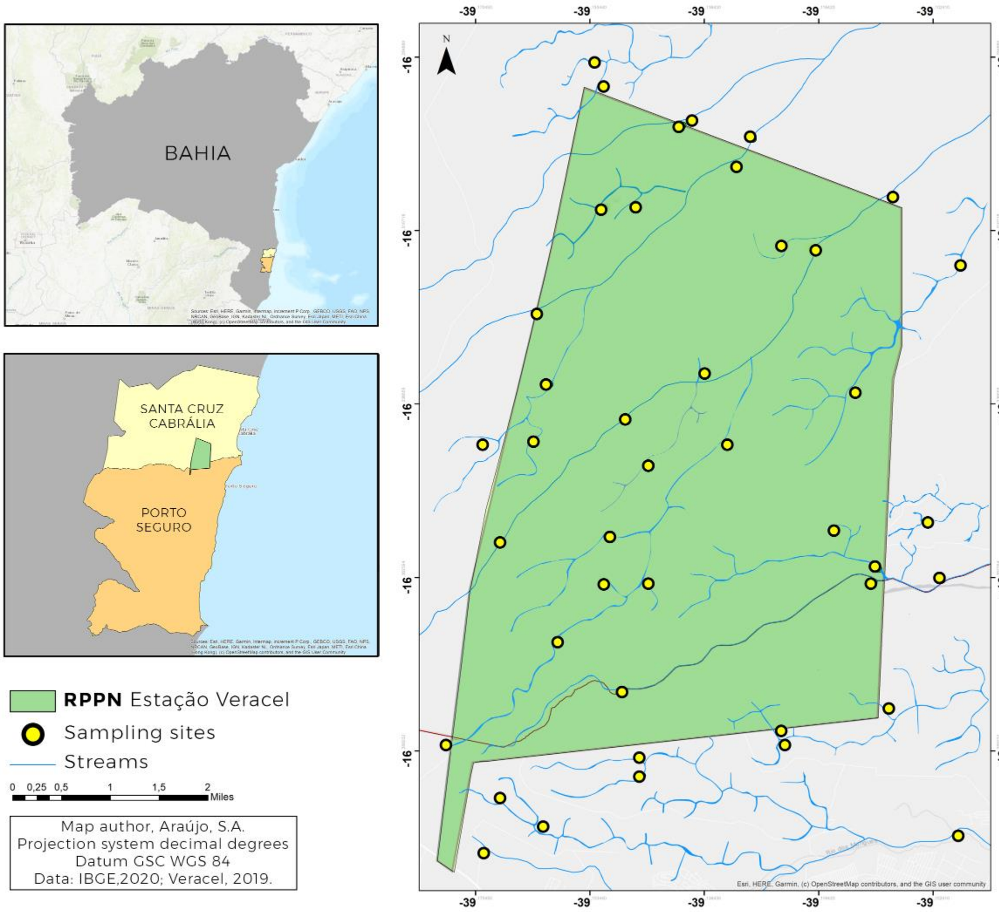

Fig. 1 Map showing the area of RPPN Estação Veracel, in the municipality of Porto Seguro, BA. The yellow dots show the sampled sites inside and outside the RPPN

The RPPN has an area of 6,069 ha. It is considered the largest private reserve in northeastern Brazil and the second largest in the Atlantic Forest biome. The area maintains characteristics of primary vegetation with high biological diversity (Veracel 2016). The streams in the RPPN have a closed and well-preserved canopy with no evidence of pollution, erosion and anthropogenic physical changes along the channels.

The areas surrounding the reserve are composed of a matrix of pasture and agriculture with agrarian settlement areas and large rural properties, whose records indicate that the soil has been exploited for these purposes since at least the 1960s. In general, the streams did not have uniform riparian vegetation (Figure 1), with a lower concentration of sediments such as leaves, pieces of logs and particulate organic matter. The riparian vegetation more frequently presented open and exposed locations to the incidence of light. At some points, the stream channel was interrupted to construct small dams for the watering of animals and/or uses on the property. At a few points, it was possible to observe a small amount of household waste.

2.2. Specimen collection

Two collections were performed, one in September 2018 (end of rainy season) and another in February 2019 (end of dry season) in order to sample the greatest representativeness of biodiversity in the area. The adults were collected with entomological nets. The collections followed a scanning methodology with fixed areas, where for each point, a 100-meter stretch of water was sampled on both banks for an hour and a half in each campaign. The collections were carried out from 9 am to 4 pm on sunny days, since the activities of adults are greater at these times and in these environmental conditions (Oliveira-Junior and Juen 2019).

In the field, the captured adult specimens were stored in paper envelopes, labeled and taken to the Laboratory of Aquatic Organisms (LOA) at the State University of Santa Cruz for later identification with the aid of identification keys (Garisson et al. 2006; Garisson et al. 2010; Lencioni 2005, 2006, 2017).

2.3. Environmental characterization

To assess each stream´s environmental integrity, the Nessimian Habitat Integrity Index (HII) (2008) was used. The HII includes 12 qualitative metrics, each composed of a scale of four to six alternatives representing different degrees of integrity, namely use of riparian land, extension and condition of the riparian forest, type of channel sediment and retention mechanisms, bank structure and erosion, substrate stream bed, presence of vegetation and aquatic debris and distribution of rapids, backwaters and meanders.

The score weights for each item in the 12 parameters used in the HII were standardized, following the methodology of Oliveira-Junior and Juen (2019). The final value of the index ranged from 0.1 to 1, with lower scores referring to environments with greater changes and higher scores to more preserved environments. A recent meta-analysis showed that this is a very important metric to explain the structuring of the aquatic insect community, especially Odonata (Brasil et al. 2020). The streams evaluated following the same classification range used by Oliveira-Junior and Juen (2019) and Oliveira-Junior et al. (2019).

2.4. Data analysis

To evaluate the distinction between stream conservation categories, the values of the 12 items of the HII that describe the prevailing environmental conditions in the study areas were summarized in the principal component analysis (PCA). To determine which principal components should be retained for analysis, the randomness obtained by the “broken-stick” model was used (Jackson 1993). To test whether the conservation categories (preserved and altered) were significantly different from each other, the scores generated by the PCA were tested using the Student test ($p < 0.05$).

For each sampled point, the assemblies were separated by suborder and the proportion of the corresponding richness and abundance at each of the sampled points was calculated. The proportion of richness and abundance found for each suborder was compared with the HII value for each point using linear regression (Zar 2010). The assumptions of normality and homoscedasticity of the residues for the richness and abundance data were tested *a priori*. The results were used to compare the proportions presented by Oliveira-Junior and Juen (2019) with the proportions found in this study. The regression line equation was used to calculate the expected values for richness and abundance by estimating how they would behave for each change in HII values in aquatic ecosystems. All statistical analyses were performed in R (R Development Core Team 2020) using the vegan package (Oksanen et al. 2019).

3. Results

At the sampled locations, HII ranged from 0.46 to 0.92. The 42 streams were classified into two categories of environmental conditions, resulting in 16 streams classified as altered environments (0.46-0.60), and 26 streams as preserved environments (0.61-0.92) The average HII score was 0.57 for streams considered altered and 0.85 for streams considered preserved.

The first two PCA axes' association represented 69.43% of the sampled environmental variation in the present study. The first axis explained 58.17% of the results (eigenvalue = 0.31). Only the first axis was analyzed because the second axis did not present an observed value greater than the estimated value when calculated using the "broken-stick" method. The PCA showed a grouping of streams that were separated by the categories "preserved" and "altered" (Figure 2). This separation in the two categories had a significant difference ($t = -15.21$, $df = 27.45$, $p = 6.615x10-15$) when compared to the values obtained by the principal components of axis 1. The preserved streams had a positive relationship with the HII index integrity variables, and they were grouped in the direction of the highest values for the surrounding types of land uses (F1), width and integrity of riparian vegetation (F2 and F3), physical channel structure (F6) and presence of aquatic plants (F11). The streams were considered altered negatively associated with the HII index integrity variables and they were characterized by significant losses and changes in the parameters of the F1, F2, F3, F6 and F11 indices (Figure 2). It is important to note that the variables that most contributed to the formation of axis 1 are closely related to the uses of the surrounding soil and the physical structure of riparian vegetation (integrity and width) and the channel (integrity) aquatic ecosystems. These variables are negatively associated with the level of conservation of these environments.

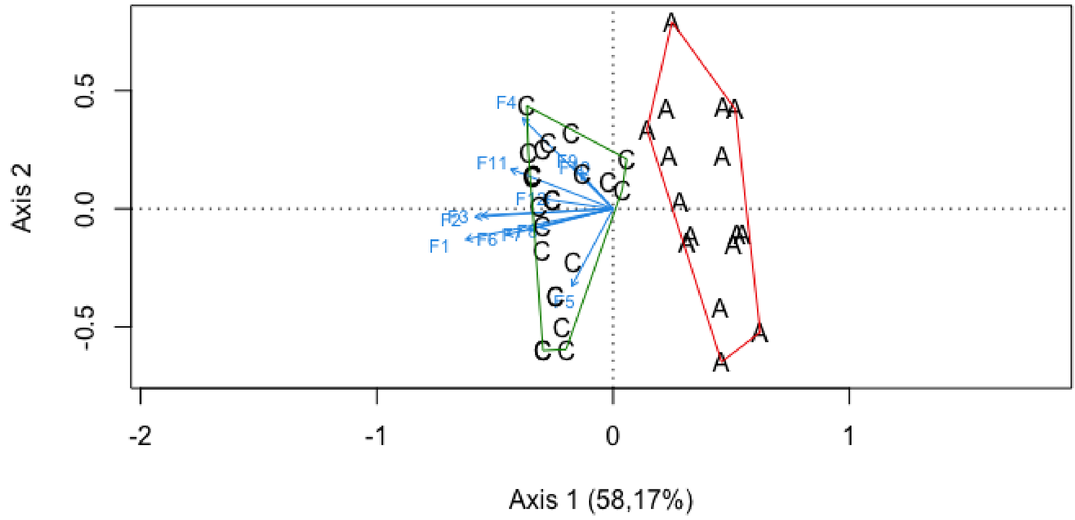

Fig. 2 Ordering environmental variables referring to the 12 parameters used by the environmental integrity index - HII, through a principal component analysis – PCA. The letter C and the green line represent the points considered Conserved. And the letter A with the red line represents the points considered Altered.

In total, 722 adult Odonata specimens were collected, representing 50 species, 35 genera and nine families. Of the specimens collected, 566 belong to the suborder Zygoptera and 156 to the suborder Anisoptera, with 22 species of Zygoptera and 28 species of Anisoptera (Appendix 1).

The regression analyses for Anisoptera richness and abundance showed that the higher the HII value, the lower the proportion of the group's richness and abundance ($R = 0.384$ and $p < 0.001$; $R = 0.37$ and $p < 0.001$, respectively). On average, with an increase of 0.1 in the environmental integrity index, there is an expected loss of 11 species and 10 specimens (Table 1, Figure 4). For Zygoptera, the analyses showed that the higher the index value, the greater the proportion of richness and abundance ($R = 0.384$ and $p < 0.001$; $R = 0.374$ and $p < 0.001$, respectively). With an increase of 0.1 in the integrity index, there is an estimated average increase of 11 species and 10 specimens (Table 1, Figure 4).

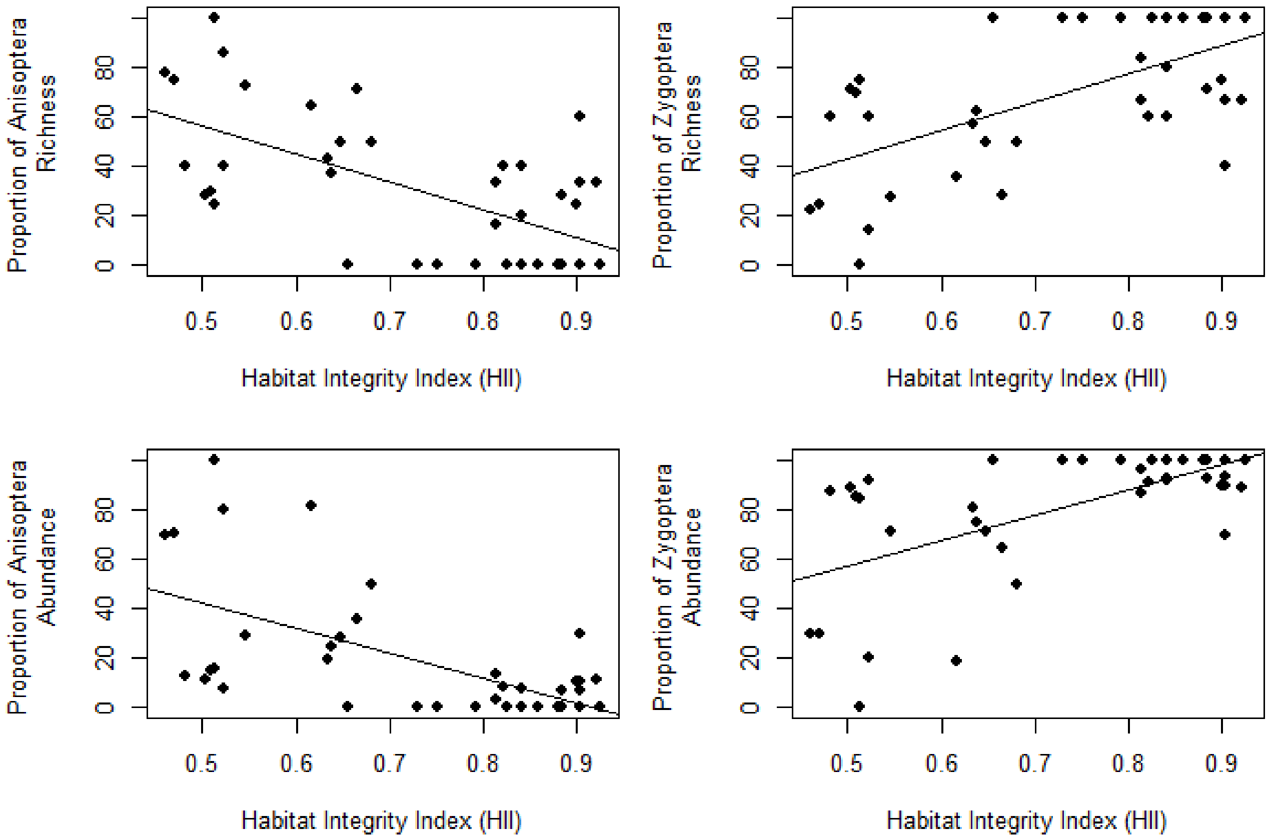

Fig. 3 Relationship between the proportion of abundance and richness of the species Odonata and the environmental integrity of streams sampled in and surrounding RPPN Estação Veracel

The proportions of abundance and richness of the species Odonata using the regression equation for each value HII were also calculated. The proportion of richness and abundance of Anisoptera was greater than 67 and 52%, respectively for streams considered altered with values IIH less than 0.4 (Table 1). On the other hand, in streams with values greater than 0.7 on the HII, considered preserved, the proportion of Zygoptera richness and abundance was bigger than 54 and 67%, respectively (Table 1).

Table 1 Relationship between the proportion of species richness and abundance and the Habitat Integrity Index (HII) of the streams sampled at the RPPN Estação Veracel. The reverse effect is observed between Anisoptera and Zygoptera according to the regression equation

	REGRESSION DATA			
	RICHNESS ANISOPTERA	ABUNDANCE ANISOPTERA	RICHNESS ZYGOPTERA	ABUNDANCE ZYGOPTERA
	R^2= 0,3987	R^2= 0,3892	R^2= 0,3987	R^2=0,3892
	R adjusted = 0,384	R adjusted = 0,3743	R adjusted = 0,384	R adjusted = 0,374
	p= 5,623*10^-6	p= 7,84*10^-6	p= 5,62*10^-6	p= 7,84*10^-6
	y= 113,39 - 113,48*X	y= 93,44 - 101,82*X	y= -13,39 + 113,48*X	y= 6,56 + 101,82*X
HII	Proportion of richness	Proportion of abundance	Proportion of richness	Proportion of abundance
0.1	102,042	83,258	-2,042	16,742
0.2	90,694	73,072	9,306	26,924
0.3	79,346	62,894	20,654	37,106
0.4	**67,998**	**52,712**	32,002	47,288
0.5	56,65	42,53	43,35	57,47
0.6	45,302	32,348	**54,698**	**67,652**
0.7	33,954	22,166	66,046	77,834
0.8	22,606	11,984	77,394	88,016
0.9	11,258	1,802	88,742	98,198
1.0	-0,09	-8,38	100,09	108,38
	Loss of + - 11	Loss of + - 10	Increase of +- 11	Increase de +- 10

4. Discussion

The use of invertebrates as biological indicators is an important tool to assist in the understanding of human threats to aquatic ecosystems (Resh 2008; Voster et al. 2020). The results in the present study show that the proportions of the Odonata suborders can be used to monitor the environmental condition of streams in the Atlantic Forest. Streams that have been altered by anthropogenic environmental degradation in riparian zones and/or along channels obtain a proportion of 67% or more for richness and 52% or more for the abundance of Anisoptera. In contrast, streams that are more preserved or with few environmental alterations have a proportion greater than 54% for richness and 67% for the abundance of Zygoptera.

The Zygoptera/Anisoptera ratio (Oliveira-Junior and Juen 2019) used in this study showed an effective potential to assess changes in aquatic ecosystems in areas of the Atlantic Forest. Moreover, the results showed that in the sampled streams, the proportions of abundance and richness of adult Odonata are closely related to the physical integrity of the channels of aquatic ecosystems and their surrounding areas. This corresponds very closely to what was proposed by Oliveira-Júnior and Juen (2019), corroborating our prediction. These results reinforce the group's role as indicators of environmental changes in aquatic ecosystems (Miguel et al. 2017), mainly in domains with an original formation in their phytophysiognomy formed by forest areas.

The change in the index integrity values is observed by a difference in response in richness and abundance proportions for the two suborders. For Anisoptera, the higher the index values, the lower the proportion of richness and abundance, while the opposite was observed for Zygoptera, which is similar to the pattern described by Oliveira-Júnior and Juen (2019). Our work showed a proportion of Anisoptera richness and abundance bigger than 67 and 52%, respectively, for streams considered altered. In the work of Oliveira-Junior and Juen (2019), the proportion bigger than 59% for both. Regarding the proportion of Zygoptera richness and abundance, a value bigger than 54 and 67%, respectively, was found for preserved streams. In the study by Oliveira-Júnior and Juen (2019), the proportion found was bigger than 54% for both. In other words, the Odonata suborders both in the Amazon Forest and in the Atlantic Forest exhibit a very similar response pattern.

The similarity of the responses between the proportions found in the two domains, Amazon Forest and Atlantic Forest, has a strong relationship with the ecophysiological and behavioral characteristics of the species of suborders found in these regions (De Marco et al., 2015). The greater richness and abundance of Anisoptera in more altered environments is related to the fact that species of these regions are adapted to more open areas or areas that have lost their natural riparian vegetation, as well as in aquatic environments with physical changes along the channel due to the construction of dams or caused by silting and reduced flow (Remsburg et al. 2008; Calvão et al. 2013; Carvalho et al. 2018; Rodrigues et al. 2018).

In relation to individuals belonging to Zygoptera, they are generally associated with healthier environments with denser riparian vegetation on the banks and a more closed canopy and environments without many physical changes along the channel (Carvalho et al. 2013; Brazil et al. 2017; Rodrigues et al. 2016; Mendes et al. 2017; Carvalho et al. 2018) and exhibit morphological and behavioral characteristics related to low dispersion capacity and greater dependence on local conditions (De Marco et al. 2015). Therefore, this group is more susceptible to local extinction due to anthropogenic changes in or around aquatic ecosystems (Rodrigues et al. 2016; Carvalho et al. 2018).

The theoretical basis of biomonitoring is the dependence of organisms on a set of conditions and specific resources for their establishment (Leibold 1995). The unavailability or modification of these variables can cause decreases in abundance or even the local exclusion of more specialized species (Oliveira-Júnior et al. 2015; Miguel et al. 2017). Thus, data on the distribution of occurrence or variation in the abundance of species can provide evidence about the environmental conditions necessary for their establishment over the landscape or time, as well as the environmental integrity of the monitored location. Biological indices are being increasingly used to complement physical and chemical data in assessments and in monitoring the quality of aquatic ecosystems, thus enabling a more careful assessment of these environments (Angermeier and Davideanu 2004; Resh 2008). An example is the Monitor Program, created by the Chico Mendes Institute for Biodiversity Conservation (ICMBio) to assess the environmental quality of streams in the Brazilian Amazon Conservation Units in a participatory manner with community members who live in the monitored areas. The program works through knowledge about the relationship/specificity of Odonata adults with the environmental impacts of deforestation in streams and streamlets (Brasil et al. 2020).

Few low-cost methods with quick and efficient responses are available to assess the quality of aquatic environments. It is of great importance to create proposals for biological indices such as the one presented by Oliveira-Junior and Juen (2019) and these indices must be widely replicated and validated for the Atlantic Forest biome as shown in the present study. This proposal brings a cost-effective approach to assess and monitor the effects of environmental changes caused by human actions in streams in the Amazon and the Atlantic Forest. With an easy-to-apply approach due to the facilitated identification of suborders in the field, it can also be adopted as an initial parameter for public environmental agencies and private companies to assess the integrity of aquatic ecosystems.

The Zygoptera/Anisoptera ratio proved to be effective for assessing changes in streams in the Atlantic Forest, with proportions similar to those recorded in the Amazon streams. The capacity to identify specimens in different Odonata suborders is practical and easy and can provide a quick answer regarding possible changes in the evaluated aquatic ecosystems. In addition, dragonflies are widely appreciated by people in general and are already being used as environmental education tools (Clausnitzer et al. 2017). The proposal should be increasingly used and tested in environments with multiple types of anthropogenic changes in different forest domains, thus providing future validation that it is safe and broad to be used as a biological index to assess physical changes in lotic ecosystems and their surroundings. Moreover, it is a tool with quick and low-cost responses that can be used in previous assessments or together with other methodologies in studies on the assessment of environmental impacts, monitoring and conservation of aquatic ecosystems.

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6. Appendices

Table 2 List of species found in the study region, separated by suborder and family

Subordem	Family/Species
	Gomphidae
	Epigomphus paludosos Hagen in Selys, 1854
	Phyllogomphoides sp Belle, 1970
	Progomphus montanos Belle, 1973
	Progomphus sp1 Selys, 1854
	Zonophora calippus spectabilis Campion, 1920
	Libellulidae
	Anatya guttata Erichson in Schomburgk, 1884
	Diastatops obscura (Fabricius, 1775)
	Elasmothemis cannacrioides (Calvert, 1906)
	Erythemis credula (Hagen, 1861)
	Erythemis vesiculosa (Fabricius, 1775)
	Erythrodiplax avithata Borror, 1942
	Erythrodiplax funera (Hagen, 1861)
	Erythrodiplax fusca (Rambur, 1842)
	Erythrodiplax leticia Machado, 1996
	Erythrodiplax lygae Ris, 1911
	Erythrodiplax paraguayensis (Foster, 1905)
Anisoptera	*Erythrodiplax umbrata* (Linnaeus, 1758)
	Micrathyria artemis Ris, 1911
	Micrathyria atra (Martin, 1997)
	Micrathyria catenata Calvert, 1909
	Micrathyria menegeri menegeri Ris, 1919
	Micrathyria ungulata Forster, 1907
	Orthemis attenuata (Erichson in Schomburgk, 1848)
	Oligoclada umbricola Borror, 1931
	Perithemis lais (Perty, 1833)
	Perithemis thais Kirby, 1889
	Planiplax phoenicura Ris, 1912
	Zenithoptera viola Ris, 1910
Zygoptera	**Coenagrionidae**
	Argia hasemani Calvert, 1909
	Acanthagrion cuyabae Calvert, 1909
	Acanthagrion gracile Rambur, 1842
	Bromeliagrion sp De Marmels, 2005 in De Marmels & Garrison, 2005
	Epipleoneura machadoi Rácenis, 1960
	Leptagrion macrurum Burmeister, 1839

	Leptagrion acutum Santos, 1961
	Telagrion longum Selys, 1876
	Telebasis corallina Selys, 1876
	Ischnura capreolus Hagen, 1861
	Neoneura sylvatica Hagen in Selys, 1886
	Nehalennia minuta Selys in Sagra, 1857
	Idioneura ancilla Selys, 1860
	Forcepsioneura sancta Hagen in Selys, 1860
	Megapodagrionidae
	Heteragrion aurantiacum Selys, 1862
	Perilestidae
	Perilestes fragilis Hagen in Selys, 1862
	Calopterygidae
	Hetaerina rosea Selys, 1853
	Hetaerina longipes Hagen *in* Selys, 1853
	Dicteriadidae
	Heliocharis amazona Selys, 1853
	Lestidae
	Lestes forficula Rambur, 1842
	Lestes tricolor Erichson in Schomburgk, 1848

Final considerations

The studies carried out by this research emphasize that land use changes also cause changes in Odonata diversity. And that protected areas play a key role in maintaining and preserving this biodiversity, especially to the species more sensitive to changes in natural environments or habitat specialists. The results also emphasize that these protected areas are of great importance for endangered species. And that without them the impacts on biodiversity would be much more tragic. Our results also show that Odonata group is a good indicator of changes in the natural environments. And that characteristics related the biology, ecology and behavior of species and which are reflected in the suborders can be used to assess changes in the natural environments. These characteristics facilitates the use of the group as a bioindicator and puts it in evidence to be used increasingly as a "surrogate" for other groups of aquatic invertebrates, especially the aquatic insects. In short, the creation, maintenance and expansion of protected areas, especially in the Atlantic Forest, can be one of the best paths for the conservation and maintenance of biodiversity.