Biota Neotropica 23(2): e20221440, 2023
www.scielo.br/bn

biotaneotropica
ISSN 1676-0611 (online edition)

Article

Ecological and biological patterns of stream fish studies from the Piracicaba-Capivari-Jundiaí Basin (PCJ Basin, SP) assessed through a systematic review

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SILVA, A.A.F., ESTEVES, K.E. **Ecological and biological patterns of stream fish studies from the Piracicaba-Capivari-Jundiaí Basin (PCJ Basin, SP) assessed through a systematic review**. Biota Neotropica 23(2): e20221440. https://doi.org/10.1590/1676-0611-BN-2022-1440

Abstract: Tropical streams are among the most threatened ecosystems in the world. As such, studies carried out and compiled over spatial and temporal scales can provide useful information to examine patterns of species diversity and threats to their survival. Here we conducted a systematic review of published research on biological and ecological aspects of stream fish fauna found in the Piracicaba-Capivari-Jundiaí Basin, an industrial watershed of São Paulo State. We aimed to detect main patterns, trends and gaps in studies related to species composition, distribution, spatial and temporal scales, as well as in the covered topics. Results were related to main land uses, biomes and Conservation Units. A constant increase in published articles occurred from 2003 until 2016 with an average of 1.8 articles/year. Twenty-six publications were considered for the present study, reporting on fish samples obtained in 67 sites and resulting in 89 species. A high proportion of studies were concentrated in the Corumbataí sub-basin, and rarefaction curves indicated that stream fish richness in the PCJ Basin may be considerably higher than that shown by the actual numbers. Basin studies were unevenly distributed and did do not include such highly preserved areas as the Camanducaia, Jaguari and Jundiaí sub-basins. We emphasize the importance of further surveys in these regions, as well as in high priority conservation areas, which may lead to new insights for developing appropriate conservation strategies for this basin.
Keywords: *Conservation; Freshwater fish; Neotropical streams; Synthesis of the literature; Spatial scale.*

Padrões ecológicos e biológicos de estudos de peixes de riachos da Bacia Piracicaba-Capivari-Jundiaí (Bacia PCJ, SP) avaliados por meio de uma revisão sistemática

Resumo: Riachos tropicais estão entre os ecossistemas mais ameaçados do mundo e a compilação de estudos temporais e espaciais pode fornecer informações úteis para examinar padrões de diversidade de espécies e ameaças nesses sistemas. Realizamos uma revisão sistemática das pesquisas publicadas sobre aspectos biológicos e ecológicos da ictiofauna de riachos da bacia do Piracicaba-Capivari-Jundiaí, uma bacia industrial do Estado de São Paulo. O objetivo foi detectar os principais padrões, tendências e lacunas em estudos relacionados à composição, distribuição de espécies, escalas espaciais, temporais e temas abordados. Os resultados foram relacionados aos principais usos do solo, biomas e Unidades de Conservação. Foi verificado um aumento constante de artigos entre 2003 e 2016, com média de 1,8 artigos/ano. Vinte e seis publicações foram consideradas para o estudo, que indicaram 67 locais amostrados, e o registro de 89 espécies. Uma alta proporção deles concentrou-se na sub-bacia de Corumbataí e curvas de rarefação indicaram que a riqueza de peixes de riacho na bacia do PCJ deve ser consideravelmente maior do que os números atuais. A distribuição desigual de estudos na bacia, que não inclui áreas altamente preservadas como as sub-bacias de Camanducaia, Jaguari e Jundiaí, enfatiza a necessidade de se obter mais informações nessas regiões, bem como em áreas de conservação de alta prioridade. Novas abordagens relacionadas a conceitos e teorias ecológicas em estudos futuros poderão fornecer informações que ajudem a desenvolver estratégias de conservação adequadas para esta bacia.
Palavras-chave: *Conservação; Peixe de água doce; Riachos neotropicais; Síntese da literatura; Escala espacial.*

Introduction

The growing biodiversity crisis has led to several global initiatives that compile datasets from studies carried out over time and space (Pereira & Cooper 2006). Although these databases have proved extremely useful and allowed major advancements in ecological research (e.g., Kendall et al. 1998; Sibly et al. 2005), few of these studies concern riverine fishes, despite several independent, often local in extent, academic research programmes (Matthews & Marsh-Matthews 2017). In Brazil, studies of stream fishes have increased in the last decades. These studies have emerged from the introduction of new sampling techniques (Alves et al. 2021) as well as the implementation of inventories aimed to characterize the biodiversity of different ecosystems, which have promoted the discussion of conservation strategies, economic potential and sustainable use (FAPESP 2016).

Despite institutional and academic monitoring efforts to produce data on freshwater fish studies, such data are dispersed and fragmented in hundreds of works and publications, often in sources that are difficult to access, and, in most cases, in a format that makes a direct application unfeasible. This poses a problem for researchers and policymakers who use scientific information on biodiversity, with the available data still being underused (Rodrigues & Bononi 2008). More specifically for stream fishes, these data need to be taxonomically verified and updated and regional databases must be compiled and maintained for the analysis of new species, long-term impacts and trends, in order to make management projects viable (Winemiller et al. 2008). Furthermore, regional databases can be combined to facilitate the analysis of patterns on a broader biogeographic scale, including regional variation in species richness and invasions by exotic species (Winemiller et al. 2008). Several of these approaches may be obtained through systematic reviews, considered a useful tool to integrate the information of a group of studies (Sampaio & Mancini 2007). Thus, causative factors of habitat loss, species introduction or chemical pollution and hybridization may be identified in freshwaters, which is not always possible due to inadequate data (Allan & Flecker 1993).

Studies in this direction were made by Dias et al. (2016). They found that research on Brazilian stream fish assemblages have been conducted mainly at small temporal and spatial scales relative to the dimension and importance of Neotropical freshwaters, but with homogeneous objectives that have varied little over the last 20 years. More recently, Junqueira et al. (2020) conducted a scientometric analysis to detected trends in published research of Brazilian stream fish assemblages. They found that the Paraná River Basin was the most studied region. Their review reveled greater financial and scientific resources available in this region as well as access to streams, owing to the high level of urban development and associated infrastructure (Dias et al. 2016).

Neotropical streams are known for their high fish biodiversity, with 70% of the 3148 species described from Brazilian freshwaters (ICMBio 2018) consisting of small fish (< 15cm) which live in small rivers and different stream types (Castro & Polaz 2020). In the State of São Paulo, intensive samplings of stream fishes have been conducted in several regions such as the northwest, including sub-basins of the Upper Paraná River (Molina et al. 2017; Zeni et al. 2019); the Paranapanema River Basin (Castro et al. 2003); the Rio Grande (Castro et al. 2004); coastal streams (Sabino & Castro 1990; Esteves & Lobón-Cerviá 2001; Gonçalves et al. 2018; Gonçalves et al. 2020) and the Piracicaba-Capivari-Jundiaí Basin (PCJ Basin). This basin coincides with important axes of economic growth, presenting increasing demands for water supply, irrigation and industry, as well as critical water quality values of the Capivari, Piracicaba and Jundiaí sub-basins (Comitês PCJ/Agência das Bacias PCJ 2020). It occupies 0.18% of the national territory, concentrating around 2.7% of the population and covering the territories of 76 municipalities, 71 of which belong to the State of São Paulo (Comitês PCJ/Agência das Bacias PCJ 2020).

Given the several knowledge gaps in relation to freshwater fishes in the State of São Paulo, especially in regions of increasing pressure to convert natural areas into urban or pasture areas (Casatti et al. 2008), literature searches may help to guide project development by indicating new directions for further investigations. With this in mind, we herein conducted a systematic review of published research on the stream fish fauna of the PCJ Basin in order to identify the main patterns, trends and gaps in studies related to species composition, distribution, spatial and temporal scales and covered topics. We then analysed the obtained results in relation to the main land uses, conservation areas and biomes with the aim of identifying regions or approaches that need greater attention, subsidizing future research and decision-making.

Material and Methods

1. Study Area

The PCJ Basin is part of the Tietê River Basin, belonging to the Upper Paraná River Basin, which covers 900 thousand km^2 and is part of the south face of the Brazilian Shield (Langeani et al. 2007) (Figure 1). It is one of the six units of Water Management of the State of São Paulo (UGHRIs), and is classified as industrial (São Paulo 2011). It has a drainage area of 14,178 km², that accommodates the Atibaia, Atibainha, Cachoeira, Camanducaia, Capivari, Corumbataí, Jaguari, Jundiaí and Piracicaba Rivers. This basin also houses 44 Conservation Units (CUs), comprising 33 categorised as Sustainable Use and 11 categorised as Integral Protection, which, together, correspond to approximately 53% of the total area of the PCJ Basins (Comitês PCJ/Agência das Bacias PCJ 2020).

Approximately 22% of the total area is covered by remnants of native vegetation, with 2% representing grassland formations and 20% forest formations. The sub-basins with the highest percentages of forest remnants are the Atibaia and Jundiaí, where Dense Ombrophilous Forest ("Atlantic Forest") occurs (Comitês PCJ/Agência das Bacias PCJ 2020). Water quality indicators show that the sub-basins of the Capivari and Piracicaba rivers have the worst quality for public supply and the highest trophic state. They also present the lowest quality of protection for aquatic fauna and flora. In contrast, the Jaguari River sub-basin stands out in terms of water quality for public supply and protection of aquatic fauna and flora, presenting a predominantly oligotrophic trophic state (Comitês PCJ/Agência das Bacias PCJ 2020).

2. Systematic review

Our systematic review followed the main steps described by Sampaio & Mancini (2007), who consider three stages: definition of the object of the review, identification of the literature and selection of the studies to be included. Potential papers were searched in the ISI Web of Science (Main Collection), as well as the Scielo and Scopus databases. The "advanced" search mode was used, which allowed to

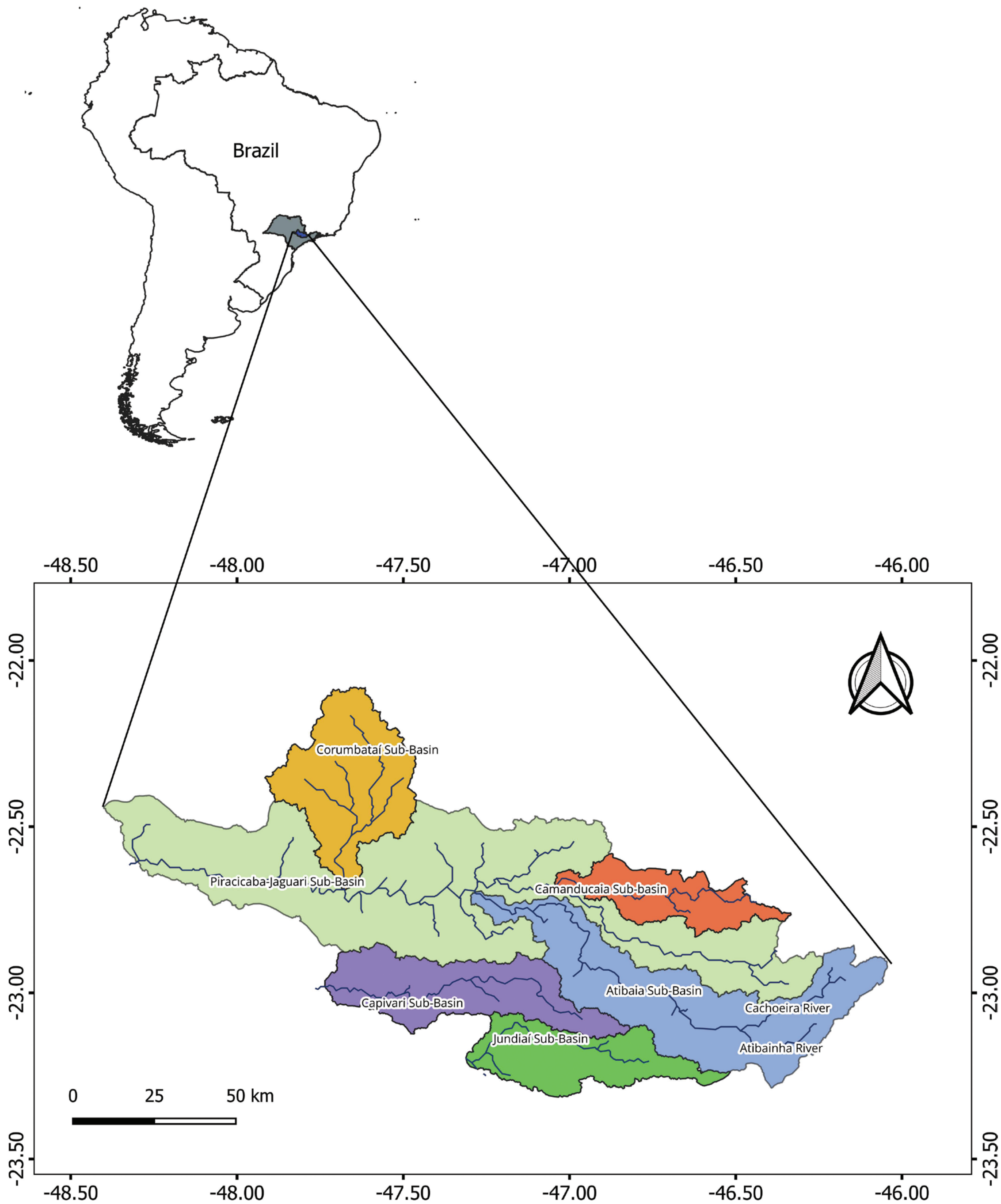

Figure 1. Location of the PCJ Basin in the State of São Paulo showing its main sub-basins.

obtain results using different keywords and their combinations. The used strings were: "*Name of the Sub-Basin*" or one of the main rivers AND (*river** OR *stream** OR *basin**) AND (*fish** OR *ichthyofauna*) for the period between 1987 and 2021. This period was chosen because a preliminary search indicated few indexed publications before 1990. The names of the main rivers and sub-basins were selected according to maps found in articles and on the official websites of the PCJ Basin Agency (https://www.comitespcj.org.br/).

After the searches were completed, all the articles were exported to the *State of the Art Throught Systematic Review (Start)* software developed at the LaPES (Software Engineering Research Laboratory of the Federal University of São Carlos, SP, Brazil). This software program divides research into three phases: planning, selection and extraction. In the first stage, a protocol with the keywords, search engines and the criteria for accepting or rejecting articles is defined. In the selection phase, articles are classified as accepted, rejected or duplicated based on the acceptance and rejection criteria defined in the protocol. Finally, in the extraction phase accepted articles are summarized and may be classified as rejected or duplicated again.

To include the studies of interest, we considered only those carried out in 1st to 4th order streams, placing emphasis on biological and ecological approaches. The subjects of interest were related to population biology, growth, diet, reproduction, morphology, ontogenetic variation, water quality and other anthropic effects on stream fishes. Studies carried out in in dams and reservoirs, large rivers as well as experimental, physiological, genetic, parasitological or systematic studies, were excluded. After reading the abstract of each article, an analysis was carried out to verify whether or not the article met the inclusion criteria. In the extraction step, a complete reading of the selected articles was performed aiming to check the inclusion criteria. To confirm the inclusion of the selected articles within the PCJ boundaries, the coordinates were previously plotted in Google Earth version 7.3.

3. Data Registration and species validation

This stage was characterized by the compilation of information obtained from the extracted articles, related to (1) species and its degree of threat (according to ICMBio 2018) (2) location (geographical coordinates) and stream order; (3) species status (native or non-native); (4) sub-basin, micro-basin and river/stream where the survey was carried out; (5) main themes (trophic ecology, growth, environmental impacts, community structure, integrated biological aspects, reproduction, riparian zone influences on the fish community/populations) and (6) temporal trends, defined as the difference between the last and the first sampling year. We considered non-native species to be those introduced outside their natural range (Garcia et al. 2021) and classified them according to Langeani et al. (2007).

To register the species, we did not consider subspecies, and all occurrences that were not identified at the species level were discarded (i.e., occurrences with genera abbreviated to sp., or species affinis commonly abbreviated as aff., and conferatum abbreviated as cf.), as suggested by Tedesco et al. (2017). Validation of the scientific names of the species was performed using the Catalog of Fishes (Fricke et al. 2021), which allowed finding valid species names and species recently described that are still not included in FishBase. The Constancy of Occurrence (Dajoz 1972), a qualitative measure that takes into account the presence or absence of the species in the samplings, was calculated for each species as $F = (P \times 100)/N$, where P is the number of samplings containing the species, and N is the total number of performed samplings. Species were then classified as constant (≥ 50.0%), Accessory (25% – 50%) or Accidental (≤ 25.0%). For these calculations, we considered each article as a sample, since species lists in some of the examined papers were pooled for the different sampling sites.

4. Species distribution, richness and environmental data

The geographical coordinates obtained for each selected article were standardized, converting them into the Universal Transverse Mercator (UTM) coordinate system. We considered the *Datum* of each article and used the Geodetic Coordinate system for Latin America (SIRGAS 2000) as the output. Spatial trends of ichthyofauna studies were plotted on maps using QGIS 3.4 (QGis Development Team 2018) overlaid on Ottocoded Hydrographic shapefiles of the PCJ Basin provided by the National Water Agency (ANA). Species distributions were also superimposed on several thematic shapefiles as biomes, priority areas for biological conservation (MMA 2018), and Conservation Units (IBGE – https://portaldemapas.ibge.gov.br/portal.php#homepage). Data on land use were acquired from the Mapbiomas Project (Collection 6, http://mapbiomas.org/) which considers 34 classes, including forests, pasture, agriculture, vegetated and non-vegetated areas and water, among others.

Land use was calculated for the years of 2010 and 2020 on the QGIS 3.4 software for the whole basin and its evolution analyzed over the period. The same procedure was used for each site, where a 500-m buffer was delimited around each (Tibúrcio et al. 2016). The area occupied by the different land use classes was calculated with geometry tools and the basic statistics interface of QGis 3.4.

Rarefaction/extrapolation curves were built to assess the sampling effectiveness, using sampled-based rarefaction. This is considered a more realistic treatment of the independent sampling units used in most biodiversity studies, and considered adequate for comparing the richness of sample sets (Gotelli & Colwell 2001). The analysis was performed for i) grouped samples of the PCJ Basin, ii) Corumbataí sub-basin and iii) all other sub-basins on PAST 4.09 software (Hammer et al. 2001), with the standard errors converted to 95 percent confidence intervals (± 95% CI).

Results

1. Search results

The original search resulted in 281 articles, and after removing duplicates, it yielded 224 studies. Subsequent screening of titles and abstracts excluded 135 studies, of which 59 were deemed potentially relevant. After a second screening, 14 articles were also classified as duplicated, resulting in 45 eligible papers that were included for further analysis. Of these, 19 were excluded because they were outside the limits of the PCJ Basin and/or were not related to the main themes, finally resulting in 26 studies.

Results indicated that the Corumbataí and Piracicaba-Jaguari sub-basins were the most studied regions, with 76.9% and 19.2% of the articles recorded respectively. In the Jundiaí and Atibaia sub-basins, the number of studies was low, while no study was performed in the Camanducaia sub-basin (Table 1). These studies were carried out in 67 different sites, most of which were located in the Corumbataí sub-basin (65.7%), followed by the Piracicaba (16.4%), Jaguari (10.4%), Atibaia (4.5%) and Jundiaí (3.0%) sub-basins.

"Trophic ecology" was addressed as the main topic (26.9 %), followed by "Environmental Impacts" (23.0%), "Community Structure" (19.2%) and "Integrated biological aspects" (15.3%). "Reproduction" and "Riparian zone influences on fish community/populations" were the least common topics (both with 7.7%) (Table 1). The publishing trend for articles is shown in Figure 2. The number of articles published per year was low (mean = 1.85; SD = 1.18). Publications started in 2003 and maintained a constant rate until 2016, after which no articles were found. However, the regression equation for the cumulative number of articles indicates a tendency toward a constant increase after 2016.

2. Species richness, distribution, land use and conservation units

We registered 89 species, of which 49.4% were Characiformes, followed by Siluriformes (34.8%), Cichliformes (5.6%), Gymnotiformes (4.5%), Cyprinodontiformes (4.5%) and Synbranchiformes (1.1%). Of these, most were autochthonous species (94.3%), 5.6 % were non-native species and 94.3% were considered least Concern (LC), while only one was classified as vulnerable (*Characidium oiticicai* Travassos, 1967) according to the Brazilian Red List (ICMBio 2018). Three

Table 1. Studies included in the systematic review (n = 26), indicating their location in the sub-basins and main covered topics.

Authors/year	Title	Sub-Basin	Main topic
Alexandre et al. (2010)	Analysis of fish communities along a ...	Piracicaba-Jaguari	Community structure
Cardone et al. (2006)	Diet and capture of *Hypostomus strigaticeps* ...	Corumbataí	Trophic ecology
Cardoso et al. (2016)	Longitudinal distribution of the ichthyofauna in ...	Corumbataí	Community structure
Carmassi et al. (2012)	Composition and structure of fish assemblage ...	Corumbataí	Community structure
Cetra & Petrere (2007)	Associations between fish assemblage and ...	Corumbataí	Riparian influences
Cetra & Petrere (2006)	Fish-assemblage structure of the Corumbatai river basin ...	Corumbataí	Environmental impacts
Esteves & Alexandre (2011)	Development of an Index of Biotic Integrity Based ...	Piracicaba-Jaguari	Environmental impacts
Ferreira & Petrere (2007)	Anthropic effects on the fish community ...	Corumbataí	Environmental impacts
Ferreira et al. (2012)	Diet of *Astyanax paranae* (Characidae) in ...	Corumbataí	Trophic ecology
Ferreira et al. (2012)	Riparian coverage affects diets of characids in ...	Corumbataí	Riparian influences
Gomiero & Braga (2003)	O lambari *Astyanax altiparanae* (Characidae) ...	Corumbataí	Trophic ecology
Gomiero & Braga (2005)	The condition factor of fishes from two river basins ...	Corumbataí	Integrated biological aspects
Gomiero & Braga (2005)	Uso do grau de preferência alimentar para a ...	Corumbataí	Trophic ecology
Gomiero & Braga (2006)	Ichthyofauna diversity in a protected area in the ...	Corumbataí	Community structure
Gomiero & Braga (2007)	Reproduction of a fish assemblage in the state ...	Corumbataí	Reproduction
Lima-Junior & Goitein (2003)	Ontogenetic diet shifts of a Neotropical catfish ...	Piracicaba-Jaguari	Trophic ecology
Lima-Junior et al. (2006)	Fish assemblage structure and aquatic pollution ...	Corumbataí	Community structure
Rondineli & Braga (2009)	Population biology of *Corydoras flaveolus* ...	Corumbataí	Integrated biological aspects
Rondineli & Braga (2010)	Reproduction of the fish community of Passa Cinco stream ...	Corumbataí	Reproduction
Rondineli et al. (2009)	Population biology of *Trichomycterus* sp.	Corumbataí	Integrated biological aspects
Rondineli et al. (2011)	Diet of fishes in Passa Cinco stream, Corumbataí ...	Corumbataí	Trophic ecology
Santos et al. (2015)	Assessing the importance of riparian zone for stream ...	Atibaia and Piracicaba-Jaguari	Environmental impacts
Santos & Esteves (2015)	A Fish-Based Index of Biotic Integrity for ...	Atibaia and Piracicaba-Jaguari	Environmental impacts
Tibúrcio et al. (2016)	Landscape effects on the occurrence of ...	Corumbataí	Environmental impacts
Villares-Junior et al. (2016)	Comparative feeding ecology of four ...	Corumbataí	Trophic ecology
Yoshida & Uieda (2014)	The importance of a Biosphere Reserve of Atlantic ...	Jundiaí	Integrated biological aspects

constant species were recorded, and most were classified as accidental (76.4 %) (Table S1).

Rarefaction curves for the sampled streams showed an increase in the number of species with the number of studies, but did not reach an asymptote, both for the individual and grouped sub-basins (Figure 3). Because of the higher number of samples in the Corumbataí sub-basin, the curve leveled the pattern of the pooled samples, while the curve obtained for the other sub-basins (Atibaia, Jundiaí and Piracicaba-Jaguari) indicated a less intensive sampling effort, which resulted in approximately 58 registered species.

Considering the 67 sampled sites with the buffer of 500m in relation to the total area of the PCJ Basin, we found that only 0.35% of the basin was sampled. The main land use in the whole basin in the period from 2010 to 2020 was “Mosaic of Agriculture and Pasture”, followed by “Forests and Pasture” and then “Sugarcane” (Figure 4A). During this period, although less representative in area, soybean plantation, citrus and coffee were the cultures that most increased. Land use of the sampled sites followed the same pattern, with the exception of coffee plantations, which increased 7100% (Figure 4B).

Most of the sampling sites were located within Conservation Units (CUs) (62.6%), especially in State Environmental Protections Areas (EPA’s) such as in the Piracicaba-Juqueri Mirim Area I, within the Corumbataí sub-basin (Figure 5A). However, no studies were performed within CUs located in the eastern part of the basin (Sistema Cantareira and Piracicaba-Juqueri Mirim Area II). Although 38.8% of sites were located within high Priority Areas for Conservation in the Cerrado (Corumbataí sub-basin; Itirapina), regions considered of extremely high importance in the western part of the basin (Barra Bonita) and in the Atlantic Forest were not studied at all (Figure 5B). The distribution of sampling sites among biomes was similar, with 55.2% of them located in the Cerrado and 44.7% in the Atlantic Forest (Figure 5B).

Psalidodon fasciatus (Cuvier, 1819), *Hypostomus ancistroides* (Ihering, 1911) and *Rhamdia quelen* (Quoy & Gaimard, 1824) were the constant species registered and occurred in sympatry in most of the sites, even though *P. fasciatus* showed a wider distribution (Figure 5C). On the other hand, all the non-native species, with the exception of *Poecilia reticulata* Peters, 1859, were accidental species. They were found mainly in the Piracicaba-Jaguari sub-basin [*Trichomycterus brasiliensis* Lütken,

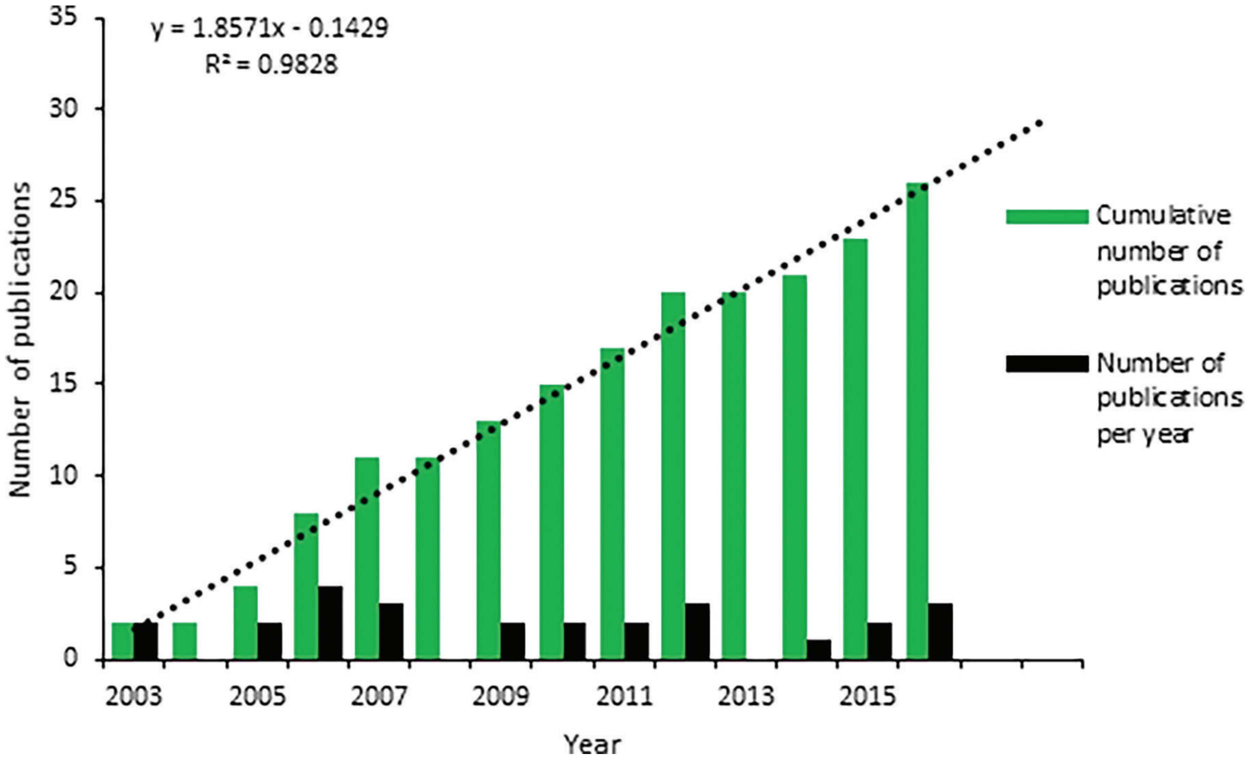

Figure 2. Number of publications per year and cumulative number of publications on stream fishes in the PCJ Basin from 2003 to 2016.

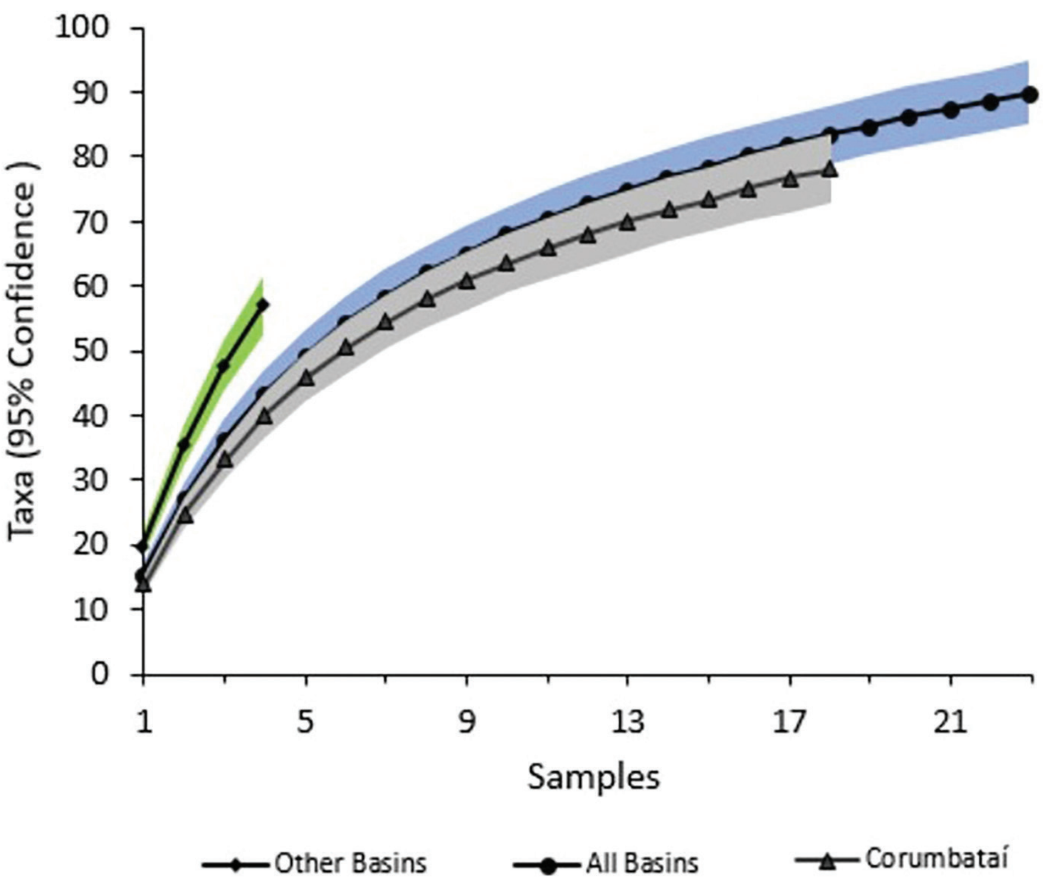

Figure 3. Sample-based rarefaction curves (Mao-Tao) for pooled sites of all studied sub-basins of the PCJ Basin, and separate curves for the Corumbataí and other sub-basins (Atibaia, Jaguari, Piracicaba and Jundiaí).

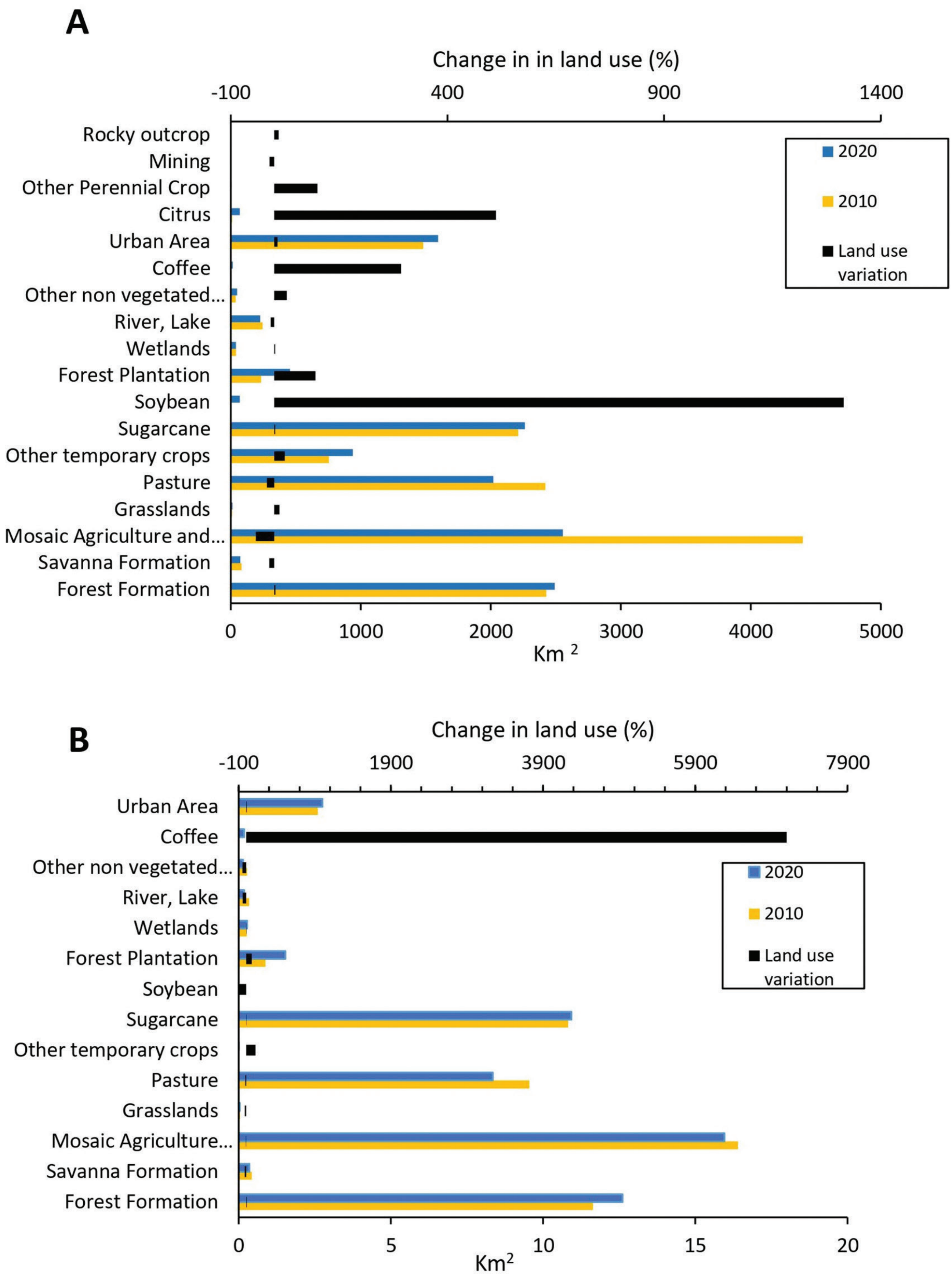

Figure 4. Land cover (km²) in the years 2010 and 2020 and changes in percentage during this period for the whole PCJ Basin (A) and considering 500 m buffers around 67 sampling sites (B).

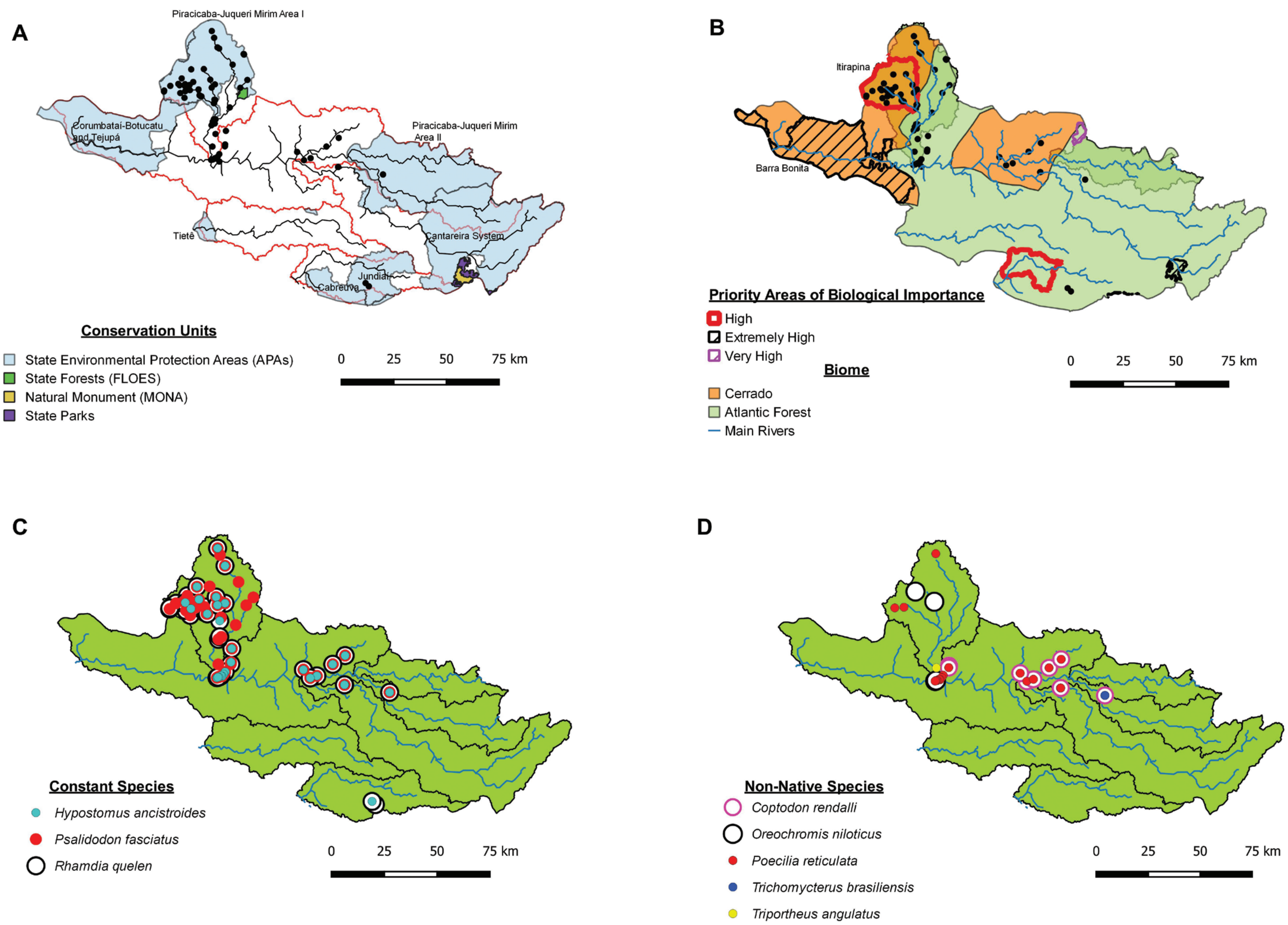

Figure 5. Distribution of 67 sampling sites in the PCJ Basin and in several Conservation Units (A); different biomes and priority Areas of Biological Importance (MMA, 2018) (B); Constant species (C) and non-native species (D). One point can represent more than one locality.

1874*, Coptodon rendalli* (Boulenger 1897) and *Oreochromis niloticus* (Linnaeus 1758)], while *Triportheus angulatus* (Spix & Agassiz 1829) was registered only in the Corumbataí sub-basin (Figure 5D).

Discussion

The present survey showed that studies related to biological and ecological aspects of streams fishes in the PCJ Basin have been carried out during the last 20 years, following the general trends verified for stream fish studies in Brazil (Dias et al. 2016). This increase from the 1990s onwards occurred as a result of the adaptation of specific collection methods for streams, such as electric fishing, as well as the influence of publications that highlighted the importance of stream fishes as a source of diversity in Brazil (Caramaschi et al. 1990). Also, the need for studies on freshwater fish species has been increasing because several assessments suggest that >30% of them are threatened (IUCN 2006).

Nevertheless, the increase in publications in the PCJ Basin has been slow and unevenly distributed among the different sub-basins, focusing mainly on the Corumbataí and Piracicaba-Jaguari sub-basins. This pattern may be related to the proximity of freshwater ecology teams operating from local research centers, as, for example the Instituto de Biociências, Universidade Estadual Paulista, located in Rio Claro, and the University of São Paulo, in the city of Piracicaba. In fact, the close relationship between the presence of universities and number of articles has been verified in other surveys on Brazilian freshwater fishes (Azevedo et al. 2010; Dias et al. 2016; Junqueira et al. 2020), all stressing that more financial and human resources as well as research facilities, have been determinant for the increase in ichthyological studies.

One of the main topic of study involved environmental impacts in particular relation to major problems of the Corumbataí sub-basin. Specifically, this basin suffers significant negative environmental impacts owing to the intensive exploitation of monocultures, especially those associated with the cultivation of sugarcane (Monteiro et al. 2008). Additionally, discharge of industrial and domestic effluents has been frequently reported, especially for the period between 1999 and 2002 (Fischer 2003). A similar situation occurs in the Piracicaba sub-basin, one of the most urbanized watersheds of the state (Comitês PCJ/Agência das Bacias PCJ 2020). Other topics such as diet, reproduction and integrated biological aspects, reflect the need to explore unknown aspects of the life-history of many species, which are fundamental at local scales, helping conservation planning.

Despite the concentration of studies in one sub-basin and the low total sampled area of the PCJ Basin (0.35%), the species database indicated that the number of species registered (89) represents 28.7% of the fish species recorded by Langeani et al. (2007) for the Upper Paraná River Basin, and 34.2% of the species for this basin in the State of São Paulo (Oyakawa & Menezes 2011). The rarefaction curves showed that sampling effort was low, even when considering all sub-basins, suggesting that stream fish richness in the PCJ Basin is considerably higher than the actual numbers. These results are similar to those for fish fauna from the Upper Paraná, which, despite having one of the most studied ichthyofaunas, show species curves that lack any stabilizing trend (Langeani et al. 2007). Thus, we recommend that the less studied areas of the PCJ Basin such as the Atibaia, Camanducaia and Jundiaí sub-basins should be more intensively sampled, especially because of their high proportion of Conservation Units, reaching 96.3% in the Camanducaia sub-basin, followed by the Jaguari (66.5%), Jundiaí (61.7%) and Atibaia (60.5%) sub-basins (Comitês PCJ/Agência das Bacias PCJ 2020).

Land use in the PCJ Basin is strongly influenced by agriculture and pasture, followed by sugarcane and forest formations, a situation also observed for the sampled sites where some crops increased along the period from 2010 to 2020. Thus, impacts on streams are expected since these activities may affect water quality, biodiversity, sedimentation and nutrient levels (Corbi et al. 2006; Riseng et al. 2011). Usually, opportunist fishes become dominant under degraded conditions by the reduction or disappearance of sensitive and specialist species (Clarke & Warwick 1994). This may be the case of the species with wide-range distributions in the basin as *Psalidodon fasciatus*, *R. quelen* and *H. ancistroides*, all of them considered tolerant species (*sensu* Karr 1981) (Alexandre et al. 2010). Nevertheless, the first two species have a wide distribution in Central and South America, while *H. ancistroides* is limited to the Upper Paraná and Tietê River Basins (Buckup et al. 2007).

The number of non-native species was low, a situation which may be related to the fact that a great proportion of the sampling sites were located within State Environmental Protection Areas (EPAs). However, a certain degree of human occupation is allowed within the EPAs, which aim to the conservation of natural processes and biodiversity, adapting the various human activities to the environmental characteristics of the area (Fundação Florestal 2022). Nevertheless, the frequency of non-native species was much lower than that documented for the Upper Paraná Basin (21.6% allochthonous and 2.6% exotic species) by Langeani et al. (2007). This difference could be explained by the habitat type considered in their study, which comprised a variety of ecosystems, including species used for food consumption, fish farming, sport fishing or as baits.

Another important aspect is that 3,700 km^2 of Priority Areas for Conservation (PAC) occur in the PCJ Basin (Comitês PCJ/Agência das Bacias PCJ 2020), but only the Corumbataí sub-basin was sampled. PACs cover areas that should be protecting biological richness, endemisms, various phytophysiognomies and ecosystem services where the existing management instruments are not enough to ensure their conservation (MMA 2018). Nevertheless, the planning of these areas is usually based on terrestrial ecosystems using phytogeographic data based on geomorphology, vegetation, soils and altitude (MMA 2007), which have limited advantages for freshwater species, as pointed out by Leal et al. (2020). According to these authors, when freshwater species are prioritized, more terrestrial species benefit than in the reverse, suggesting that a terrestrial-freshwater conservation approach is recommended.

Incorporating data on freshwater stream fishes into the planning of conservation areas may bring promising approaches for stream fish conservation, as conservation planning relies fundamentally on spatial information about the distribution of biodiversity which is still very limited (Margules & Pressey 2000). Besides recommending further studies in the several sub-basins of the PCJ Basin, new approaches can be used to support the establishment of public policies aimed at the conservation and restoration of the remaining biodiversity. These may include studies aimed to test hypotheses related to ecological concepts and theories, such as landscape ecology, macroecology, macroevolution and climatic changes. Finally, substantial freshwater gains in the PCJ Basin could also benefit from the planning of conservation areas based on integrated terrestrial-freshwater approaches, because of maximum achievable benefits related to this approach.

Supplementary Material

The following online material is available for this article:

Table S1 – List of fish species registered in streams of the Piracicaba, Capivari and Jundiaí River Basins (PCJ), SP, indicating their origin (N – Native; NN – Non Native), Conservation Status (LC – Least Concern; DD – Data Deficient; VU – Vulnerable), and Constancy of Occurrence according to Dajoz (1972). Taxonomic classification based on Fricke et al. (2021).

Acknowledgments

We are grateful to the Instituto de Pesca/APTA/SAA for providing access to the databases and one anonymous reviewer for valuable suggestions on this manuscript. The first author received a Scientific Initiation grant (PIBIC/CNPq) (award number nº 122333/2021 – 7).

Author Contributions

Alexia Almeida Ferraz da Silva: contributed to data collection, data analysis and interpretation and manuscript preparation.

Katharina Eichbaum Esteves: contributed to the concept and design of the study, project supervision, data analysis and manuscript preparation, adding intellectual content.

Conflicts of Interest

The authors declare that they have no conflict of interest related to the publication of this manuscript.

Data Availability

Supporting data are available at <https://doi.org/10.48331/scielodata.ROG2NF>.

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Received: 08/11/2022
Accepted: 12/05/2023
Published online: 09/06/2023

Biota Neotropica 23(2): e20221454, 2023
www.scielo.br/bn

biotaneotropica
ISSN 1676-0611 (online edition)
Article

March or Die: road-killed herpetofauna along BR-040 highway, an ancient road on the Atlantic Forest from Southeastern Brazil

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GOMES, D.F., BUENO, C., PINNA, P.H., WOITOVICZ-CARDOSO, M., PASSOS, P. **March or Die: road-killed herpetofauna along BR-040 highway, an ancient road on the Atlantic Forest from Southeastern Brazil**. Biota Neotropica 23(2): e20221454. https://doi.org/10.1590/1676-0611-BN-2022-1454

Abstract: The construction of highways is responsible for access to previously protected areas, resulting in changes in landscape and dynamics of the animal populations that live in these areas. These enterprises are the major responsible for the mortality of wild animals, surpassing hunting and even the trafficking of animals. The objective of this study was to make a list that reflects the diversity of amphibians and reptile's road-killed along the BR-040, a highway that crosses the threaten lowland Atlantic Forest in Southeastern region of Brazil, including the use of microhabitats, lifestyle, activity pattern, reproductive cycles, and possible rare or endangered species. The study area consists of 180,4 km of highways. Monitoring began in 2006 and continues to the present day. A total of 1,410 individuals from 60 species were recorded in this study. The reptiles were more frequent in number of individuals and species. The commonest species recorded were *Crotalus durissus* and *Dipsas mikanii*. We have registered a single endangered species: *Ranacephala hogei*. The highest rates of road-kill were recorded during the wet season. Road-kills of fauna is a major threat to species, studies are of great importance to define plans that seek to mitigate the effects generated by these enterprises.
Keywords: *Animal-vehicle collision; Roads; Road-kill mitigation; Road ecology; Reptiles; Amphibians.*

Marche ou Morra: herpetofauna atropelada ao longo da rodovia BR-040, uma antiga estrada na Mata Atlântica do Sudeste do Brasil

Resumo: A construção de rodovias é responsável pelo acesso a áreas anteriormente protegidas, resultando em alterações na paisagem e na dinâmica das populações animais que vivem nessas áreas. Esses empreendimentos são os maiores responsáveis pela mortalidade de animais silvestres, superando a caça e até mesmo o tráfico de animais. O objetivo deste estudo foi realizar uma lista que reflita a diversidade de anfíbios e répteis atropelados ao longo da BR-040, uma rodovia que atravessa a ameaçada Mata Atlântica na região Sudeste do Brasil, incluindo o uso de microhabitats, estilo de vida, padrão de atividade, ciclos reprodutivos, e possíveis espécies raras ou ameaçadas. A área de estudo é constituída por 180,4 km de rodovias. O monitoramento começou em 2006 e segue até os dias atuais. Ao todo 1.410 indivíduos de 60 espécies foram registrados nesse estudo. Os répteis foram mais frequentes, em número de indivíduos e espécies. As espécies mais comumente registradas foram *Crotalus durissus* e *Dipsas mikanii*. Registramos uma espécie ameaçada de extinção: *Ranacephala hogei*. A maior taxa de atropelamento foi registrada durante a estação chuvosa. O atropelamento de fauna é uma grande ameaça as espécies, sendo de grande importância estudos para definição de planos que busquem mitigar os efeitos gerados por esses empreendimentos.
Palavras-chave: *Colisão animal-veículo; Rodovias; Mitigação de atropelamentos; Ecologia de estradas; Répteis; Anfíbios.*

Introduction

As a rule, roads make a major contribution to the high levels of biodiversity loss around the world (Coffin, 2007; Jochimsen et al., 2014; Van der Ree et al., 2015), being road-kills one of the main causes of direct death of wild vertebrate species, overcoming the impacts generated by hunting and mortality rates from natural causes (Seiler & Helldin, 2006; Valadão et al., 2018; Hill et al., 2019). Every second 15 wild animals die on Brazilian roads, and these numbers can reach 1.3 million per day and exceed 475 million per year with extrapolated data

(CBEEE, 2022). However, the actual number may be even higher, since several deaths are not recorded and road impacts go beyond collisions between wild animals and vehicles (Casella et al., 2006; Van der Ree et al., 2015; Boyle et al., 2019).

Due to the great urgency and growth of the road network around the world, road-kills and other direct and indirect road impacts have received attention in many studies (Bager & Rosa, 2010; Almeida, 2013; Vélez, 2014). Among the various threats to wildlife are, in addition to direct collisions with vehicles, the increase in the level of air and noise pollution, the rise in temperature and the emergence of urban agglomerations on the roadside (Gomes et al., 2007; Bager & Rosa, 2010; Vélez, 2014; Shannon et al., 2016). To complicate matters, roads potentially reduce the size of natural populations, affecting their long-term persistence (Fahrig et al., 2003; Bueno et al., 2013; Gonçalves et al., 2018), by separating habitats by reducing their size, configuration and quality (McKinney, 2002; Fahrig, 2003; McKinney, 2006; Maynard et al., 2016), acting as barriers to dispersion (Parris & Schneider, 2008, Ware et al., 2015), limiting gene flow (Ascensão et al., 2017). Additionally, roads affect individual survival, and provide humans with easy access to previously difficult-to-reach areas, thereby increasing the negative pressure on wildlife (Laurance et al., 2009).

Nonetheless, wildlife road-kill's do not occur randomly (Sosa & Schalk, 2016; Filius et al., 2020). Several factors favor certain species to be road-killed more than others, such as biological characteristics (body size and diet; Barthelmess & Brooks, 2010), the characteristics of the landscape and the road itself (Bueno et al., 2013, 2015), as well as seasonal variations in temperature and rainfall (Bueno & Almeida, 2010; Santana, 2012; Santos & Carvalho, 2012). The vehicular traffic, as well as the vehicles' speed are also important determining characteristics that lead to the collision of vehicles with animals (Cunha et al., 2010; Lester, 2015). Various taxonomic groups are affected distinctly by vehicle collisions around the world, including mammals (Grilo et al., 2020; Hill et al., 2021; Navas-Suárez et al., 2022), birds (Bujoczek et al., 2011; Rosa & Bager, 2015 Grilo et al., 2020; Medrano-Vizcaíno et al., 2022), reptiles (Aresco, 2005; Shepard et al., 2008a; Hallisey et al., 2022), amphibians (Fahrig et al., 2003; Hels & Buchwald, 2001; Glista et al., 2008; Hallisey et al., 2022) and invertebrates (Seibert & Conover, 1991; McKenna et al., 2001). Ectotherms (amphibians and reptiles), despite being underrepresented in the literature on road ecology (Guns et al., 2011; Popp & Boyle, 2017), had a higher probability to be road-killed (D'Amico et al., 2015). This is probably because their metabolism causes slowness in amphibians (Hels & Buchwald, 2001; Puky, 2005), the behavioral freezing responses to threats (Andrews et al., 2005; Lima et al. 2015) and, mainly, due to characteristic basking behavior for reptile's thermoregulation (Ashley & Robinson, 1996; Tanner & Perry, 2007; Jochimsen et al. 2014; Andrews et al. 2015; D'Amico et al. 2015; Schalk & Saenz, 2016). In addition, there are other more intricate reasons, as such the cultural aversion to reptilian Bauplan in the Western civilization, mostly in the case of snakes and other limbless Squamata (Davey, 1994; Fernandes-Ferreira et al., 2011; Ceríaco, 2012; Castilla et al., 2020; Silva et al., 2021). Amphibians and reptiles are vulnerable to road-kills when they travel on roads that cross their area of origin, or when they are attracted by the resources available in the area surrounding road edges, often because they are not seen by drivers (Laurance et al., 2009; Bueno & Almeida, 2010; Carvalho et al., 2015). Sometimes, however, when stigmatized animals are in sight of drivers on the road, some swerve the vehicle ever so slightly to run over them or simply do not try to swerve the vehicle to avoid hitting them (Ashley et al., 2007; Beckmann & Shine, 2012; Mesquita et al., 2014; Secco et al., 2014; Assis et al., 2020).

There is global concern about the road-kill threats in animal conservation (Freitas, 2015; Adárraga-Caballero & Gutiérrez-Moreno, 2019; Jarvis et al., 2019, Grilo et al., 2021). Although road ecology is a recent topic of interest in temperate and tropical regions (Rosa & Bager, 2013; Pereira et al., 2017), especially in the New World, the visibility of this theme has increased rapidly with the public becoming aware of its relevance to the protections of wild animal populations (Attademo et al., 2011). Nevertheless, there is still a paucity of accurate information on the spatial and temporal distribution of road-kill's. Understanding the dynamics of wildlife-vehicle collisions allows us to find alternative solutions to increase safety on the roads, reduce the impacts on humans and wildlife, reduce costs, and invest in mitigation measures aimed at conservation of biodiversity (Forman, 1998; Czech et al., 2000; Rytwinski et al., 2016; Abra et al., 2019; Ascensão et al., 2021; Silva et al., 2021). The objective of this study was to make a list of the diversity of amphibians and reptiles' road-killed along the BR-040, a highway that crosses the threaten Atlantic Forest in the Southeastern region of Brazil, including information on the use of microhabitats, lifestyle, activity pattern, reproductive cycles, and possibly rare or endangered species.

Material and Methods

1. Study area and source of data

The database used in the study come from the monitoring of the fauna road-killed along a 180 km stretch on the BR-040 (from km 125.2 in the municipality of Duque de Caxias, state of Rio de Janeiro to km 773.5 in the municipality of Juiz de Fora, state of Minas Gerais) (Figure 1). The project "Caminhos da Fauna" (free translation, Wildlife Pathways) started in 2006, is still in progress, and comprises the pioneering study in the monitoring of road-killed animals in the state

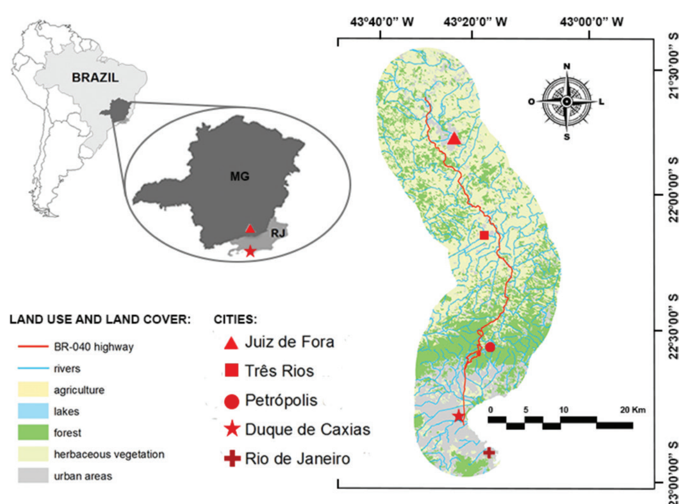

Figure 1. Land cover map for the selected area of surroundings from the BR-040 highway stretches where road-kill were recorded, from km 125.2 in the municipality of Duque de Caxias (red star), state of Rio de Janeiro, to the Km 773.5 in the municipality of Juiz de Fora (red triangle), state of Minas Gerais.

of Rio de Janeiro. In the present study, we analyzed the records from April 2006 to June 2022, comprising both the specimens discarded after identification at the lowest taxonomic level possible and those preserved for scientific purposes and deposited in the Amphibians and Reptiles collections of the Museu Nacional, Universidade Federal do Rio de Janeiro (MNRJ). These specimens comprise an important source of data in the amphibians and reptiles collections. The list of deposited specimens is in the appendix and the institutional abbreviation followed is as detailed in Sabaj (2020).

The area of study crosses the Biodiversity Corridor of the Serra do Mar National Park, whose main native vegetation cover is composed of tropical rain forest (Veloso et al., 1991). The topography varies from the lowlands in the municipality of Duque de Caxias (22°90’46"S, 43°18'43"W; 19 m above sea level, hereafter asl), through the mountain range (about 1,000 m asl) near the municipality of Petrópolis (22°30'18"S, 43°10'44"W; 838 m asl), up to the municipality of Juiz de Fora (21°41'20"S, 43°20'40"W; 715 m asl) (Figure 2). Since 1996, the BR-040 stretch from Rio de Janeiro to Juiz de Fora has been under the authority of a private company, CONCER. The mean traffic volume on this road is 37,000 vehicles/day (CONCER, 2020). Within this entire range, the road has 2 paved lanes in each direction, and for the stretch crossing the mountain range, the 2-lanes going up and the 2-lanes going down run separately.

2. *Sampling design*

The collection protocol is based on standard forms and techniques developed for the project Caminhos da Fauna, which includes taking pictures, removing carcasses, storing them in freezers, and recording their location, date and time of collection. The project has promoted the installation of three freezers located at the 104 km, 45 km and 816 km marks of BR-040 highway to provide a better preservation of the collected carcasses. Twice a month, the carcasses accumulated in the freezers were taken to the laboratory, at Veiga de Almeida University, in the municipality of Rio de Janeiro. After that, the specimens were donated to MNRJ, where they were defrosted, weighted and measured (in the case of slightly damaged animals) and sampled for genetic material (muscle tissue was taken from most of reptiles specimens and for selected amphibians specimens). Carcass collections are carried out in partnership with the CONCER concessionaire throughout the week for 24 hours. The monitoring speed is 50 km/h which allows a best visualization of the road-killed animals (small reptiles and amphibians) along the entire highway. The data were converted to road-kill rate (number of individuals/km/day). For each record, a field form is filled out with: mileage, direction, location on the road, sex, taxonomic group of the road-killed animal, local speed limit, weather for the day of collection, presence of water nearby, surrounding vegetation, in addition all occurrences were georeferenced and made available in decimal degrees decimals. Unidentified species at least at gender level were not considered for further analyses.

Carcass collections are included in the SISBIO License Number: 30727-9. The animal carcasses used in this study meet and are in accordance with operation license No. 1187/2013 and authorization for capture, collection and transport of biological material - Abio (1st Renewal and 3rd Rectifier) 514/2014.

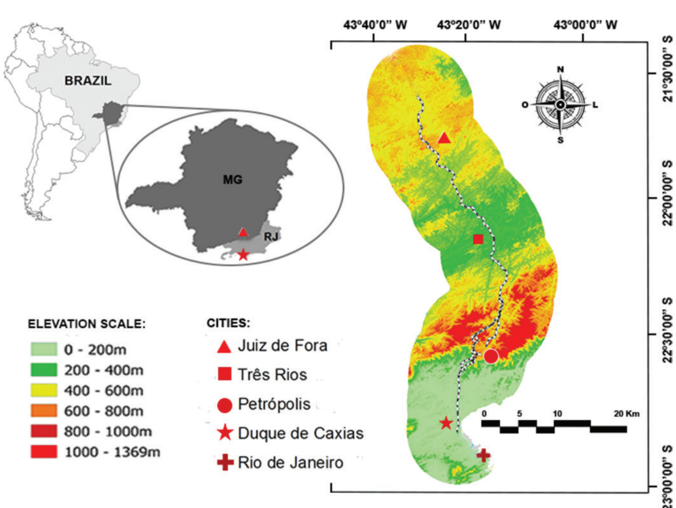

Figure 2. Elevation map for the selected area of surroundings from the BR-040 highway stretches where road-kill were recorded, from km 125.2 in the municipality of Duque de Caxias (red star), state of Rio de Janeiro, to the Km 773.5 in the municipality of Juiz de Fora (red triangle), state of Minas Gerais.

3. *Species identification*

The collected carcasses were identified by experts at the lowest taxonomic level possible using the relevant taxonomic literature, as well as by means of direct comparison with the specimens from the MNRJ collections of Amphibians and Reptiles. After identification, they are fixed in formalin solution and preserved in ethanol 70ºGL and incorporated to the respective collection. The photographs aided in the taxonomic identification, but they were not considered alone for the species identification. Data regarding activity patterns, foraging, microhabitat selection and reproductive cycles were based on the available literature for each taxonomic group (e.g., Haddad et al., 2013; Marques et al., 2019) and are summarized in Tables 1–2, indicating the specific source of each natural history information.

Results

We recorded 1,411 road-killed individuals, being 934 reptiles (66.19%) of 45 species and 478 amphibians (33.81%) of 15 species (Figures 3–10). However, due to the poor morphological condition of some specimens, it was not possible to identify them to the level of species. In these cases, specimens were identified up to the generic level (records for 41 reptiles and 46 amphibians) or remained without identification (17 amphibians and 12 reptiles) (Tables 1–2).

Considering only road-killed reptiles, Serpentes was the most recorded group, corresponding to 72.91% (N = 681) of the entire sampling, followed by lizards with 24.19% (N = 226), Amphisbaenia 0.96% (N = 9), Testudines 0.42% (N = 4), and Crocodylia 0.32% (N = 3); without identification 1.20% (N = 12).

The most representative of the snakes (N = 681) were the Rattlesnake, *Crotalus durissus* Linnaeus, 1758 corresponding to 19.23% (N = 130) followed by the Neuwied’s Tree Snake, *Dipsas neuwiedi* (Ihering, 1911) (11.45%; N = 78), and the Lancehead, *Bothrops jararaca* (Wied-Neuwied, 1824) (7.48%; N = 51). These three species together correspond to 38.16% of the sample for the snakes group.

The most commonly found lizards were the White Tegu, *Salvator merianae* Duméril & Bibron, 1839 (45.13%; N = 102) and the

Table 1. Complete list of road-killed amphibians on highway BR-040. **Abbreviations:** N = Sample number; No = Nocturnal; Di = Diurnal; Arb = Arboreal; Cry = Cryptozoic; Ter = Terrestrial; Art = Arthropods; Mol = Mollusks; Anu = Anura; S = Small; M = Medium; L = Large; REP. MODE = Reproductive Mode; LC = Least Concern; DD = Data Deficient; **REP. MODE:** (1) Direct development of terrestrial eggs; (2) Eggs and exotrophic tadpoles in still or running water; (3) Eggs on wet rocks, rock crevices, exotrophic semi-terrestrial tadpoles; (4) Eggs and exotrophic tadpoles in still water or eggs and early larval stages in natural or constructed basin; (5) Eggs and exotrophic tadpoles in running water or eggs and early larval stages in natural or constructed basin; (6) Eggs and exotrophic tadpoles in still water; (7) Foam nest with eggs and early larval stages in underground constructed chamber; (8) Foam nest floating on still water; **HABITS:** (1) Forest floor; (2) Swamp or pond; (3) Rock wall; (4) River or stream backwaters.

Taxa	N	Activity	Habit	Diet	REP. MODE	Calling site	Size	ICMBio	IUCN
CLASS AMPHIBIA									
ORDER ANURA									
without identification	20								
BRACHYCEPHALIDAE									
Ischnocnema guentheri (Steindachner, 1864)	3	No.	Arb./Cry.	Art./Mol.	1	1	S	LC	LC
BUFONIDAE									
Rhinella diptycha (Cope, 1862)	1	No.	Ter.	Art.	2	2 or 4	L	LC	DD
Rhinella icterica (Spix, 1824)	157	No.	Ter.	Art.	2	2 or 4	L	LC	LC
Rhinella ornata (Spix, 1824)	10	No.	Ter.	Art.	2	2 or 4	M	LC	LC
Rhinella sp.	35								
CYCLORAMPHIDAE									
Thoropa miliaris (Spix, 1824)	21	No.	Ter.	Art./Mol.	3	3	S	LC	LC
CRAUGASTORIDAE									
Haddadus binotatus (Spix, 1824)	2	No.	Cry.	Art.	1	1	S	LC	LC
HYLIDAE									
Boana faber (Wied-Neuwied, 1821)	20	No.	Arb.	Art.	4	2	M	LC	LC
Boana semilineata (Spix, 1824)	1	No.	Arb.	Art./Mol./ Anu.	2	2 or 4	M	LC	LC
Bokermannohyla circumdata (Cope, 1871)	4	No.	Arb.	Art./Mol.	5	2	S	LC	LC
Dendropsophus elegans (Wied-Neuwied, 1824)	2	No.	Arb.	Art./Mol.	6	2	S	LC	LC
Scinax eurydice (Bokermann, 1968)	4	No.	Arb.	Art./Mol.	6	2	M	LC	LC
Trachycephalus mesophaeus (Hensel, 1867)	1	No./Di.	Arb.	Art./Mol.	6	2	M	LC	LC
LEPTODACTYLIDAE									
Leptodactylus fuscus (Schneider, 1799)	2	No.	Ter.	Art./Mol.	7	2	M	LC	LC
Leptodactylus gr. latrans (Steffen, 1815)	4	No.	Ter.	Art./Mol.	8	2	M	LC	LC
Leptodactylus labyrinthicus (Spix, 1824)	15	No.	Ter.	Art./Mol.	8	2	L	LC	LC
Leptodactylus latrans (Steffen, 1815)	168	No.	Ter.	Art./Mol.	8	2	M	LC	LC
Leptodactylus sp.	11								

Amazon Lava Lizard, *Tropidurus torquatus* (Wied-Neuwied, 1820) (32.30%; N = 73). Together, these two species correspond to 77.43% of the sample for lizards.

The most frequently road-killed species of amphibians' was the Butter Frog, *Leptodactylus latrans* (Steffen, 1815) (N = 168; 35.14%), followed by the Yellow Cururu Toad, *Rhinella icterica* (Spix, 1824) (N = 157; 32.84%), and Military River Frog, *Thoropa miliaris* (Spix, 1824) (N = 21; 4.39%). These three species together correspond to 72.37% of the amphibian sample.

During the more than 15 years of sampling (2006 to 2022), the year with the highest number of records was 2014, with 462 road-kills of wildlife (32.77%), followed by 2015 (N = 301; 21.35%) and 2016 (N = 195; 13.83%). In 2014, the highest rate of accidents occurred in the rainy season, in the months of October (N = 49; 10.60%), November (N = 48; 10.39%), December (N = 45; 9.74%), January (N = 76; 16.45%), February (N = 65; 14.07%) and March (N = 59; 12.77%). These six months together corresponded to 74.02% of the trampling of wildlife of the year sampled.

Table 2. Complete list of road-killed reptiles on highway BR-040. **Abbreviations:** N = Sample number; No = Nocturnal; D = Diurnal; A = Arboreal; C = Cryptozoic; T = Terrestrial; Fo = Fossorial; Aq = Aquatic; Sa = Saxicola; M = Mammals; B = Birds; Ar = Arthropods; Mol = Mollusks; An = Anura; F = Fish; L = Lizard; E = Earthworm; Sn = Snake; G = Generalist; PMa = Plant material; Sm = Small; M = Medium; L = Large; REP. MODE = Reproductive Mode; S = Seasonal; C = Continuous; Bo = Both; V = Viviparous; O = Oviparous; D = Dry; R = Rainy LC = Least Concern; DD = Data Deficient; CR = Critically Endangered.

Taxa	N	Activity	Habit	Diet	REP. MODE	Reproduction	Season	Size	ICMBio	IUCN
CLASS REPTILIA										
WITHOUT IDENTIFICATION	58									
ORDER SQUAMATA										
SERPENTES										
BOIDAE										
Boa constrictor Linnaeus, 1758	1	No/D	T/A	M/B	V	S	D	L	LC	LC
Corallus hortulana (Linnaeus, 1758)	4	No	A	M/B	V	S	R	L	LC	LC
COLUBRIDAE										
Chironius bicarinatus (WIED-NEUWIED, 1820)	26	D	T/A	An	O	S	R	L	LC	LC
Chironius exoletus (LINNAEUS, 1758)	1	D	T/A	An	O	S	R	M	LC	LC
Chironius fuscus (LINNAEUS, 1758)	12	D	T/A	An	O	C	Bo	M	LC	LC
Chironius laevicollis (WIED-NEUWIED, 1824)	8	D	T/A	An	O	S	R	L	LC	LC
Chironius sp.	7									
Leptophis ahaetulla (LINNAEUS, 1758)	5	D	T/A	An/L	O	S	D	M	LC	LC
Spilotes sulphureus (WAGLER, 1824)	21	D	T/A	M/B	O	S	R	L	LC	LC
Spilotes pullatus (LINNAEUS, 1758)	22	D	T/A	M/B	O	S	R	L	LC	LC
DIPSADIDAE										
Atractus zebrinus (JAN, 1862)	21	No	C/Fo	E	O	S	R	Sm	LC	LC
Dipsas sp.	1									
Dipsas mikanii SCHLEGEL, 1837	2	No	T	Mo	O	S	R	Sm	LC	LC
Dipsas neuwiedi (IHERING, 1911)	78	No	T/A	Mo	O	S	R	Sm	LC	LC
Elapomorphus quinquelineatus (RADDI, 1820)	14	D	C	Sn	O	S	Dry	M	LC	LC
Erythrolamprus aesculapii (LINNAEUS, 1758)	8	D	T	Sn	O	C	Bo	M	LC	LC
Erythrolamprus miliaris (LINNAEUS, 1758)	15	No/D	T/Aq	F/An	O	S	R	M	LC	LC
Erythrolamprus poecilogyrus (WIED-NEUWIED, 1824)	7	No/D	T	An	O	C	Bo	M	LC	LC
Erythrolamprus reginae (LINNAEUS, 1758)	1	D	T/Aq	F/ An/L	O	C	Bo	Sm	LC	LC
Helicops carinicaudus (WIED-NEUWIED, 1824)	2	No/D	T/Aq	F/An	V	S	R	M	LC	LC
Hydrodynastes sp.	2									
Leptodeira annulata (LINNAEUS, 1758)	4	No	T/A	An	O	S	D	Sm	LC	LC
Oxyrhopus clathratus DUMÉRIL, BIBRON & DUMÉRIL, 1854	47	No	T	Sn/L	O	S	R	Sm	LC	LC
Oxyrhopus petolarius (LINNAEUS, 1758)	42	No	T	M/L	O	S	R	M	LC	LC
Philodryas olfersii (LICHTENSTEIN, 1823)	14	D	T/A	M/An	O	S	R	M	LC	LC
Pseudablades patagoniensis (GIRARD, 1858)	44	D	T	An/L	O	S	R	M	LC	LC
Pseudoboa nigra (DUMÉRIL, BIBRON & DUMÉRIL, 1854)	2	No	T	L	O	C	Bo	L	LC	LC
Siphlophis compressus (DAUDIN, 1803)	20	No	T/A	L	O	S	R	M	LC	LC
Dibernardia affinis (GÜNTHER, 1858)	2	D	T/C	An/L	O	S	R	Sm	LC	LC

Continue...

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Taxa	N	Activity	Habit	Diet	REP. MODE	Reproduction	Season	Size	ICMBio	IUCN
Thamnodynastes sp.	1									
Tropidodryas sp.	4									
Xenodon neuwiedi GÜNTHER, 1863	5	D	T	An	O	C	Bo	M	LC	LC
ELAPIDAE										
Micrurus corallinus (MERREM, 1820)	3	D	C	Sn	O	S	R	M	LC	LC
VIPERIDAE										
Bothrops jararacussu LACERDA, 1884	8	No/D	T	M	V	S	R	L	LC	LC
Bothrops jararaca (WIED-NEUWIED, 1824)	51	No	T/A	M	V	S	R	M	LC	LC
Crotalus durissus LINNAEUS, 1758	128	No	T	M	V	S	D	M	LC	LC
SAURIA										
DIPLOGLOSSIDAE										
Ophiodes cf. fragilis (RADDI, 1820)	5	Di.	C	Ar/Mo	V	S	R	M	LC	DD
Ophiodes fragilis (RADDI, 1820)	7	D	C	Ar/Mo	V	S	R	M	LC	DD
Ophiodes sp.	25							M	LC	DD
Ophiodes striatus (SPIX, 1824)	12	D	C	Ar/Mo	V	S	R	M	LC	DD
SCINCIDAE										
Mabuya dorsivittata (COPE, 1862)	2	D	Sa	Ar	V	S	R	Sm	LC	LC
TEIIDAE										
Salvator marianae DUMÉRIL & BIBRON, 1839	102	D	T	G	O	S	R	L	LC	LC
TROPIDURIDAE										
Tropidurus torquatus (WIED-NEUWIED, 1820)	73	D	T	Ar/Mo	O	S	D	S	LC	LC
AMPHISBAENA										
AMPHISBAENIDAE										
Amphisbaena alba LINNAEUS, 1758	4	D	Fo	Ar	O	S	R	L	LC	LC
Leposternon microcephalum WAGLER, 1824	5	D	Fo	Ar	O	S	R	Sm	LC	LC
CROCODYLIA										
ALLIGATORIDAE										
Caiman latirostris (DAUDIN, 1801)	3	D	T/Aq	G	O	S	R	M	LC	LC
TESTUDINE										
CHELIDAE										
Mesoclemmys hogei (MERTENS, 1967)	1	D	T/Aq	Ar/Mo/F/An	O	S	R	S	CR	CR
Phrynops geoffroanus (SCHWEIGGER, 1812)	1	D	T/Aq	Ar/Mo/F/An	O	S	D	M	LC	NE
TESTUDINIDAE										
Chelonoidis carbonarius (SPIX, 1824)	2	D	T	PMa	O	S	R	M	LC	NE

The road-kill rate for the stretch of highway studied was 0.04 road-kill's per kilometer per month. It was possible to observe a higher number of road-kills in the stretches where the speed limit is higher. The three stretches with the highest incidence of road-kills have speed limits of 110 km/h (N = 698; 49.50), 70 km/h (N = 533; 37.80%), and 90 km/h (N = 145; 10.28%). The remaining road-kills (2.42%) occurred on stretches with speed limit between 30 and 60 km/h. The higher number of road-kills occurred on two-lane and one-lane stretches of the highway. Together, these stretches corresponded to 96.74% of all road-kills. The sections with three and four lanes had respectively 36 (2.55%) and 10 (0.71%) records of road-kills. The number of tracks showed that the stretches with 2 (N = 508; 36.03%) and 4 (N = 812; 57.59%) lanes were the most susceptible to trampling, together corresponding to 93.62% of the road-kill events.

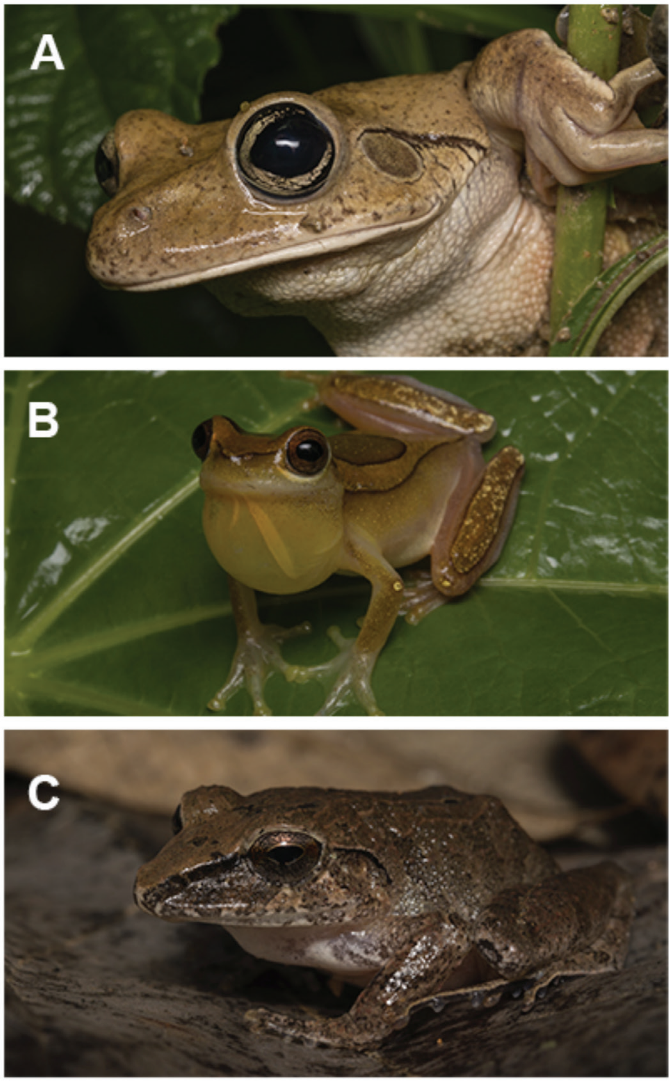

Figure 3. Road-kill species registered in the monitored stretch. A – *Boana faber*, municipality of Bandeira, state of Minas Gerais, Brazil; B – *Dendropsophus elegans*, municipality of Bandeira, state of Minas Gerais, Brazil; and C – *Haddadus binotatus*, municipality of Bandeira, state of Minas Gerais, Brazil. Photos by Teles, A.

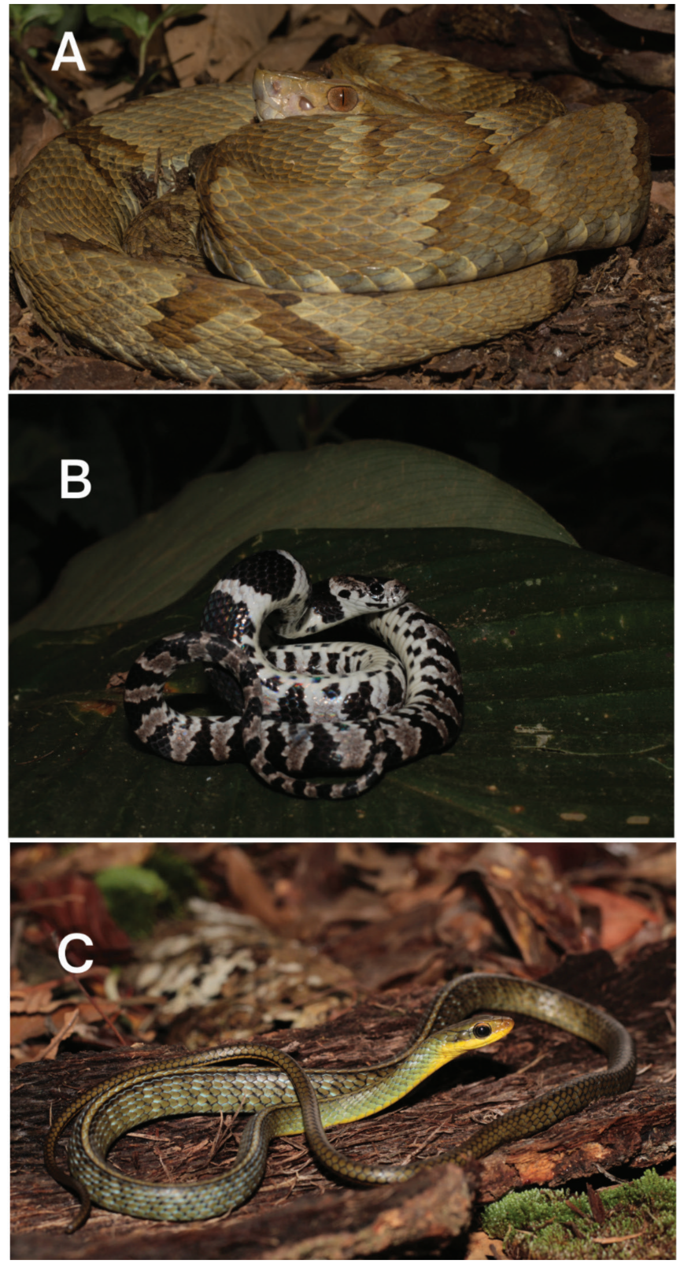

Figure 4. Road-kill species registered in the monitored stretch. A – *Bothrops jararaca,* municipality of Bandeira, state of Minas Gerais, Brazil; B – *Dipsas mikanii,* Serra do Caraça, state of Minas Gerais; and C – Brazil *Chironius bicarinatus,* Serra do Caraça, state of Minas Gerais, Brazil. Photos by Soares, M.

Among the 60 species identified throughout the study, 52 species were classified as LC (Least Concern) for both red lists consulted. Five species were classified as LC for ICMBio (Instituto Chico Mendes de Conservação da Biodiversidade) and DD (Data Deficient) for IUCN (International Union for Conservation of Nature). Only one species was listed as CR (Critically Endangered, in both lists) and two species had no information (NE, Not Evaluated) for the IUCN list (LC in ICMBio list) (see Tables 1 and 2).

No difference in the impact of road-kills was observed between the patterns of activity reported for the group of reptiles: diurnal (N = 439; 47%), nocturnal (N = 403; 43.14%) and those active in both periods (N = 33; 3.53%). For snakes, we observed a higher number of road-kill on species that presented predominantly nocturnal activity (N = 403; 59.17%), species with diurnal activity recorded 28.92% (N = 197) while species active in both periods 4.84% (N = 33). For reptiles considered as a whole, the three lifestyles attributed to the most recorded animals were terrestrial (N = 495; 52.99%) followed by semi-arboreal (N = 276; 29.55%) and cryptozoic (N = 64; 6.85%), these three totaling 89.39% of the records. The other lifestyles: semi-aquatic (N = 25; 2.67%), saxicolous (N = 2; 0.21%), fossorial (N = 9; 0.96%), and arboreal (N = 4; 0.42%), together totaled 4.26% of the sample. For snakes we noticed the same pattern observed above, species with terrestrial (N = 293; 43.02%), semi-arboreal (N = 276; 40.52%) and cryptozoic (N = 40, 5.87%) lifestyles were the most affected by road-kill, totaling 89.41% of the records. Snakes that

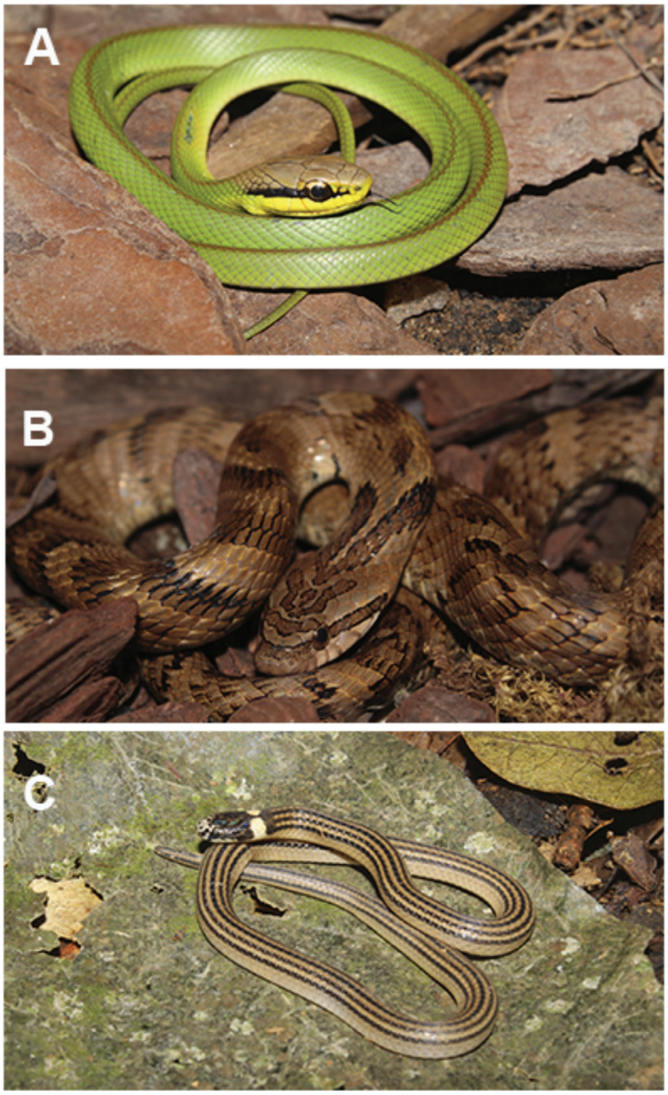

Figure 5. Road-kill species registered in the monitored stretch. A – *Philodryas olfersii*, Serra do Mendanha, state of Rio de Janeiro, Brazil; B – *Xenodon neuwiedii*, municipality of Rio de Janeiro, state of Rio de Janeiro, Brazil; and C – *Elapomorphus quinquelineatus*, municipality of Simão Pereira, state of Minas Gerais, Brazil. Photos by Soares, M (A – B) and Silva, F (C).

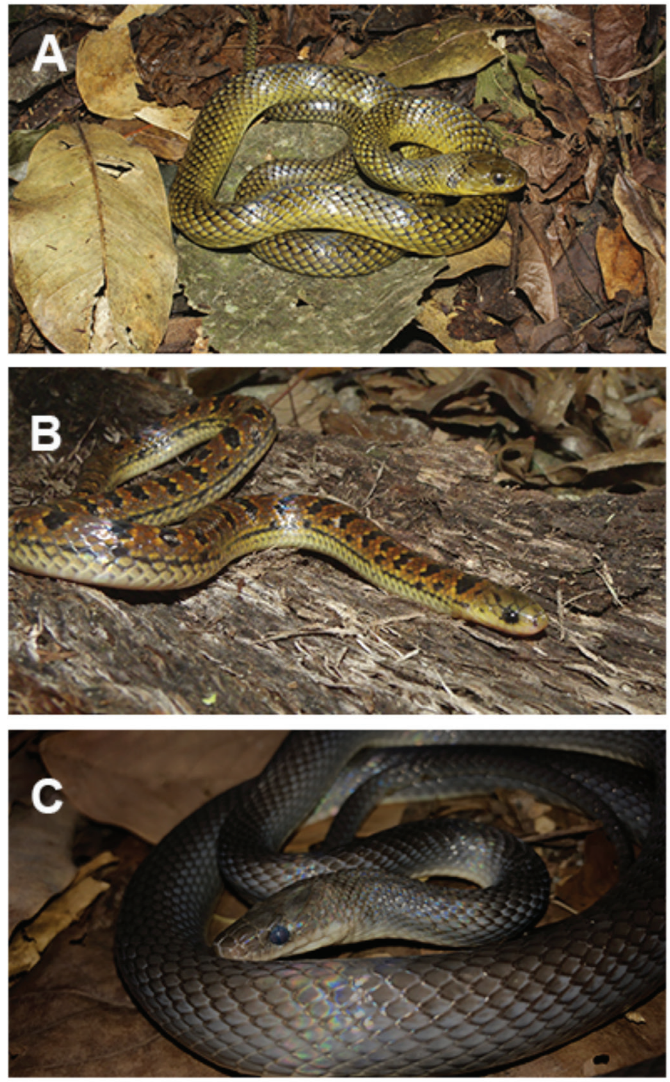

Figure 6. Road-kill species registered in the monitored stretch. A – *Erythrolamprus miliaris*, Serra do Caraça, state of Minas Gerais, Brazil; B – *Atractus zebrinus*, Serra do Caraça, state of Minas Gerais, Brazil; and C – *Pseudablades patagoniensis*, state of Rio de Janeiro, Brazil. Photos by Silva, F (A) and Ferreira-Cunha, L (B – C).

feed exclusively on mammals or that combine mammals and other taxonomic groups (i.e., generalist), were the most sampled (N = 282) corresponding to 41.40% of road-kill's. Followed by snakes that feed exclusively on anurans or that combine anurans and other taxonomic groups (i.e., generalist; N = 155), corresponding to 22.76% of road-kill's. Reptiles with seasonal reproduction (N = 777; 95.67%) were more road-killed than those with continuous reproduction throughout the year (N = 35; 4.33%). Animals with reproduction in the wet season had 545 records (67.45%), while in the dry season 228 records (28.22%). For reptiles, medium-sized individuals (500 < CRC < 1000 mm) were more commonly found on the highway (N = 403; 48.49%), followed by small animals (CRC < 500 mm) (N = 231; 27.79%) and finally large animals (CRC > 1000 mm) (N = 197; 23.70%). In a more directed view, for the snake's group, we found the same pattern, the species most hit were the medium-sized ones (500 < CRC < 1000 mm) (N = 373; 60.85%), followed by the small ones (CRC < 500 mm) (N = 150; 24.47%) and large ones (CRC > 1000 mm) (N = 90; 14.68%).

For amphibians, the lifestyle attributed to the animals most hit was the terrestrial (N = 378; 92.42%), followed by arboreal (N = 26; 6.36%) and cryptozoic (N = 5; 1.22%) individuals. Regarding habitat, the two most frequently found groups were animals with habits strictly associated with swamp or pond (N = 216; 52.55%) or swamp or pond and river or stream backwaters (N = 169; 41.12%), together amounting to 93.7%. The other two habits, Forest floor (N = 5) and Rock wall (N = 21), together corresponded to 6.3% of the road-kills. Large individuals (CRC > 100 mm) were the most found (N = 193; 46.96%), followed by medium-sized (50 < CRC < 100 mm) (N = 186; 45.26%) and small-sized (CRC < 50 mm) (N = 32; 7.79%).

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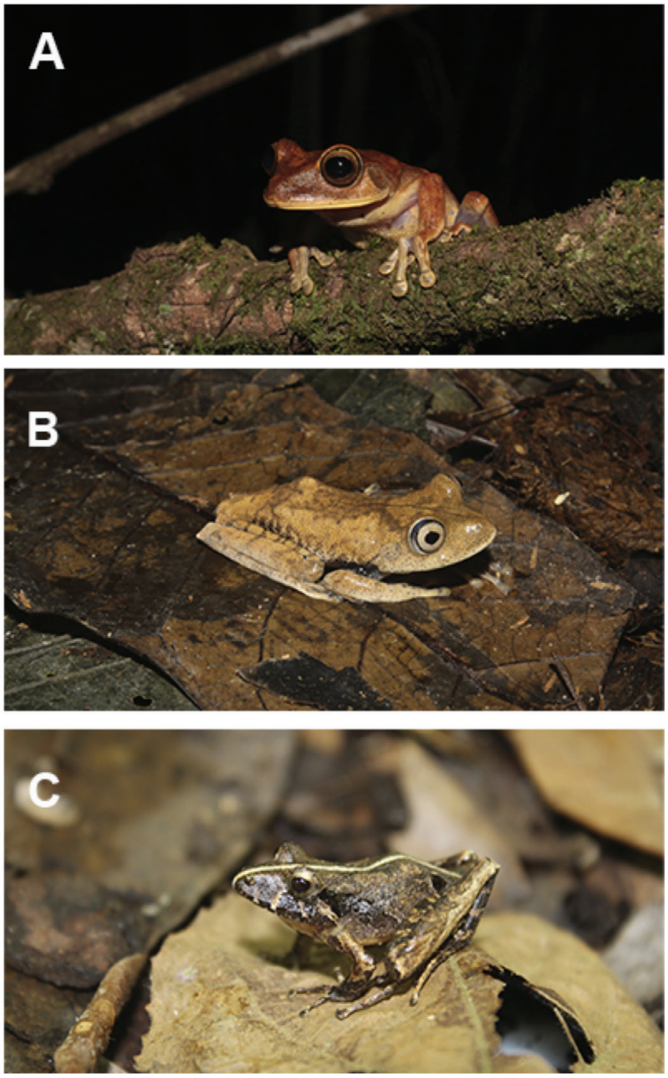

Figure 7. Road-kill species registered in the monitored stretch. A – *Bokermannohyla circumdata*, Serra do Caraça, state of Minas Gerais, Brazil; B – *Boana semilineata*, Simão Pereira, state of Minas Gerais, Brazil; and C – *Ischnocnema guentheri*, Parque Nacional da Serra dos Órgãos, state of Rio de Janeiro, Brazil. Photos by Soares, M (A) and Silva, F (B – C).

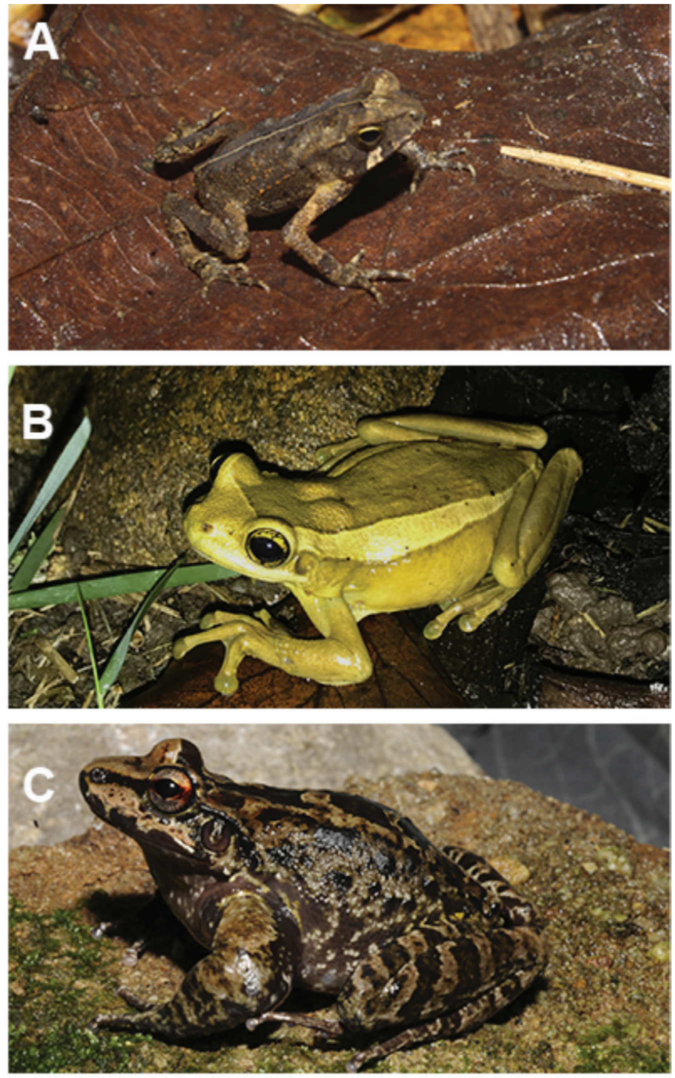

Figure 8. Road-kill species registered in the monitored stretch. A – *Rhinella ornata*, Serra do Caraça, state of Minas Gerais, Brazil; B – *Trachycephalus mesophaeus*, Serra do Caraça, state of Minas Gerais, Brazil; and C – *Thoropa miliaris*, Rebio do Tinguá, state of Rio de Janeiro, Brazil. Photos by Silva, F (A) and Ferreira-Cunha, L (B – C).

Discussion

Road ecology is a recent topic of interest in evolutionary biology, initiated in Brazil in the late 1980s (see Novelli et al., 1988) with the objective of understanding the patterns and processes related to the interactions between the road network and the ecosystems, establishing effective mitigation measures for the negative effects of roads on wildlife (Huijser et al., 2009; Rosa & Bager, 2013). However, these recent studies still show aggregations of trampling in Brazil and the World (Cáceres et al. 2012; Teixeira et al., 2013; Carvalho-Roel et al., 2019; Miranda et al., 2020; Spanowicz et al., 2020; Ascensão et al., 2021). In the present study we observed an increase in the number of road-killed during the rainy season, which indicates a seasonal pattern of trampling, as observed in other studies (Bencke & Bencke, 1999; Seibert & Conover, 1991; Machado et al., 2015; Garriga et al., 2017). This period is usually associated with the reproductive season of many groups (e.g., amphibians and reptiles) (Toledo et al., 2003; Jochimsen, 2005; Zina et al., 2007; Shepard et al., 2008a) and the increased availability of food at foraging sites. These factors stimulate the greater movement of animals, thus increasing the chance of trampling of the fauna (Forman & Alexander, 1998; Smith & Dodd, 2003; Jochimsen, 2005; Pinowski, 2005).

For instance, some studies point to ectotherms (amphibians and reptiles) as the largest victims of road-kills in wet areas (Ashley & Robinson, 1996; Glista et al., 2008; Shepard et al., 2008b) because they are strongly influenced by environmental conditions in terms of humidity and temperature (Zug et al., 2001). This pattern can be observed here, for both groups, where the trampling peak occurred during the rainy season (hot moments), coinciding with the time of greatest activity for foraging and reproduction. Due to aspects intrinsic

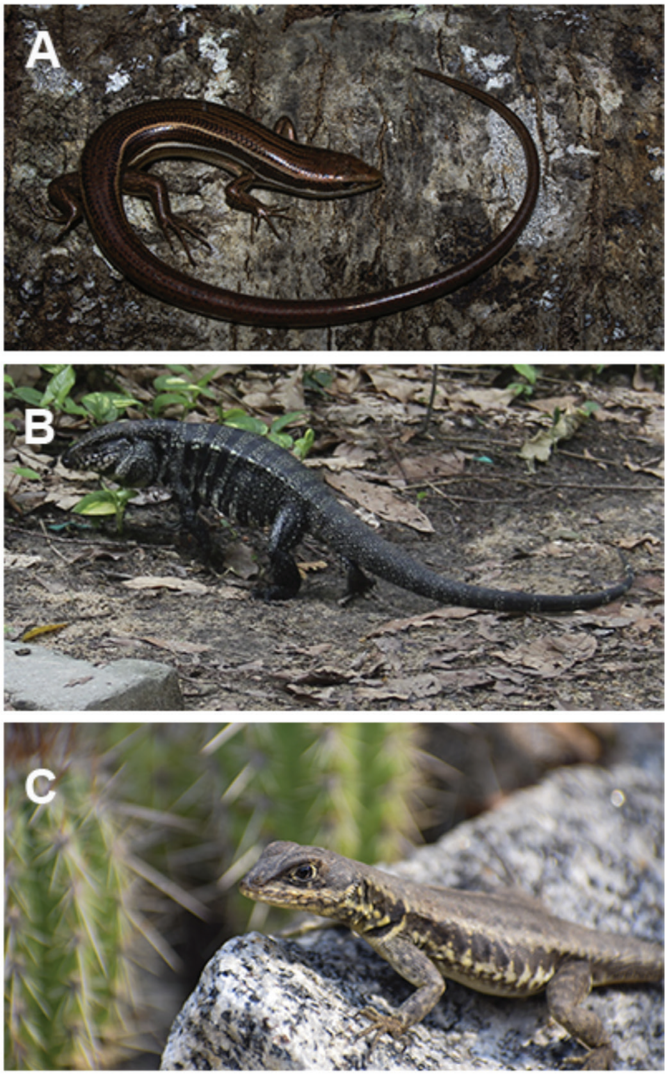

Figure 9. Road-kill species registered in the monitored stretch. A – *Aspronema dorsivittata*, Serra do Caraça, state of Minas Gerais, Brazil; B – *Salvator merianae*, municipality of Rio de Janeiro, state of Rio de Janeiro, Brazil; and C – *Tropidurus torquatus*, Serra do Caraça, state of Minas Gerais, Brazil. Photos by Andrade-Jr, A (A), Ferreira-Cunha, L (B) and Carvalho, B (C).

to each species (e.g., biological cycle, population density, speed of movement and use of surrounding areas close to highways) (Steen & Gibbs, 2004; Aresco, 2005) monitoring protocols should be established targeting the study area and taxonomic group of interest (Glista et al., 2008; Attademo et al., 2011).

As a rule, road-kills are concentrated in a few species of the faunal elements in a given region, usually species presenting generalist habits (non-specialized diet and microhabitats), relatively abundant population density, with high mobility and that use the highways (primarily or secondarily) as a source of resources (e.g., food intake and/or thermoregulation opportunity) (Forman et al., 2003, Secco et al., 2014). Not surprising, the group most affected by trampling in our study were reptiles, especially snakes. We raised four, not mutually exclusive, possibilities that could contribute to this high rate, as such: (i) use of the road to maximize thermoregulatory behavior at night and in cold days (Sullivan, 1981; Mccardle & Fontenot, 2016; Gonçalves et al., 2018); (ii) motionlessness as a defensive tactic used by some species (Andrews & Gibbons, 2005); (iii) intentional road-killing predominantly of snakes by cultural motivation (Secco et al., 2014; Assis et al., 2020); and (iv) the scavenging behavior of some species that are attracted to carcasses on highways (Schwartz et al., 2018; Muszynska et al. 2022).

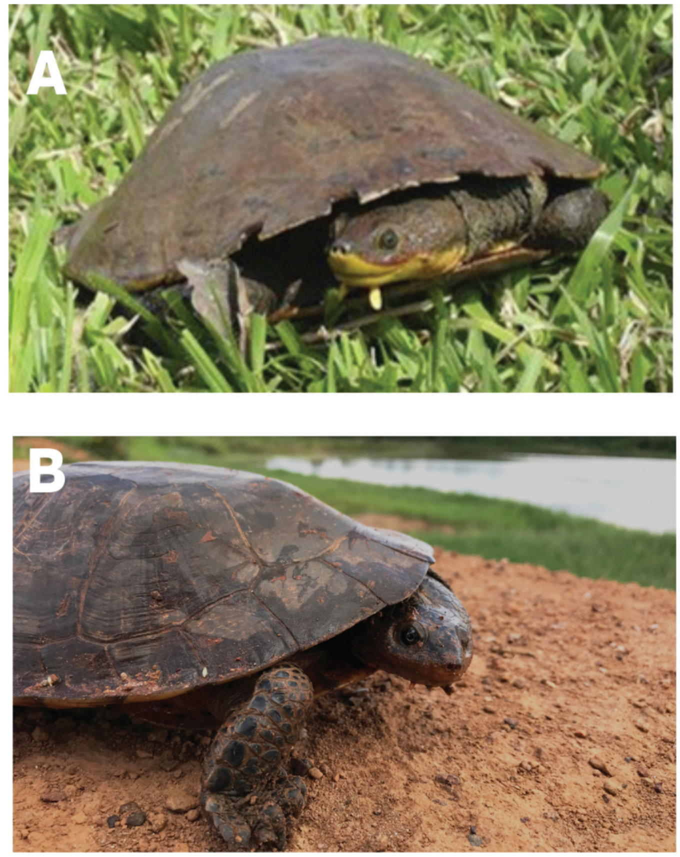

Figure 10. Road-kill species registered in the monitored stretch. A – *Phrynops geoffroanus*, municipality of Paracatu, state of Minas Gerais, Brazil; and B – *Mesoclemmys hogei*, municipality of Faria Lemos, state of Minas Gerais, Brazil. Photos by Silva, F (A) and Carrara, R (B).

The high number of road-killed *Crotalus durissus* species (N = 128) and *Salvator merianae* (N = 102) can be explained by the fact that they are species commonly found and adapted to open and anthropized areas, such as residential and commercial regions along roadsides. On the other hand, as in several other studies carried out in Brazil (Coelho et al., 2008; Kunz & Ghizoni-Jr, 2009; Turci & Bernarde, 2009; Abra et al., 2019; Ascensão et al., 2021) larger animals were more represented in our records of road-kills and this effect can be explained by the monitoring speed (50 km/h) and the means of transport used for monitoring (car assistance), a general standard used in our collection methodology and in several other studies (Enge & Wood, 2002; Taylor & Goldingay, 2004; Coleman et al., 2008; Delgado et al., 2019). These choices likely result in lower detection of small animals as reported in other studies that used bicycles and/or lower speed during monitoring (Pracucci et al., 2012; Rosa et al., 2012; Pinheiro & Turci, 2013; Santos et al., 2016; Wang et al., 2022), indicating that monitoring carried out with the help of cars can generate biased results for large animals.

Considering the presence of amphibians (mostly nocturnal animals) in the sample, it is believed that most of the road-kills occurred between 18:00 p.m. and 07:00 a.m. (Silva et al., 2007). This period is off-peak road traffic activity, which usually occurs in the beginning of the day and in the end of the afternoon, so that, even with this asynchronism with the peak moment on the highways, amphibians are greatly affected by trampling of wildlife, although they are still poorly sampled in studies on this topic (Glista et al., 2008). Previous studies indicated that frogs of the genus *Rhinella* are among the amphibians most road-killed (Dornas et al., 2017), result also observed in the present study: *Rhinella* spp. were the amphibian most road-killed with 203 register (42.47%). One possible explanation for the high rate of road-kills of individuals of this genus is that they are commonly found foraging around light poles (Coelho et al., 2012; Bastos et al., 2019). In addition to that, *Rhinella* species are explosive breeders, that dislocate to breeding areas during the reproductive season (the rainy season) (*e.g., Rhinella ornata*, Dixo et al., 2009). *Leptodactylus latrans* is another very abundant species in records of road-kills, as it is a species frequently recorded in areas modified by humans (Bastos et al., 2019).

Another point to be discussed is that small vertebrates, such as frogs, are usually less visualized on highways than large animals, such as some representatives of the mammal group (Santos et al., 2016; Filius et al., 2020), with this more than half of these small animals that road-killed easily go unnoticed in monitoring (Delgado et al., 2019). Due to this fact, slower research methods employing bike or walking and with more than one agent are encouraged for better visualization of smaller animals as they can result in detectability up to 8.4 times greater than using a car (Medrano-Vizcaíno et al, 2022; Wang et al., 2022). This may explain in parts the low number of road-kills recorded for the amphibian group in the present study when compared to the work performed by Filius et al. (2020), in which monitoring with bicycle and walking was carried out. Another two points that can help explain this low number of records for the amphibians are (i) smaller animals can more easily be thrown off the road and even get stuck in tires and (ii) the shorter persistence time of reptile and amphibian carcasses on highways, especially for smaller representatives (Santos et al., 2016; Filius et al., 2020).

As proposed by Sosa & Schalk (2016), our results suggest that roads can act as a barrier to the dispersion of amphibians and reptiles, especially for fossorial and arboreal species (snakes) and small species (some amphibians and lizards), since the members of this guild are more unfeasible, either by the style of movement and the crossing time, or by the lack of connectivity between the forest areas on each side of the road. Despite the high number of species found in our study, we believe that this number may be underestimated. Due to two factors: (i) species that were not yet recorded in our dataset of road-killed animals, but are expected for the region (e.g., *Dactyloa punctata; Hemidactylus mabouia; Gymnodactylus darwinii; Echinanthera cephalostriata*), (ii) some species may have been thrown out of the track, or even, if they took refuge in the forest and later died outside the area of collection, in addition to the possibility that they served as a food source for carnivorous and scavenger animals (Rodrigues et al., 2002; Bagatini, 2006; Silva et al., 2007; Pracucci et al., 2012; Ratton et al., 2014; Machado et al., 2015). In fact, the accumulation of carrion along the highways attracts animals, which consequently may be also road-killed (Muszynska et al. 2022). The behavior of scavenging is quite reported for the group of mammals (Gonzáles-Suarez et al., 2018). Although the scavenging behavior is not commonly recorded among snakes, as theoretically they have the preference for live prey (Sazima & Strussmann, 1990; Greene, 1997), there are some records in the literature reinforcing such behavior in the group (see Sazima & Strussmann, 1990; Lillywhite et al., 2002; Gomes et al., 2017; Marques et al., 2017). In this way, some snakes can lead two stages in what we call "the cycle of road-kills", constituting of two main steps: (i) as a source of food for scavengers animals (attracting other carnivorous animals like birds and mammals) and/or (ii) as carrion consumers of amphibians (e.g., *Chironius* spp.) or other vertebrates (e.g., *Philodryas* spp.) along the roads. Another very important point when we talk about road ecology is the rate of decomposition of carcasses along the roads and the difficulties generated by this factor. The estimate for the disappearance of carcasses in the snake group is that approximately 50% disappear within the first 8 to 24 hours (de Gregorio et al., 2011; Santos et al., 2016; Cabrera-Casas et al., 2020), depending on traffic and time of year. We extrapolate that for most amphibians and other small reptiles (several lizards and small snakes), this rate should be even higher due to the smaller body size, directly affecting the number of recorded individuals. However, this is not the only problem caused by the decomposition of carcasses, another known issue is the difficulty for the identification of very damage specimens (Bastos et al., 2019). For this reason, 20 amphibians and 58 reptiles could not be identified to the specific level. In addition, some specimens have been identified only to the generic level, such as *Tropidodryas* sp. due to the fact that more than one species occurs sympatrically in the region in combination with lacking preservation of key characters for diagnosing between congeners.

About road-kill rates, most information available were also estimated for the entire vertebrate clade, not for specific taxonomic groups, or as the number or record per kilometer, which is affected by the duration of the study. In Brazil, vertebrate road-kill rates varied from 0.18 road-kills/km/month in Pantanal wetlands (Fischer, 1997), 0.19 road-kills/km/month in stretches of Cerrado (Prada, 2004), and 0.21 and 0.46 road-kills/km/month in two roads in the sandy and wet restinga (Coelho et al., 2008), important remnants of Atlantic Forest in the south of Brazil. The rate found in the section analyzed by us (0.04 road-kill's/km/month) can be considered high, since it is relative only to the herpetofauna and for a stretch of highway. Several concepts about the ecology of roads were not and still are not taken into account in the environmental licensing process (Machado et al., 2015), causing the various ecological problems presented and discussed throughout the present study. Despite a myriad of mitigating measures to road-kills—such as the construction of ecological corridors, bridges, fences, and catwalks—are constantly encouraged to prevent animals from being road-killed when crossing the roads, some of these are criticized for their efficiency (e.g., isolated use of signs) and sometimes related to an increase in the rate of predation, hunting and trafficking of animals with economic interest (e.g., tunnels and underpasses) (Smith & Dodd, 2003).

Future Directions

The use of continuous fences and tunnel system for fauna (eco-passages or wildlife culverts) are currently the most recommended strategies for mitigate the impact of road-kills of amphibians and reptiles (Schmidt & Zumbach 2008; Lesbarreres & Fahrig, 2012; Beebee, 2013;

Yue et al., 2019) and can be used by other taxonomic groups, including invertebrates and small mammals.

Mitigation strategies focused on one taxonomic species or group need to be beneficial, or at least not bring negative effects, to other animals present in the study region (Jarvis et al., 2019). With this, to reduce the road-kills of amphibians and reptiles in the stretch analyzed by us we recommend, in addition to speed reducers, fauna-signaling plates and environmental education campaigns, the addition of continuous fences (no spaces for the animal to pass through it) and tunnels for fauna prioritizing the highest and well-preserved areas in order to mitigate damage to populations of more vulnerable and fragile species to automotive enterprises. We also recommend the preparation of further studies along the stretch aimed at detecting hotspots and the proposal of new strategies that help in conservation of local species.

Supplementary Material

The following online material is available for this article:

Appendix – List of specimens deposited in the Amphibians and Reptiles collections of the Museu Nacional, Universidade Federal do Rio de Janeiro (MNRJ).

Acknowledgments

We are grateful to CONCER for help with data collection. We are deeply indebted to Albedi Andrade-Jr., André Teles, Bárbara Carvalho, Fernanda Silva, Francielly Reis, Larissa Ferreira-Cunha, and Rodrigo Carrara for providing photographs of the species. This study was supported by Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES; #2022-1001), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq; #307631/2021-4), and Fundação Carlos Chagas Filho de Amparo à Pesquisa do Estado do Rio de Janeiro (FAPERJ; #E-26/202.737/2018).

Associate Editor

Pedro Nunes

Author Contributions

Daniel Faustino Gomes: contribution to conceptualization; methodology; writing – original draft.

Cecília Bueno: contribution to conceptualization; methodology; resources writing – review & editing.

Pedro H. Pinna: contribution to resources; methodology; resources writing – review & editing.

Manoela Woitovicz-Cardoso: contribution to resources; methodology; resources writing – review & editing.

Paulo Passos: contribution to conceptualization; methodology; resources writing – review & editing.

Conflicts of Interest

The authors declares that they have no conflict of interest related to the publication of this manuscript.

Ethics

This study did not involve human beings and/or clinical trials that should be approved by one Institutional Committee.

Data Availability

The data used in our analysis is available at Zenodo Dataverse <https://doi.org/10.5281/zenodo.7459911>.

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Received: 19/12/2022
Accepted: 08/05/2023
Published online: 30/06/2023

Biota Neotropica 23(2): e20221419, 2023
www.scielo.br/bn

biotaneotropica
ISSN 1676-0611 (online edition)
Article

Taxonomic study and local environmental conditions of occurrence of Chlorophyceae (Chlorophyta) from subtropical lotic environments, Paraná, Brazil

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PILATTI, M.C., SILVA, T.T., BORTOLINI, J.C., MEDEIROS, G., AMARAL, M.W.W., NARDELLI, M.S., BUENO, N.C. **Taxonomic study and local environmental conditions of occurrence of Chlorophyceae (Chlorophyta) from subtropical lotic environments, Paraná, Brazil.** Biota Neotropica 23(2): e20221419. https://doi.org/10.1590/1676-0611-BN-2022-1419

Abstract: Lotic environments are subjected to the impacts of human activities in an intense way in urban regions and one of the ways to assist in the environmental diagnosis is through the knowledge of the composition of bioindicator organisms, including microalgae. The objective of this work was to qualitatively characterize the Chlorophyceae Class Wille, providing descriptions and meristic data of the specimens as well as the environmental conditions in which the taxa were recorded. Water and phytoplankton samplings were carried out quarterly in 2020, in nine supply rivers, distributed in three river basins in the western region of Paraná (Paraná III basin, Piquiri basin and Baixo Iguaçu basin). The studied rivers were classified as oligotrophic or mesotrophic and the taxa were mostly rare. Thirty-six taxa belonging to the Chlorophyceae class were recorded, distributed in five families: Hydrodictyaceae Dumortier, Neochloridaceae Ettl & Komárek, Radiococcaceae Fott ex P.C.Silva, Scenedesmaceae Oltmanns, Selenastraceae Blackman & Tansley. These taxa have mainly cenobial representatives, with about 70% of the individuals in this thallus configuration, followed by 22% colonies and 8% unicellular thallus. Among the identified species, five occurred only in mesotrophic sites, warning for environments with tendencies to elevate their trophic, since they are genera previously associated with these conditions. Ten new citations were recorded for the State of Paraná, namely: *Pseudopediastrum boryanum* var. *longicorne* (Reinsch) P.M.Tsarenko, *Radiococcus skujae* I.Kostikov, T.Darienko, A.Lukesová & L.Hoffmann, *Desmodesmus perforatus* (Lemmermann) E.Hegewald, *Desmodesmus subspicatus* (Chodat) E.Hegewald & A.W.F.Schmidt, *Scenedesmus indicus* Philipose ex Hegewald, Engelberg & Paschma, *Ankistrodesmus bernardii* Komárek, *Monoraphidium capricornutum* (Printz) Nygaard, *Monoraphidium caribeum* Hindák, *Raphidocelis danubiana* var. *elegans* (Playfair) Taşkin & Alp, *Selenastrum rinoi* Komárek & Comas. Taxonomic studies, such as this one, are an important tool for understanding the flora, and in addition to contributing to the registration of species in aquatic ecosystems, they serve as a basis for ecological studies and other approaches used to preserve biodiversity in these places.
Keywords: *bioindicators; green algae; phytoplankton; rivers; taxonomy.*

Estudo taxonômico e condições ambientais locais da ocorrência de Chlorophyceae (Chlorophyta) de ambientes lóticos subtropicais, Paraná, Brasil

Resumo: Ambientes lóticos são ecossistemas muito vulneráveis aos impactos das atividades humanas, especialmente em regiões urbanas, e uma das formas para auxiliar no diagnóstico ambiental é utilizando o conhecimento da composição dos organismos bioindicadores, dentre eles as microalgas. O objetivo foi caracterizar qualitativamente as microalgas enquadradas na Classe Chlorophyceae Wille, fornecendo

descrições e dados merísticos dos espécimes bem como as condições ambientais em que os táxons foram registrados. Foram realizadas amostragens de água e de fitoplâncton trimestralmente no ano de 2020, em nove rios de abastecimento, distribuídos em três bacias hidrográficas da região oeste do Paraná (bacia do Paraná III, bacia do Piquiri e bacia do Baixo Iguaçu). Os rios estudados foram enquadrados como oligotróficos ou mesotróficos e os táxons apresentaram em sua maioria ocorrência rara. Foram registrados 36 táxons pertencentes a classe Chlorophyceae distribuídos em cinco famílias: Hydrodictyaceae Dumortier, Neochloridaceae Ettl & Komárek, Radiococcaceae Fott ex P.C.Silva, Scenedesmaceae Oltmanns, Selenastraceae Blackman & Tansley. Esses táxons possuem representantes principalmente cenobiais, apresentando cerca de 70% dos indivíduos nessa configuração de talo, seguido por 22% de colônias e 8% de talos unicelulares. Entre as espécies identificadas, cinco ocorreram somente em locais mesotróficos, advertindo para ambientes com tendências a elevar sua trofia, visto que são gêneros já associados anteriormente a essas condições. Foram registradas 10 novas citações para o Estado do Paraná, sendo estas: *Pseudopediastrum boryanum* var. *longicorne* (Reinsch) P.M.Tsarenko, *Radiococcus skujae* I.Kostikov, T.Darienko, A.Lukesová & L.Hoffmann, *Desmodesmus perforatus* (Lemmermann) E.Hegewald, *Desmodesmus subspicatus* (Chodat) E.Hegewald & A.W.F.Schmidt, *Scenedesmus indicus* Philipose ex Hegewald, Engelberg & Paschma*, Ankistrodesmus bernardii* Komárek, *Monoraphidium capricornutum* (Printz) Nygaard*, Monoraphidium caribeum* Hindák, *Raphidocelis danubiana* var. *elegans* (Playfair) Taşkin & Alp, *Selenastrum rinoi* Komárek & Comas. Trabalhos taxonômicos, como este, *são uma importante ferramenta para o* conhecimento da flora, e além de contribuir no registro das espécies nos ecossitemas aquáticos, servem como base para estudos ecológicos e demais abordagens utilizadas na preservação da biodiversidade nesses locais.
Palavras-chave: *algas verdes; bioindicadores; fitoplâncton; rios; taxonomia.*

Introduction

The Chlorophyceae class is part of the "UTC clade" (Ulvophyceae, Trebouxiophyceae and Chlorophyceae) within the Chlorophyta division, and stands out for having its undeniable monophyly, supported by molecular and ultrastructural data (Fučíková et al. 2019). Owing to the high number of species, it is considered one of the most abundant and diverse group in Brazilian continental waters (Rodrigues et al. 2010), grouping around 563 genera and 3.797species (Guiry & Guiry 2023). The morphology of these organisms ranges from flagellated unicellular to unicellular devoid of locomotion organelles, motile or non-motile colonies, filaments and pseudoparenchyma structures (Wehr et al. 2015). The species of the Class Chlorophyceae present a wide morphometric and ecophysiological variability, being able to develop in different habitats, being influenced especially by the light exposure, availability of reactive soluble phosphorus and mixing of the water column (Happey-Wood 1988).

Increasingly, lotic environments are degraded due to urbanization and intense anthropic activities (Li et al. 2022). In this context the water for public supply is deteriorated, besides all aquatic biota that suffer the consequences of inadequate management of these environments (Peres et al. 2022). In this sense the taxonomic composition of the Class Chlorophyceae is an important tool to assist in environmental diagnosis, since the species are often associated with environments with organic pollution and potential eutrophication (Wijeyaratne & Nanayakkara 2020). The literature for the State of Paraná, mainly studies in lotic environments, is scarce when compared to lentic environments, in addition to all the richness of species that have not yet been described. Among the main works referring to the Chlorophyceae Class in rivers, we can mention: Oliveira et al. (1994) with 46 taxa distributed in 25 genera in the Paraná River; Bittencourt-Oliveira (1997) with 24 taxa distributed in the Chlorococcales, Oedogoniales and Volvocales orders in the Tibagi River; Medri et al. (2002) with the flora of the Tibagi River; Biolo et al. (2009) with the identification of 21 taxa distributed in six families in the São Francisco Falso River.

Among the most recent works we can still cite: Bortolini et al. (2010) with 28 taxa distributed in the families Hydrodictyaceae, Oocystaceae and Scenedesmaceae in the São João River; Aquino et al. (2014) with thirty taxa, distributed in six families and 16 genera in the Cascavel River; Medeiros et al. (2021) recorded 26 taxa in a subtropical river in the State of Paraná and Aquino et al. (2022) with a book chapter that synthesizes the taxa of green microalgae described in the works for western Paraná.

Thus, our study aimed to carry out a taxonomic survey of phytoplanktonic chlorophyceans in lotic environments with different physical and chemical conditions of the water of the western region of Paraná; Provide descriptions, illustrations, morphometric and meristic data of the species found along the environments; Contribute to the registration of species found on the UNOPA (herbarium of UNIOESTE – Universidade Estadual do Oeste do Paraná) species Link platform.

Material and Methods

1. Selection, location and characterization of study sites

We selected nine rivers used to capture water for public supply in the western region of Paraná, along the Lower Iguaçu River, Paraná III and Piquiri river basins (Table 1), which were distributed in nine municipalities: Guaraniaçu, Catanduvas, Três Barras do Paraná, Boa Vista Aparecida, Foz do Iguaçu, Medianeira, Santa Tereza do Oeste, Cascavel and Toledo (Figure 1).

The water sampling for physicochemical and biological analyses was performed in two sites in each river. These sites are similar in terms of flow, riparian vegetation and human influence, however, they were

Table 1. Herbarium sample number (UNOPA), weather station, geographic coordinates, river and watershed of the water samples for microalgae analysis.

UNOPA	Weather station	Geographic coordinates	River	Watershed	UNOPA	Weather station	Geographic coordinates	River	Watershed
6799 6989 7066 7242	Summer Autumn Winter Spring	25°40'56"S 52°53'29"W	Baú	Piquiri	6817 7007 7084 7260	Summer Autumn Winter Spring	25°32'13"S 54°31'25"W	Tamanduá	Lower Iguaçu River
6801 6991 7068 7244	Summer Autumn Winter Spring	25°40'27"S 52°53'20"W	Baú	Piquiri	6819 7009 7086 7262	Summer Autumn Winter Spring	25°18'35"S 54°30'31"W	Alegria	Lower Iguaçu River
6803 6993 7070 7246	Summer Autumn Winter Spring	25°11'13"S 53°08'18"W	Passo Liso	Paraná III	6821 7011 7088 7264	Summer Autumn Winter Spring	25°17'30"S 54°40'35"W	Alegria	Lower Iguaçu River
6805 6995 7072 7248	Summer Autumn Winter Spring	25°12'38"S 53°07'51"W	Passo Liso	Paraná III	6823 7013 7090 7266	Summer Autumn Winter Spring	25°20'29"S 53°35'20"W	Gonçalves Dias	Lower Iguaçu River
6807 6997 7074 7250	Summer Autumn Winter Spring	25°26'11"S 53°11'17"W	Itaguaçu	Paraná III	6825 7015 7092 7268	Summer Autumn Winter Spring	25°30'47"S 53°36'14"W	Gonçalves Dias	Lower Iguaçu River
6809 6999 7076 7252	Summer Autumn Winter Spring	25°26'21"S 53°10'50"W	Itaguaçu	Paraná III	6827 6981 7094 7270	Summer Autumn Winter Spring	52°53'29"S 53°26'06"W	Cascavel	Lower Iguaçu River
6811 7001 7078 7254	Summer Autumn Winter Spring	25°25'17"S 53°25'46"W	Jacutinga	Paraná III	6829 6983 7096 7272	Summer Autumn Winter Spring	52°53'20"S 53°26'19"W	Cascavel	Lower Iguaçu River
6813 7003 7080 7256	Summer Autumn Winter Spring	25°25'46"S 53°26'17"W	Jacutinga	Paraná III	6831 6985 7098 7274	Summer Autumn Winter Spring	24°45'49"S 53°39'50"W	Toledo	Lower Iguaçu River
6815 7005 7082 7258	Summer Autumn Winter Spring	25°30'26"S 54°31'50"W	Tamanduá	Lower Iguaçu River	6833 6987 7100 7276	Summer Autumn Winter Spring	24°43'51"S 53°42'40"W	Toledo	Lower Iguaçu River

selected taking into consideration the characteristic of lotic systems, where the water flow carries dissolved materials including pollutants, making a comparison between the two possible (Vannote et al. 1980). Sampling was carried out during the year 2020 in all four seasons. All samples were deposited in the herbarium of UNIOESTE – Universidade Estadual do Oeste do Paraná – UNOPA, Campus Cascavel, connected to the Brazilian Network of Herbaria and the data were computerized and made available on speciesLink (www.splink.cria.org.br).

2. *Sampling and analysis of environmental variables in rivers*

Data were obtained on water temperature (Temp – °C), dissolved oxygen (DO – mg L^{-1}), pH, electrical conductivity (Condut – mS/cm^{-1}) and turbidity (Turb – NTU), measured at the moment of the samples through the multiparameter probe Horiba U-5000. The data referring to the flow (m^3 s) and maximum depth were collected with the aid of a ruler, measuring tape and a floating object, considering the multiplication between the average speed resulting from the displacement of the object and the cross-sectional area at the site to calculate the flow, measured *in situ*.

For chemical analysis, water samples were collected by subsurface immersion of polyethylene bottles, being kept properly refrigerated and in the dark until their destination. Estimates of concentrations of nitrate (NO_3 – mg L^{-1}), ammonia nitrogen (N-NH3 – mg L^{-1}), total phosphorus (TP- mg L^{-1}), chlorophyll *a* (CL*a* – mg L^{-1}), were performed following the standardized methods in Standard Methods (APHA 2017).

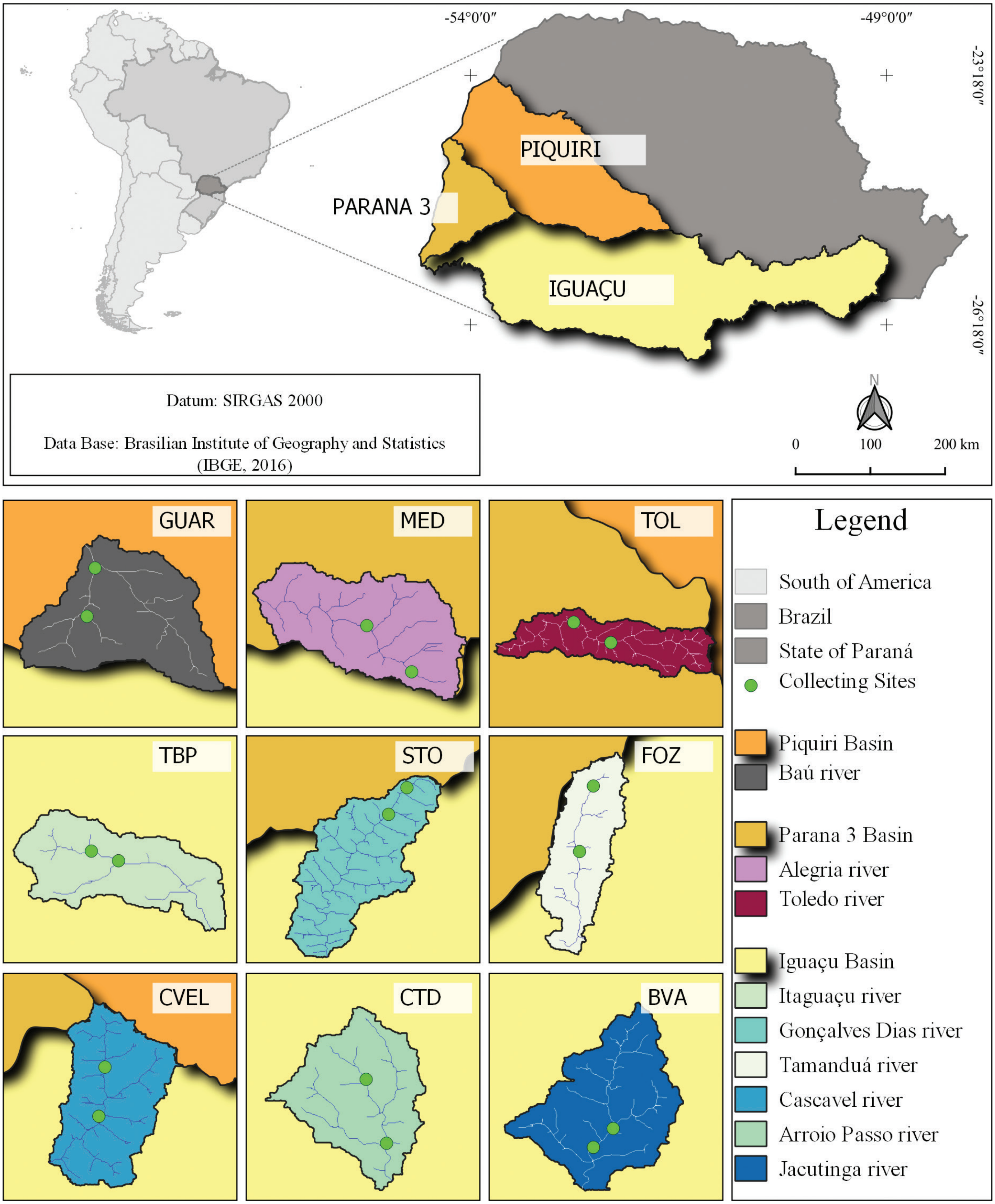

Figure 1. Municipalities in western Paraná, Brazil, selected for a taxonomic study of microalgae belonging to the Class Chlorophyceae. Abbreviations of the municipalities: GUAR: Guaraniaçu; MED: Medianeira; TOL: Toledo; TBP: Três Barras do Paraná; STO: Santa Tereza do Oeste; FOZ: Foz do Iguaçu; CVEL: Cascavel; CTD: Catanduvas; BVA: Boa Vista Aparecida.

3. Phytoplankton community

For the qualitative analysis, phytoplankton samples were obtained using a 25 µm mesh size plankton, were preserved in Transeau solution (Bicudo & Menezes 2017) in order to concentrate the phytoplanktonic material. The qualitative study of phytoplankton was carried out using an Olympus CX41 photomicroscope, coupled with an Olympus SC30 camera, and the morphometry of the taxa was performed at 40× and 100× magnification. The taxa were identified based on specialized literature, classification used follows Krienitz & Bock (2012). In order to verify the occurrence of taxa in the Paraná State, we considered only taxonomic studies with description, measures and/or illustrations, in lotic and lentic environments. The measurements (in µm) are represented by L – length; W – width; T – thorn; D – diameter; De – dent; Co – coenobium; P – process. Constancy is a measure of species occurrence (C) and was expressed as follows: constant (C ≥ 70%), common (30% ≥ C ≤ 70%), sporadic (10% ≥ C ≤ 30%) and rare (C ≤ 10%) (Dajoz 2005).

4. TSI – Trophic State Index

The Trophic State Index presented and used in the calculation of VAT (Index of Preservation of Aquatic Life), was composed by the Trophic State Index for phosphorus – TSI(PT) and the Trophic State Index for chlorophyll *a* – TSI(CL), modified by Lamparelli (2004), being established to lotic environments, according to the equations:

Rivers

$$TSI\ (CL) = 10 \times (6-((-0{,}7-0{,}6 \times (\ln CL)) / \ln 2)) -20$$

$$TSI\ (PT) = 10 \times (6-((0{,}42-0{,}36 \times (\ln PT)) / \ln 2)) -20$$

Where: PT: total phosphorus concentration measured at the water surface, in µg L^{-1}; CL: chlorophyll *a* concentration measured at the water surface, in µg L^{-1}; ln: natural logarithm. To classify the Trophic State for rivers, the Carlson Index (1977) modified by Toledo et al. (1983).

Results

Were registered 36 taxa belonging to the class Chlorophyceae Wille, distributed in five families: Hydrodictyaceae Dumortier, Neochloridaceae Ettl & Komárek, Radiococcaceae Fott ex P.C.Silva, Scenedesmaceae Oltmanns, Selenastraceae Blackman & Tansley and 16 genera : *Ankistrodesmus* Corda, *Coelastrum* Nägeli, *Comasiella* E.Hegewald, M.Wolf, A.Keller, Friedl & Krienitz, *Desmodesmus* (R.Chodat) S.S.An, T.Friedl & E.Hegewald, *Golenkinia* Chodat, *Kirchneriella* Schmidle, *Lacunastrum* H.A.McManus, *Monoraphidium* Komárková-Legnerová, *Pediastrum* Meyen, *Pseudopediastrum* E.Hegewald, *Radiococcus* Schmidle, *Scenedesmus* Meyen, *Stauridium* Corda, *Selenastrum* Reinsch, *Tetrallantos* Teiling, *Westella* De Wildeman (Figures 2–4).

FAMILY HYDRODICTYACEAE

Lacunastrum gracillimum (West & G.S.West) H.A.McManus., J. Phycol., 47(1): 123-130, 2011. Basionym: *Pediastrum duplex* var. *gracillimum* West & G.S.West, J. Bot., 33:52, 1895.

Figure 2: A

Flat coenobium, with circular to oval shape; formed by 16 cells, clathrated; marginal cells in an asymmetrical "H" shape, concave base, two slender, long processes of equal length, ending in a slightly rectused papilla, deeply excavated "U" incision; inner cells similar to outer ones; chloroplast with the shape of the cell, one central pyrenoid.

Morphometric data: Co = 32.5-62.5 µm; L = 12.5-15.0 µm; W = 5.0-10.0 µm.

Paraná State citation: Aquino et al. (2014) as *Pediastrum duplex* var. *gracillimum* West & G.S.West, Aquino et al. (2022).

Taxonomic remarks: molecular studies, associated with cell wall scan data, considering the differential characteristics of the coenobium, elongated cell lobes and smooth cell wall, made it possible to transfer the species *Pediastrum gracillimum* (West & G.S.West) Thunmark to the genus *Lacunastrum* H.A.McManus (McManus et al. 2011). Some specimens in this work presented larger dimensions than those recorded in Aquino et al. (2014), however they are in agreement with their description and illustration.

Occurrence in samples: UNOPA 6985, 6987, 6995, 7001

Frequency of occurrence: rare

Pediastrum duplex Meyen, Nova Acta Phys.-Med. Acad. Caes. Leop.-Carol. Nat., 14: 768-778, 1829.

Figure 2: B

Circular coenobium; formed by 16 to 32 cells (sometimes four, eight or 64 cells) arranged concentrically; intercellular spaces present; polygonal marginal cells joined at the base; square to angled inner cells; chloroplast with the shape of the cell; pyrenoid not observed.

Morphometric data: Co = 32.5-50.0 µm; L = 7.5-15.0 µm; W = 7.5-15.0 µm.

Paraná State citation: Picelli-Vicentim (1987), Rodrigues & Train (1993), Oliveira et al. (1994), Bittencourt-Oliveira (1997), Picelli-Vicentim et al. (2001), Train et al. (2001), Borges et al. (2003), Biolo et al. (2009), Bortolini et al. (2010), Felisberto & Rodrigues (2010, 2012), Aquino et al. (2014), Medeiros et al. (2021) and Aquino et al. (2022).

Occurrence in samples: UNOPA 6983, 6985, 6987, 7094

Frequency of occurrence: rare

Pseudopediastrum boryanum* var. *longicorne (Reinsch) P.M.Tsarenko, Algae of Ukraine: diversity, nomenclature, taxonomy, ecology and geography, 3: 280-355, 2011.

Basionym: *Pediastrum boryanum* f. *longicorne* Reinsch, Algenfl. Franken, 96, 1866.

Figure 2: C

Circular to oval coenobium; formed by 16 to 32 cells arranged in concentric rings without intercellular space; marginal cells extended into two longer processes, ending in swollen, stubby spines; polygonal inner cells with straight sides; cell wall usually granulated or smooth; parietal chloroplast, with one pyrenoid.

Morphometric data: Co = 32.5-41.0 µm; L = 7.5-18.0 µm; W = 5.0-13.0 µm; P = 7.0-10.0 µm.

Paraná State citation: first record.

Taxonomic remarks: the lineage of *Pseudopediastrum boryanum* (Turpin) E.Hegewald is divided into varieties through a set of morphological characters, such as the number of coenobium cells, the size and shape of the marginal cells, the absence of perforations between the cells and the density of the granules from the surface (Lenarczyk & Saluga 2018). The specimens of this work were identified as

P. boryanum var. *longicorne* (due to the size of the marginal cells, which are longer than the type species, resembling a "U" shape, and are in agreement with the specialized literature (Rai & Misra 2013).
Occurrence in samples: UNOPA 6805, 6815, 6995
Frequency of occurrence: rare

Stauridium tetras (Ehrenberg) E.Hegewald, J. Phycol., 41: 1039-1054, 2005.
Basionym: *Micrasterias tetras* Ehrenberg, Infus., 155, 1838.
Figure 2: D-F
Rectangular, oval or circular coenobium; formed by four, eight or 16 cells without intercellular spaces; marginal cells divided into two lobes, by a deep linear incision, from the outside reaching the middle of the cell; internal cells formed by four to six sides with a single linear incision; smooth cell wall, parietal chloroplast; pyrenoid not observed.
Morphometric data: D = 22.5-35.0 μm; L = 7.5-12.5; W = 7.5-13.5 μm.
Paraná State citation: Cited as *Pediastrum tetras* (Ehrenberg) Ralfs in Lozovei & Luz (1976), Lozovei & Hohmann (1977), Picelli-Vicentim (1986), Rodrigues & Train (1993), Oliveira et al. (1994), Train et al. (2001), Train et al. (2003), Algarte et al. (2006), Borges et al. (2008) and Felisberto & Rodrigues (2012), Biolo et al. (2009), Bortolini et al. (2010), and as *Stauridium tetras* in Menezes et al. (2011), Aquino et al. (2014), Aquino et al. (2022).
Taxonomic remarks: in the species proposition, some specimens from the sampling had been identified as *Pediastrum tetras*, however, Buchheim et al. (2005) proposed the transference of *Pediastrum tetras* to the genus *Stauridium* Corda from molecular analyses. Despite being a well-defined species, it can present considerable morphological variation (Ramos et al. 2016), and therefore the dimensions of the specimens also vary, as verified in Aquino et al. (2022).
Occurrence in samples: UNOPA 6799, 6981, 6983, 6995, 7072, 7096, 7272
Frequency of occurrence: rare

FAMILY NEOCHLORIDACEAE

Golenkinia radiata Chodat, J. Bot., 8: 305-308, 1894.
Figure 2: G
Spherical cells, isolated; with numerous long and delicate spines (12-14); parietal and single chloroplast; an elliptical to reniform pyrenoid.
Morphometric data: D = 13 .5- 15.0 μm; T = 19.0-25.0 μm.
Paraná State citation: Cecy et al. (1976), Lozovei & Luz (1976), Stankiewicz et al. (1981), Oliveira et al. (1994), Train et al. (2000), Bittencourt-Oliveira (2002), Train et al. (2003), Perbiche-Neves et al. (2007), Borges et al. (2008), Felisberto & Rodrigues (2010), Menezes et al. (2011), Aquino et al. (2014), Riediger et al. (2014), Medeiros et al. (2021) and Aquino et al. (2022).
Taxonomic remarks: the dimensions analyzed in this study are smaller than in Aquino et al. (2022), however they are in agreement with the specimens by Tucci et al. (2014), and in both works the description and illustration are also in agreement.
Occurrence in samples: UNOPA 6805, 6821, 6991, 6995
Frequency of occurrence: rare

FAMILY RADIOCOCCACEAE

Radiococcus skujae I.Kostikov, T.Darienko, A.Lukešová & L.Hoffmann, Algol. Stud., 104:40, 2002.
Figure 2: H
Spherical colonies; tetrahedrally or octahedrally arranged cells; sporangial cell wall fragments at the periphery of the mucilage; pyrenoid not observed.
Morphometric data: D = 4.0 – 6.0 μm.
Paraná State citation: first record.
Taxonomic remarks: in the specialized literature, some authors identified *R. skujae* as *Thorakochloris nygaardii* Komárek, however this taxon was transferred to the genus *Hindakochloris nygaardii* (Komárek) Comas. Kostikov et al. (2002) considered this genus as a synonym of *R. skujae*, due to the cell shape and reproductive behavior, with *Radiococcus* being the most used nowadays.
Occurrence in samples: UNOPA 6981
Frequency of occurrence: rare

FAMILY SCENEDESMACEAE

Coelastrum astroideum De Notaris, Desmidacée delle Val Itrasca, pp. 1-84,1867.
Figure 2: I
Spherical coenobium; formed by eight to 32 ovoid-shaped cells; intercellular spaces present, quadrangular; smooth cell wall, often with apical thickening; parietal chloroplast, with one pyrenoid.
Morphometric data: Co = 15.0-25.0 μm; D = 5.0-7.0 μm.
Paraná State citation: Biolo et al. (2009) and Aquino et al. (2022).
Taxonomic remarks: *Coelastrum astroideum* De Notaris can be confused with other species, such as *Coelastrum microporum* Nägeli, however it differs in that its cells are ovoid in lateral view.
Occurrence in samples: UNOPA 6805, 6809, 6981
Frequency of occurrence: rare

Coelastrum microporum Nägeli, Algarum unicellularium genera nova et minus cognita praemissis verificationibus de algis unicellularibus in genere, p.70, fig. 6, 1855.
Figure 2: J
Spherical coenobium; formed by eight to 32 cells joined directly by the cell wall; spherical cells, without connective processes; small triangular or rectangular intercellular spaces; smooth cell wall, without apical thickening; parietal chloroplast, one central pyrenoid.
Morphometric data: Co = 22.5-25 μm; D = 4-6 μm.
Paraná State citation: Picelli-Vicentim (1987) and Picelli-Vicentim et al. (2001).
Taxonomic remarks*:* *Coelastrum microporum* can be confused with *Coelastrum astroideum*, however, it differs in that its cells are spherical in both lateral and apical views. The specimens in this study have relatively smaller dimensions than those analyzed by Ramos et al. (2015), in comparison with Tucci et al. (2019) the dimensions are larger, however, the description and morphology are in agreement with both works.
Occurrence in samples: UNOPA 6805, 6829, 7096
Frequency of occurrence: rare

Coelastrum proboscideum Bohlin, Algae aquae dulcis exsiccatae praecipue scandinavicae quas adjectis algis marinis chlorophyllaceis et phycochromaceis, 1201-1400, 1896.
Figure 2: K-L
Tetrahedral coenobium; formed by four to 32 triangular cells, in lateral view; outer poles with crown-like thickening; quadratic intercellular spaces; single parietal chloroplast.
Morphometric data: Co = 15.5 μm; L = 4.0-8.0 μm.
Paraná State citation: Lozovei & Luz (1976), Lozovei & Hohmann (1977), Picelli-vicentim (1986), Aquino et al. (2014) and Aquino et al. (2022)
Taxonomic remarks: the specimens in this study have smaller dimensions than those analyzed by Tucci et al. (2019), but they are in agreement with other studies as in Aquino et al. (2022), in addition to having illustration and description according to these works.
Occurrence in samples: UNOPA 6995, 7274
Frequency of occurrence: rare

Coelastrum pulchrum Schmidle, Ber. Deutsch. Bot. Ges., 10: 206-211, 1892.
Figure 3: A-B
Spherical coenobium; formed by eight to 32 cells; intercellular spaces present; octagonal cells, joined by conical-truncated processes, facing the periphery of the coenobium; thickened apices; parietal chloroplast with one pyrenoid.
Morphometric data: Co = 30.0-62.5 μm; D = 5.0 -7.5 μm.
Paraná State citation: Oliveira et al. (1994), Picelli-Vicentim et al. (2001), Biolo et al. (2009), Bortolini et al. (2010), Menezes et al. (2011), Aquino et al. (2014), Medeiros et al. (2021) and Aquino et al. (2022).
Occurrence in samples: UNOPA 6827, 6829, 6981, 6983, 7094, 7270
Frequency of occurrence: rare

Coelastrum reticulatum* var. *cubanum Komárek, Preslia, 47: 277, 1975.
Figure 3: C
Spherical coenobium found singly or in multiples; formed by 16 to 32 spherical cells connected to each other by cylindrical processes; intercellular spaces present; parietal chloroplast with one pyrenoid.
Morphometric data: Co = 10.0-35.0 μm; D = 4.0-7.5 μm .
Paraná State citation: Rodrigues & Train (1993), Aquino et al. (2014), Medeiros et al. (2021) and Aquino et al. (2022).
Occurrence in samples: UNOPA 6805, 6827, 6829, 6833, 6981, 6983, 7005, 7094, 7096
Frequency of occurrence: sporadic

Comasiella arcuata* var. *platydisca (G.M.Smith) E.Hegewald & M.Wolf, Phycologia, 49 (4): 325-335, 2010.
Basionym: *Scenedesmus arcuatus* var. *platydiscus* G.M.Smith, Trans. Wis. Acad. Sci. Arts Lett., 18: 451, 1916.
Figure 3: D-E
Flat coenobium; formed by four to eight reniform cells with rounded poles; cells arranged in double, alternating series, sheathed with inconspicuous mucilage; outer cells not fully aligned; parietal chloroplast with one pyrenoid.
Morphometric data: Co = 14.0-23.0; L = 9.0-13.0 μm; W = 4.0-5.0 μm.
Paraná State citation: Menezes et al. (2011) cited as *Scenedesmus arcuatus* var. *platydiscus*, Aquino et al. (2014) and Aquino et al. (2022).
Occurrence in samples: UNOPA 6981, 6983 7072, 7094
Frequency of occurrence: rare

Desmodesmus armatus (Chodat) E.H.Hegewald, Algol. Stud., 96: 1-18, 2000.
Basionym: *Scenedesmus hystrix* var. *armatus* Chodat, Algues vertes de la Suisse, p.25, 1902.
Figure 3: G
Flat coenobium; formed by two to four cells arranged linearly; ellipsoid inner cells; ellipsoid to arcuate outer cells, often with frontal ribs; main spines in linear arrangement; parietal chloroplast with one pyrenoid.
Morphometric data: L = 9.0-13.0 μm; W = 3.0-4.0; De = 0.8-1.2 μm; T = 13.0-14.0 μm.
Paraná State citation: in the work of Lozovei & Luz (1976) still cited as *Scenedesmus quadricauda* Chod., Moresco & Bueno (2007), Biolo et al. (2009), Bortolini et al. (2010), Felisberto & Rodrigues (2010), Menezes et al. (2011), Felisberto & Rodrigues (2012), Aquino et al. (2014) and Aquino et al. (2022).
Taxonomic remarks: some specimens have smaller cell dimensions than those reported by Tucci et al. (2019), however they are in agreement with those found in Aquino et al. (2022).
Occurrence in samples: UNOPA 6805, 6995, 7005
Frequency of occurrence: rare

Desmodesmus brasiliensis (Bohlin) E.Hegewald, Algol. Stud., 96: 1-18, 2000.
Basionym: *Scenedesmus brasiliensis* Bohlin, Die Algen der ersten Regnell'schen Expedition, p.22, 1897.
Figure 3: H
Flat coenobium; formed by four cells arranged linearly; elliptical or oblong cells, up to cylindrical; attenuated ends with slightly rounded poles; ribs present in all cells, which can unite and form one to three teeth at the poles of the cells; parietal chloroplast with one pyrenoid.
Morphometric data: L = 15.0 μm; W = 7.9 μm; De = 2.0 μm.
Paraná State citation: Moresco & Bueno (2007); Biolo et al. (2009); Bortolini et al. (2010) and Aquino et al. (2022).
Taxonomic remarks: according to Biolo et al. (2009) and Tucci et al. (2019) the lateral projections are called teeth already in Bortolini et al. (2010) and Aquino et al. (2022) are called thorns, concluding that both denominations are correct.
Occurrence in samples: UNOPA 6831
Frequency of occurrence: rare

***Desmodesmus communis* (**E.Hegewald) E.Hegewald, Algol. Stud., 96:1-18, 2000.
Basionym: *Scenedesmus communis* E.Hegewald, Algol. Stud., 151, 1977.
Figure 3: I
Flat coenobium; formed by four to eight cells arranged linearly; internal cells oblong, with rounded poles, without ornamentation; trapezoidal outer cells, with a slightly convex outer margin and a long spine at each pole; smooth cell wall; parietal chloroplast with one pyrenoid.
Morphometric data: L = 10.0-22.5 μm; W= 5.0-12.5 μm; T = 7.5-15.0 μm.

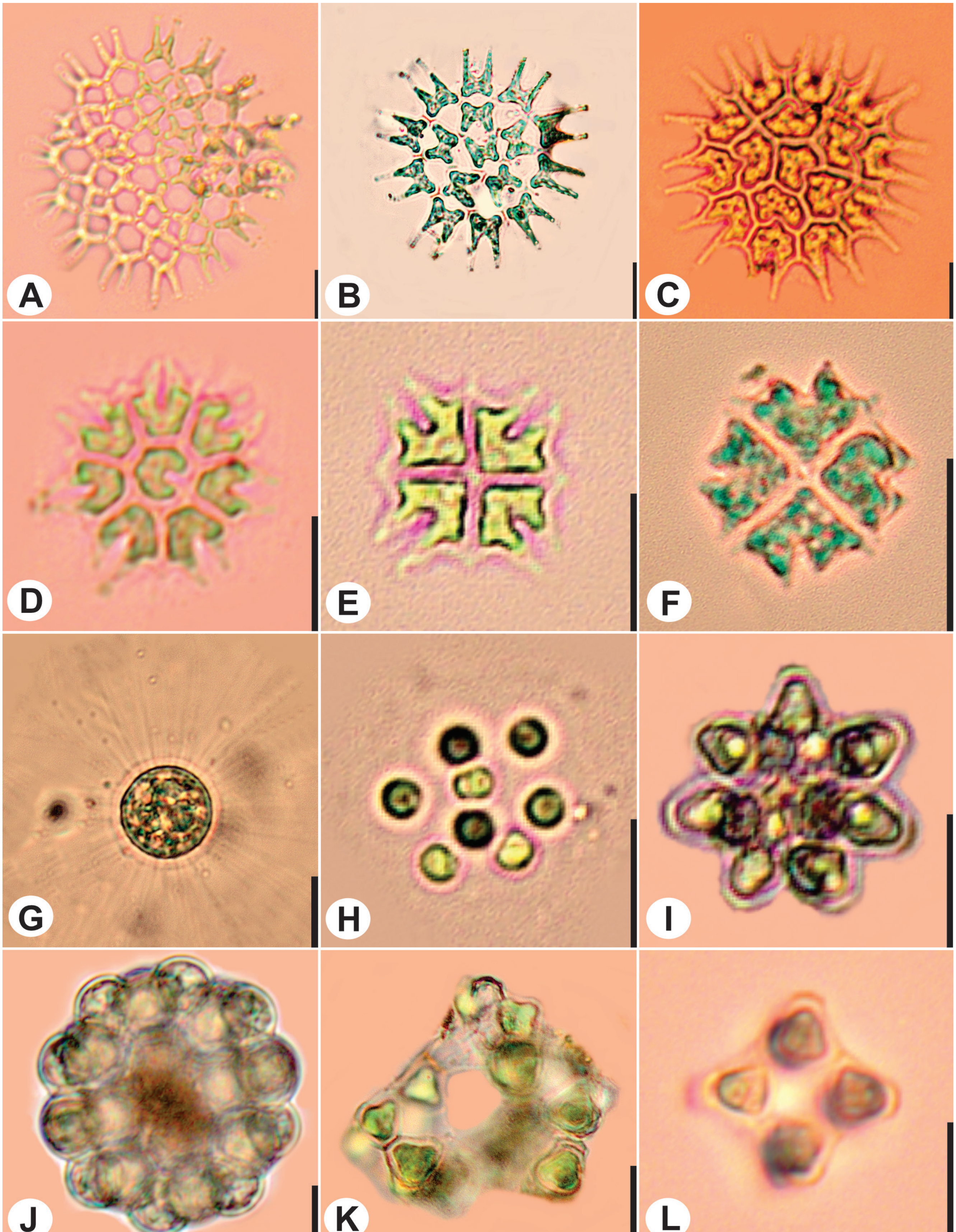

Figure 2. A. *Lacunastrum gracillimum.* B. *Pediastrum duplex.* C. *Pseudopediastrum boryanum* var. *longicorne.* D-F. *Stauridium tetras.* G. *Golenkinia radiata.* H. *Radiococcus skujae.* I. *Coelastrum astroideum.* J. *Coelastrum microporum.* K-L. *Coelastrum proboscideum.* Scales =10 µm.

 https://doi.org/10.1590/1676-0611-BN-2022-1419

Paraná State citation: Picelli-Vicentim (1985, 1987), Rodrigues & Train (1993), Oliveira et al. (1994), Picelli-Vicentim, (2001), Moresco & Bueno (2007), Biolo et al. (2009), Bortolini et al. (2010), Felisberto & Rodrigues (2010), Menezes et al. (2011), Aquino et al. (2014), Medeiros et al. (2021) and Aquino et al. (2022).
Occurrence in samples: UNOPA 6819, 6829, 6831, 6833, 6987, 6995, 6997, 7001, 7074, 7094
Frequency of occurrence: sporadic

Desmodesmus denticulatus (Lagerheim) S.S.An, T.Friedl & E.Hegewald, Plant Biol., 1:427, 1999.
Basionym: *Scenedesmus denticulatus* Lagerheim, Öfversigt af Kongl. Vetenskaps-Akademiens Förhandlingar Arg., 39(2): 47-81, 1882.
Figure 3: J
Flat coenobium; formed by four cells arranged alternately; oval inner cells, without ornamentation; outer cells elliptical, with one or two short spines at each pole; parietal chloroplast with one pyrenoid.
Morphometric data: L = 7.5-22.5 µm; W = 7.5-12.5 µm; De = 2.5 µm.
Paraná State citation: Moresco & Bueno (2007), Biolo et al. (2009), Bortolini et al. (2010), Felisberto & Rodrigues (2010), Menezes et al. (2011), Felisberto & Rodrigues (2012), Aquino (2014) and Aquino et al. (2022), still cited as *Scenedesmus denticulatus* (Lagerh) in the studies of Picelli-Vicentim (1987), Oliveira et al. (1994), Train et al. (2001), Train et al. (2003) and Algarte et al. (2006).
Occurrence in samples: UNOPA 6833, 6995, 7072
Frequency of occurrence: rare

Desmodesmus intermedius* var. *acutispinus (Roll) E.Hegewald, Algol. Stud., 96: 1-18, 2000.
Basionym: *Scenedesmus quadricauda* var. *acutispinus* Roll, Russkii Arkhiv Protistologii 4(3-4): 137-152, 1925.
Figure 4: B
Flat coenobium; formed by two to four oblong cells, arranged linearly; outer cells with a long spine on only one of the apices, distributed diagonally in the coenobium, parietal chloroplast with one pyrenoid.
Morphometric data: L = 6.6-7.0 µm; W = 2.5-3.0 µm; T = 6.0-8.0 µm.
Paraná State citation: Moresco & Bueno (2007) and Aquino et al. (2022).
Taxonomic remarks: the variety *acutispinus* differs from the type species in terms of the number and diagonal arrangement of spines at the apices of the outer cells. In the variety there are only two spines, and in the type species there are four spines.
Occurrence in samples: UNOPA 6981, 6983
Frequency of occurrence: rare

Desmodesmus opoliensis (P.G.Richter) E.Hegewald, Algol. Stud., 96: 1-18, 2000.
Basionym: *Scenedesmus opoliensis* P.G.Richter, *Scenedesmus opoliensis* P. Richt, nov. sp. Zeitschrift für angewandte Mikroskopie, 1: 3-7, 1895.
Figure 3: K
Flat coenobium; formed by four cells arranged linearly; internal fusiform cells, with attenuated and rounded poles, without ornamentation; trapezoidal outer cells, with truncated poles at the base of spine insertion and slightly convex outer margin; parietal chloroplast with one pyrenoid.
Morphometric data: L = 15.0-17.0 µm; W = 6.0 µm; T = 14.0-16.0 µm.
Paraná State citation: Moresco & Bueno (2007), Aquino et al. (2022), and still cited as *Scendesmus opoliensis* in the works of Rodrigues & Train (1993) and Picelli-Vicentim (2001).
Taxonomic remarks: the dimensions recorded in the specimens of this study are relatively smaller than those of Tucci et al. (2019), however they are in agreement with other works as in Aquino et al. (2022).
Occurrence in samples: UNOPA 6805
Frequency of occurrence: rare

Desmodesmus perforatus (Lemmermann) E.Hegewald, Algol. Stud., 96: 1-18, 2000.
Basionym: *Scenedesmus perforatus* Lemmermann, *Zeitschrift für Fischerei und deren Hilfswissenschaften* 11: 73-123, 1903.
Figure 3: L
Flat coenobium; formed by four biconcave cells in a linear arrangement; external cells with concave internal face and convex external face; curved spines, presence of microtubules and sometimes presence of frontal ribs; parietal chloroplast with one pyrenoid.
Morphometric data: L = 14.5-16.0 µm; W = 5.5-6.0 µm; T = 6.0-10.0 µm.
Paraná State citation: first record.
Taxonomic remarks: Tucci et al. (2019) have larger dimensions than those recorded in this study, but in Souza & Felisberto (2014) the specimens are also small, thus being in agreement with the population observed in our work.
Occurrence in samples: UNOPA 6827, 6829, 6981, 6983, 7094, 7270
Frequency of occurrence: rare

Desmodesmus serratus (Corda) S.S.An, Friedl & E.Hegewald, Plant Biol., 1: 418-428, 1999.
Basionym: *Arthrodesmus serratus* Corda, Almanach de Carlsbad, 9: 213-244, 1839.
Figure 4: A
Flat coenobium; formed by four cells arranged linearly, without main spines; oblong cells, punctuated rib running through each cell; frequent presence of one to three teeth at the cell poles; outer cells with a row of spinules along their entire length; parietal chloroplast with one pyrenoid.
Morphometric data: L = 7.0-15.0 µm; W = 2.5-5.0 µm T = 2.0-3.0 µm.
Paraná State citation: Moresco & Bueno (2007), Bortolini et al. (2010) and Aquino et al. (2022).
Occurrence in samples: UNOPA 6831, 6833, 7094
Frequency of occurrence: rare

Desmodesmus subspicatus (Chodat) E.Hegewald & A.W.F.Schmidt, Algol. Stud., 96: 1-18, 2000.
Basionym: *Scenedesmus subspicatus* Chodat, Schweizerische Zeitschrift für Hydrologie, 3: 71-258, 1926.
Figure 4: C
Flat coenobium; formed by two or four oblong cells arranged linearly; frequent presence of secondary spine on inner cells and one or two lateral spines on outer cells; main spines in linear arrangement; parietal chloroplast with one pyrenoid.
Morphometric data: L = 7.0 µm; W = 2.0 µm; T = 4.0-6.0 µm.
Paraná State citation: first record.
Taxonomic remarks: *D. subspicatus* can be found in older literature (Rosini et al. 2013), as *Scenedesmus quadricauda* (Turpin) Brébisson,

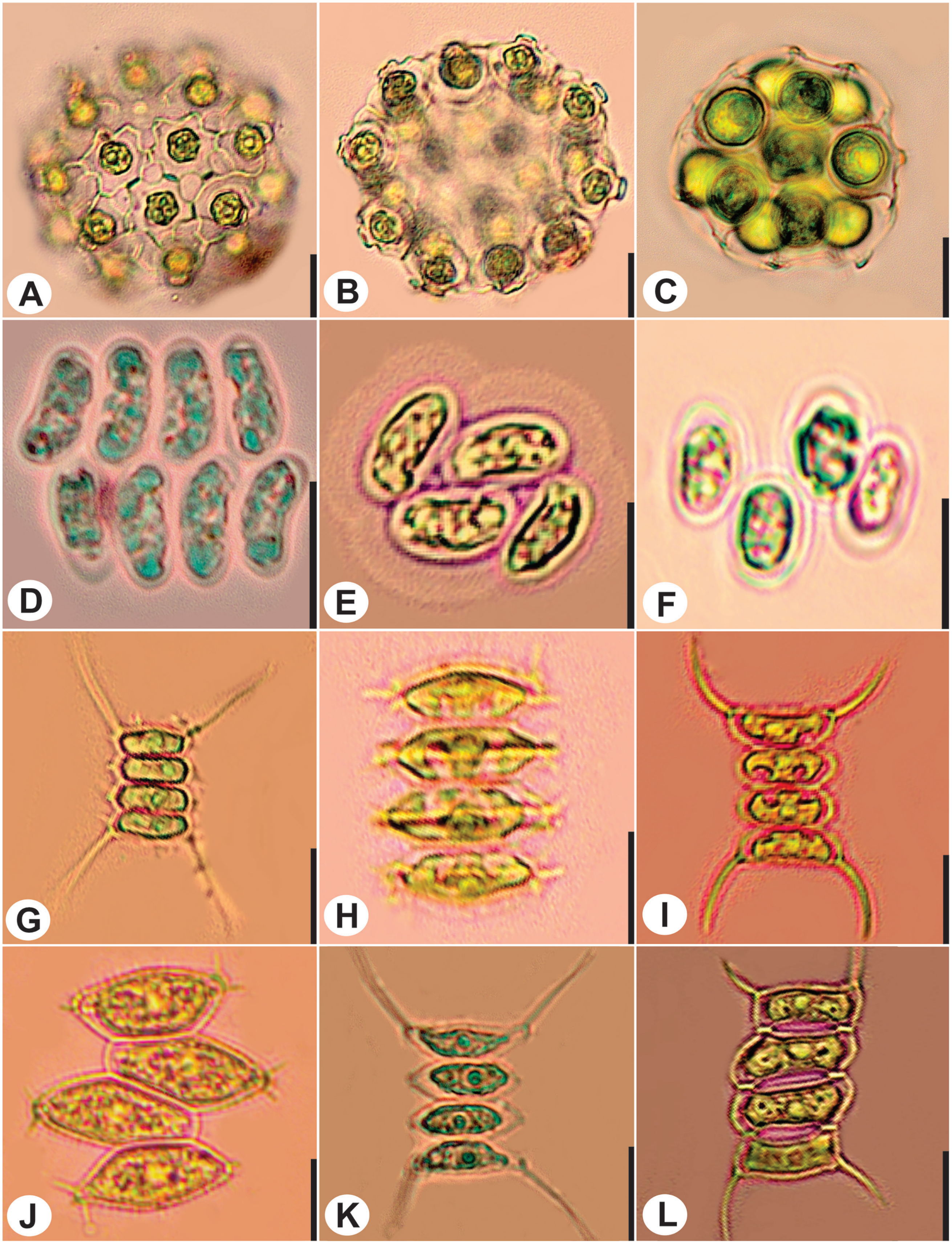

Figure 3. A-B. *Coelastrum pulchrum.* C. *Coelastrum reticulatum* var. *cubanum.* D-E. *Comasiella arcuata* var. *platydisca.* F. *Scenedesmus obtusus.* G. *Desmodesmus armatus.* H. *Desmodesmus brasiliensis.* I. *Desmodesmus communis.* J. *Desmodesmus denticulatus.* K. *Desmodesmus opoliensis.* L. *Desmodesmus perforatus.* Scales =10 µm.

which refers to its holotype, however, currently the current name is *Desmodesmus subspicatus* as seen in Tucci et al. (2019).
Occurrence in samples: UNOPA 6833
Frequency of occurrence: rare

Scenedesmus indicus Philipose ex Hegewald, Engelberg & Paschma, Nova Hedwig., 47 (3/4): 497-533, 1988.
Figure 4: I
Flat and linear coenobium; formed by four cells arranged alternately; outer cells arcuate with rounded, dilated or swollen poles; oblong inner cells with swollen poles; parietal chloroplast with one pyrenoid.
Morphometric data: L = 11.0 – 15.0 µm; W = 3.5 – 4.0 µm.
Paraná State citation: first record.
Taxonomic remarks: *Scenedesmus indicus* is easily differentiated from the other species due to the morphology of the coenobium, in which the cells are arranged in an alternating manner.
Occurrence in samples: UNOPA 6805, 6995, 7276
Frequency of occurrence: rare

Scenedesmus obtusus Meyen, Nova Acta Phys.-Med. Acad. Caes. Leop. -Carol. Nat., 14: 768-778, 1829.
Figure 3: F
Flat coenobium; formed by four or eight alternating cells; ovate-cylindrical cells with rounded poles; outer cells usually oblique; straight inner cells; slightly thickened cell wall; parietal chloroplast with one pyrenoid.
Morphometric data: L = 11.0-13.0 µm; W = 7.0 µm.
Paraná State citation: Picelli-Vicentim (1987); Bortolini et al. (2010) and cited as *Scenedesmus graevenitzii* in Moresco & Bueno (2007).
Occurrence in samples: UNOPA 7082
Frequency of occurrence: rare

Tetradesmus dimorphus (Turpin) M.J.Wynne, Feddes Repert., 126: 83-86, 2016.
Basionym: *Achnanthes dimorpha* Turpin, Mém. Mus. natl. hist. nat., 16: 313, 1828.
Figure 4: D
Flat coenobium; formed by four to eight fusiform cells with acute poles; arranged in a linear or alternating manner; external cells markedly concave, reaching straight or slightly convex; almost straight inner cells; parietal chloroplast; pyrenoid not observed.
Morphometric data: L = 19.0-22.0 µm; W= 3.0-5.0 µm; Co = 18.0 – 22.0 µm.
Paraná State citation: cited as *Tetradesmus dimorphus* in Aquino et al. (2022), cited as *Scenedesmus obliquus* var. *dimorphus* (Turpin) Hansgirg in Bortolini et al. (2010), Menezes et al. (2011), Aquino et al. (2014), and cited as *Scenedesmus acuminatus* (Lagerh.) and/or *Scenedesmus acutus* (Meyen) in Picelli-Vicentim (1987), Rodrigues & Train (1993), Oliveira et al. (1994), Bittencourt-Oliveira (1997), Train et al. (2001), Borges et al. (2003), Train et al. (2003), Algarte et al. (2006), Moresco & Bueno (2007), Borges et al. (2008), Felisberto & Rodrigues (2010) and Felisberto & Rodrigues (2012).
Occurrence in samples: UNOPA 6827, 6981, 7272
Frequency of occurrence: rare

Tetrallantos lagerheimii Teiling, Svensk Bot. Tidskr., 10 (1):59-66, 1916.
Figure 4: K
Coenobium formed by up to four cells; two are in the same plane and two are arranged vertically, joined by the poles; lunate or approximately reniform cells with rounded apices; parietal chloroplast with one pyrenoid.
Morphometric data: L = 10.0-12.5 µm; W = 5.0-7.5 µm.
Paraná State citation: Rodrigues & Train (1993).
Occurrence in samples: UNOPA 7015, 7274, 7276
Frequency of occurrence: rare

Westella botryoides (West) De Wildeman, Bull. Herb. Boissier., 5:532, 1897.
Basionym: *Tetracoccus botryoides* West, J. R. Microsc. Soc., 735, 1892.
Figure 4: J
Quadrangular coenobium; formed by four cells arranged in a cruciate manner; these cells form sincenobia with eight or 16 cells joined by the rest of the maternal cell wall by threads of mucilage; globular to subtriangular cells; parietal chloroplast with one pyrenoid.
Morphometric data: Co = 15.0 µm; D = 5.0 -7.5 µm.
Paraná State citation: Picelli-Vicentim (1987).
Occurrence in samples: UNOPA 6991, 6995
Frequency of occurrence: rare

FAMILY SELENASTRACEAE

Ankistrodesmus bernardii Komárek, Nova Hedwigia 37: 65-180, 1983.
Figure 4: E
Fasciculate colony; formed by approximately eight to 10 spindle cells with a sigmoid curve in the median region; longer than wide; united and intertwined in the middle region of the colony, tapering gradually towards the apex; pyrenoid not observed.
Morphometric data: L = 60.0 µm; W = 1.8-2.6 µm.
Paraná State citation: first record.
Taxonomic remarks: *Ankistrodesmus bernardii* Komárek can be confused with *Ankistrodesmus densus* Korshikov, as both form colonies with many cells that are variable in length but differ in that *A. densu*s has curved or sigmoid cells throughout.
Occurrence in samples: UNOPA 6827
Frequency of occurrence: rare

Ankistrodesmus densus Korshikov, The Freshwater Algae of the Ukrainian SSR. p. 300, 1953.
Figure 4: F
Colony with many fasciculate cells; usually formed by 16 densely packed cells; cells longer than wide, fusiform, curved or sigmoid throughout their length; gradually taper towards the apex; superimposed on each other; parietal chloroplast without pyrenoids.
Morphometric data: L = 35.0-55.0 µm; W = 2.5 µm.
Paraná State citation: Picelli-Vicentim (1987), Rodrigues & Train (1993), Oliveira et al. (1994), Biolo et al. (2009), Bortolini et al. (2010) and Aquino et al. (2022).
Occurrence in samples: UNOPA 6829, 7082
Frequency of occurrence: rare

Ankistrodesmus falcatus (Corda) Ralfs, The British Desmidieae, p. 180, 1848.
Basionym: *Micrasterias falcatus* Corda, Almanach de Carlsbad, 5: 206, 1835.
Figure 4: G
Starry colony formed by one to four fascicles; cells arranged irregularly; cells are long, fusiform, slightly curved, falcate, longer than wide, joined by the medial convex region, gradually tapering towards the apex; parietal chloroplast without pyrenoids.
Morphometric data: L = 37.5-50.0 µm; W = 2.5µm.
Paraná State citation: Andrade & Rachou (1954), Picelli-Vicentim (1986), Rodrigues & Train (1993), Oliveira et al. (1994), Bittencourt-Oliveira (1997), Train et al. (2001), Bittencourt-Oliveira (2002), Algarte et al. (2006), Felisberto & Rodrigues (2010), Aquino et al. (2014) and Aquino et al. (2022).
Taxonomic remarks: the specimens in this work have larger dimensions than those recorded in Aquino et al. (2022), however they are in agreement with the specimens observed in Ramos et al. (2012).
Occurrence in samples: UNOPA 6829, 6981
Frequency of occurrence: rare

Ankistrodesmus fusiformis Corda, Almanach de Carlsbad, 8: 179-198, 1838.
Figure 4: H
Starry colony formed by two to four cells; cells cruciate, fusiform, from straight to arcuate; tapered towards the apex, crossing over each other; pointed poles, longer than wide; pyrenoid not observed.
Morphometric data: L = 30.0 µm; W = 1.4-2.5 µm.
Paraná State citation: Picelli-Vicentim (1987), Rodrigues & Train (1993), Train et al. (2001), Algarte et al. (2006), Bortolini et al. (2010), Felisberto & Rodrigues (2010), Menezes et al. (2011), Felisberto & Rodrigues (2012), Aquino et al. (2014) and Aquino et al. (2022).
Taxonomic remarks: in Aquino et al. (2022) the registered specimens with four cells, whereas those observed in this study have only two, but they are in agreement with Ramos et al. (2012) who also recorded this morphological variation in their population.
Occurrence in samples: UNOPA 7082, 7268
Frequency of occurrence: rare

Kirchneriella lunaris (Kirchner) Möbius, Abh. Senckenb. Nat. Gesell., 18: 309-350, 1894.
Basionym: *Rhaphidium convolutum* var. *lunare* Kirchner, Kryptogamen-Flora von Schlesien, 114, 1878.
Figure 4: L
Colonies formed by four to 16 cells, sickle-shaped lunate; wrapped in a mucilaginous sheath; sometimes lonely; parietal chloroplasts close to the cell wall; pyrenoid not observed.
Morphometric data: L = 5.0-10.0 µm.
Paraná State citation: Picelli-Vicentim (1987), Rodrigues & Train (1993) and Oliveira et al. (1994).
Occurrence in samples: UNOPA 6981, 6995, 7005, 7082
Frequency of occurrence: rare

Monoraphidium capricornutum (Printz) Nygaard, Biol. Skr., 21(1): 1-107, 1977.
Basionym: *Selenastrum capricornutum* Printz, Skr. Norske Vidensk. -Akad. Oslo, Mat. -Naturvidensk. Kl., 6:92, 1914.
Figure 4: N
Spindle cells; arched in semicircles, slightly tapered at the ends; parietal chloroplast without pyrenoids.
Morphometric data: L = 10.0-12.5 µm; W = 2.5 µm.
Paraná State citation: first record.
Taxonomic remarks: the specimens in this study have relatively larger dimensions than those recorded in Ramos et al. (2012) and Nandi et al. (2017) with regard to the length of the cells, however the width, description and illustration are in agreement with the works.
Occurrence in samples: UNOPA 6827
Frequency of occurrence: rare

Monoraphidium caribeum Hindák, Algol. Stud., 1:7-32, 1970.
Figure 4: O
Solitary cells, arcuate in a semicircle, slightly tapering at the ends; parietal chloroplast without pyrenoids.
Morphometric data: L = 17.5 µm; W = 2.5 µm.
Paraná State citation: first record.
Taxonomic remarks: *M. caribeum* can be confused with *Ankistrodesmus arcuatus* Korshikov, however the species differ by the size of the cells, where *A. arcuatus* has larger dimensions.
Occurrence in samples: UNOPA 7100
Frequency of occurrence: rare

Raphidocelis danubiana* var. *elegans (Playfair) Taşkin & Alp, Türkiye suyosunlari listei [Turkey algae list]. p.804, 2019.
Basionym: *Kirchneriella elegans* Playfair, Proc. Linn. Soc., 41: 838, 1917.
Figure 4: M
Colony formed by four cells irregularly arranged in mucilage; curved cylindrical cells with rounded apices, in the same plane; parietal chloroplast without pyrenoid.
Morphometric data: L = 4.0 µm (distance between apexes); W = 2.0 µm.
Paraná State citation: first record.
Taxonomic remarks: recently the genus *Kirchneriella contorta* var. *elegans* (Playfair) Komárek changed to the taxon *Raphidocelis danubiana* var. *elegans*, which can be found in the literature in the first way.
Occurrence in samples: UNOPA 7005
Frequency of occurrence: rare

Selenastrum bibraianum Reinsch, Abh. Senckenb. Nat. Gesell., 3(2): 1- 238, 1866.
Figure 4: P
Coenobium formed by four to 16 lunate or semicircular cells; ventral margins strongly convex towards the center of the colony, tapering towards the cell apex; single parietal chloroplast occupying the entire intercellular space; pyrenoid not observed.
Morphometric data: L = 15.0 µm; W = 4.0 µm.
Paraná State citation: Cited as *Ankistrodesmus bibraianus* (Reinsch) Koršikov in Bortolini et al. (2010); Aquino et al. (2014).
Occurrence in samples: UNOPA 6827
Frequency of occurrence: rare

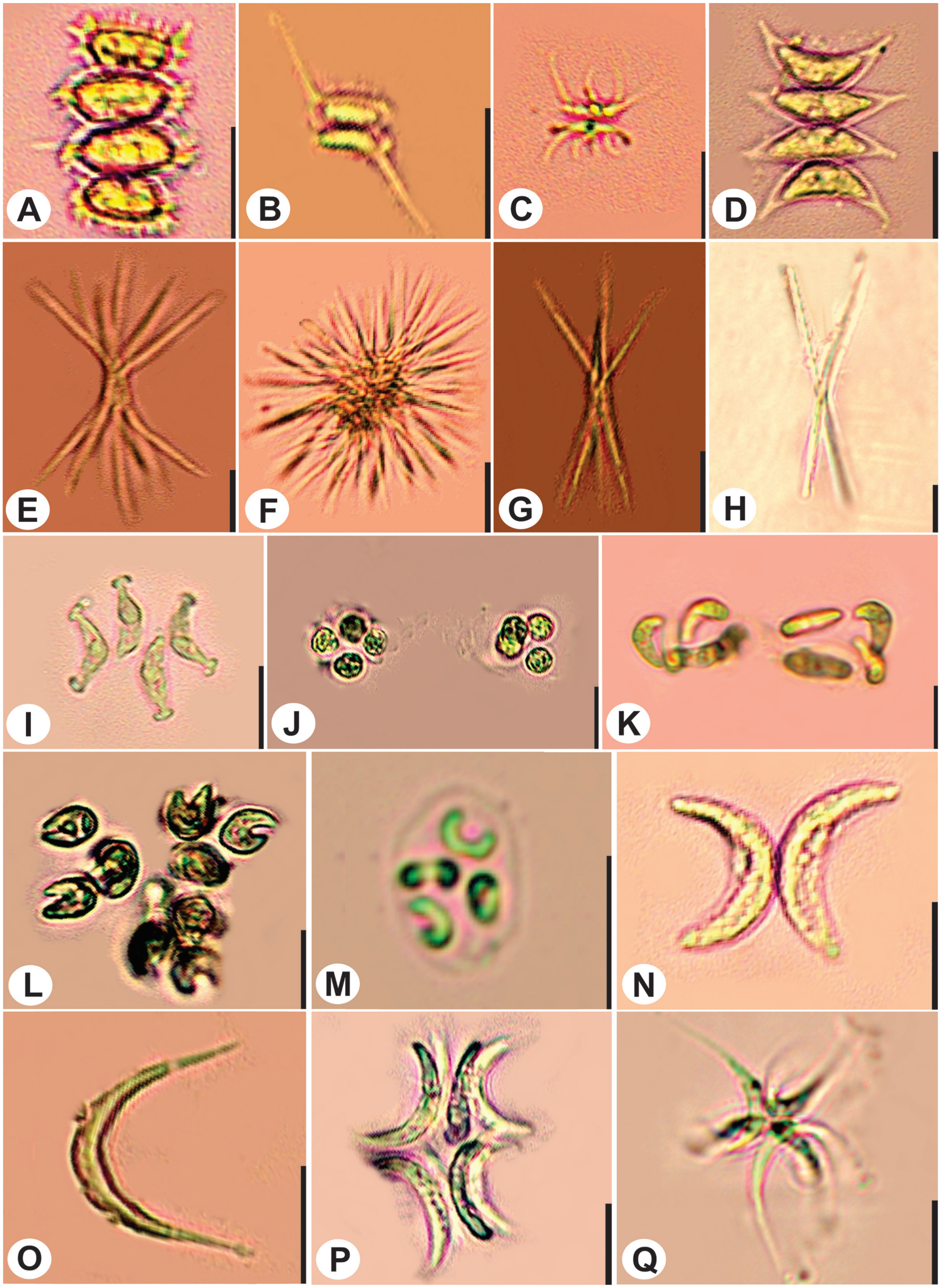

Figure 4. A. *Desmodesmus serratus.* B. *Desmodesmus intermedius* var. *acutispinus.* C. *Desmodesmus subspicatus.* D. *Tetradesmus dimorphus.* E. *Ankistrodesmus bernardii.* F. *Ankistrodesmus densus.* G. *Ankistrodesmus falcatus.* H. *Ankistrodesmus fusiformis.* I. *Scenedesmus indicus.* J. *Westella botryoides.* K. *Tetrallantos lagerheimii.* L. *Kirchneriella lunares.* M. *Raphidocelis danubiana* var. *elegans.* N. *Monoraphidium capricornutum.*O. *Monoraphidium caribeum.* P. *Selenastrum bibraianum.* Q. *Selenastrum rinoi.* Scales =10 µm.

Selenastrum rinoi Komárek & Comas, 272, 276, figure 10, 1982.
Figure 4: Q
Colonies formed by four moon cells; convex side of cells oriented towards the center; apexes tapered; chloroplast without pyrenoid.
Morphometric data: L= 16.0 μm; W = 5.0 μm.
Paraná State citation: first record.
Taxonomic remarks: *Selenastrum rinoi* can be confused with *Monoraphidium capricornutum*, however, it differs in that it has smaller cells, with more distant apices, in addition to having an irregular arrangement of cells in the colony.
Occurrence in samples: UNOPA 6827
Frequency of occurrence: rare

The trophic state index indicated that seven of the nine sampled municipalities fall into oligotrophic conditions, while the remaining two are considered mesotrophic. Regarding the limnological conditions we observed a variation in the flow of the sampled rivers, where the lowest flow recorded is in Rio Tamanduá (0.09 m^3 s) and the highest is in Rio Toledo (1.39 m^3 s). Water temperature varied from 15°C to 20°C in Baú and Jacutinga Rivers respectively. The pH value in all sampled rivers was considered neutral (6). The electrical conductivity varied from (0.02 mS cm^{-1}) in the Toledo River to (0.08 mS cm^{-1}) in the Baú River. As for turbidity, the lowest value recorded was in Rio Baú (6.41 NTU) and the highest in Rio Jacutinga (23.35 NTU). Dissolved oxygen varied from (6.39 mg L^{-1}) in Gonçalves Dias River to (14.95 mg L^{-1}) in Baú River. The lowest value of ammonia nitrogen (0.01 mg L^{-1}) was constant in four rivers, namely Baú, Itaguaçu, Gonçalves Dias, and Jacutinga, while the highest value (0.14 mg L^{-1}) was registered in the Cascavel River. The values of total phosphorous were similar in all sampled rivers. Nitrate varied from (0.53 mg L^{-1}) at Gonçalves Dias to (1.83 mg L^{-1}) at Toledo River (Table 2). All sampled rivers had at least two species recorded, however, approximately 70% of the occurrences were in oligotrophic environments. The rivers with higher occurrences recorded were Cascavel (31%), Passo Liso (23%) and Toledo (17%), the first two being oligotrophic and the third mesotrophic.

Table 2. Occurrence of species, ecological data and trophic state. GUAR: Guaraniaçu, MED: Medianeira, TOL: Toledo, TBP: Três Barras do Paraná, CVEL: Cascavel, FOZ: Foz do Iguaçu, STO: Santa Tereza do Oeste, CTD: Catanduvas, BVA: Boa Vista da Aparecida. OLIG: oligotrophic, MESO: mesotrophic.

Watershed	**Piquiri**	**Paraná III**		**Lower Iguaçu River**					
River	**Baú**	**Alegria**	**Toledo**	**Itaguaçu**	**Cascavel**	**Tamanduá**	**Gonçalves Dias**	**Passo Liso**	**Jacutinga**
Occurrence in samples	**GUAR**	**MED**	**TOL**	**TBP**	**CVEL**	**FOZ**	**STO**	**CTD**	**BVA**
Family Hydrodictyaceae									
Lacunastrum gracillimum			x					x	x
Pediastrum duplex			x		x				
Pseudopediastrum boryanum var. *longicorne*						x		x	
Stauridium tetras	x				x			x	
Family Neochloridaceae									
Golenkinia radiata	x	x						x	
Family Radiococcaceae									
Radiococcus skujae					x				
Family Scenedesmaceae									
Coelastrum astroideum				x	x			x	
Coelastrum microporum					x			x	
Coelastrum proboscideum			x					x	
Coelastrum pulchrum					x				
Coelastrum reticulatum var. *cubanum*			x		x	x		x	
Comasiella arcuata var. *platydisca*					x			x	
Desmodesmus armatus						x		x	
Desmodesmus brasiliensis			x						
Desmodesmus communis		x	x	x	x			x	x
Desmodesmus denticulatus			x		x			x	
Desmodesmus intermedius var. *acutispinus*					x				
Desmodesmus opoliensis								x	
Desmodesmus perforatus					x				
Desmodesmus serratus			x		x				
Desmodesmus subspicatus			x						
Scenedesmus indicus			x					x	
Scenedesmus obtusus						x			
Tetradesmus dimorphus					x				
Tetrallantos lagerheimii			x				x		
Westella botryoides	x							x	

Continue...

...Continuation

Watershed	Piquiri	Paraná III		Lower Iguaçu River					
River	**Baú**	**Alegria**	**Toledo**	**Itaguaçu**	**Cascavel**	**Tamanduá**	**Gonçalves Dias**	**Passo Liso**	**Jacutinga**
Occurrence in samples	**GUAR**	**MED**	**TOL**	**TBP**	**CVEL**	**FOZ**	**STO**	**CTD**	**BVA**
Family Selenastraceae									
Ankistrodesmus bernardii					x				
Ankistrodesmus densus					x	x			
Ankistrodesmus falcatus					x				
Ankistrodesmus fusiformis						x	x		
Kirchneriella lunaris					x	x		x	
Monoraphidium capricornutum					x				
Monoraphidium caribeum			x						
Raphidocelis danubiana var. elegans						x			
Selenastrum bibraianum					x				
Selenastrum rinoi					x				
Ecological data									
Flow (m^3 s)	0.14	0.14	1.39	0.20	0.41	0.09	0.29	0.22	0.51
Water Temperature (°C)	15.83	19.23	17.87	18.18	18.27	19.46	19.54	17.22	20.36
pH	6.8	6.99	6.33	6.74	6.27	6.40	6.89	6.71	6.95
Conductivity (mS cm^{-1})	0.08	0.03	0.02	0.07	0.05	0.04	0.03	0.07	0.07
Turbidity (NTU)	6.41	9.30	19.63	9.44	8.29	18.18	9.84	7.40	23.35
Dissolved oxygen (mg L^{-1})	14.95	6.77	8.88	7.84	8.77	7.19	6.39	8.09	9.97
Ammoniac nitrogen (mg L^{-1})	0.01	0.02	0.03	0.01	0.14	0.08	0.01	0.02	0.01
Total phosphorous (mg L^{-1})	0.02	0.02	0.04	0.03	0.02	0.04	0.02	0.03	0.02
Nitrate (mg L^{-1})	0.74	1.02	1.86	1.7	1.3	1.51	0.53	1.1	1.07
Trophic state	OLIG	OLIG	MESO	OLIG	OLIG	MESO	OLIG	OLIG	OLIG

Discussion

In our study we recorded 36 taxa of chlorophyceans belonging to the order Sphaeropleales. The Scenedesmaceae family was the most representative with 20 taxa (55%), followed by Selenastraceae with 10 taxa (27.7%) and Hydrodictyaceae with 4 taxa (11%). We also highlight the record of 10 new citations for the State of Paraná, thus expanding the knowledge of the flora of Chlorophyceae for lotic environments: *Ankistrodesmus bernardii*, *Desmodesmus perforatus*, *Desmodesmus subspicatus*, *Monoraphidium capricornutum*, *Monoraphidium caribeum*, *Pseudopediastrum boryanum* var. *longicorne*, *Raphidocelis danubiana* var. *elegans*, *Radiococcus skujae*, *Scenedesmus indicus* and *Selenastrum rinoi*. The class Chorophyceae develops in wide environmental variation, but they are important in oligotrophic aquatic environments and described as cosmopolitan, being bioindicators of water quality (D'Alessandro & Nogueira 2017).

The genus with the highest number of taxa was *Desmodesmus* with 9 species (25%), followed by *Coelastrum* with 5 species (13%) and *Ankistrodesmus* with 4 species (11%). The higher occurrence of *Desmodesmus* compared to the others may be associated with the fact that this genus is common in most aquatic environments, from eutrophic to oligotrophic (Borges et al. 2008, Hentschke & Torgan 2010, Domingues & Torgan 2012, Rosini et al. 2012, Aquino et al. 2014), which can be observed in the Cascavel (Municipality of Cascavel) and Arroio Passo Liso (Municipality of Catanduvas) rivers, both in the watershed of the lower Iguaçu River, considered oligotrophic where high contributions of the genera *Coelastrum* sp. and *Desmodesmus* sp. were observed.

Among the 36 taxa identified in this study, five species occurred only under mesotrophic conditions, namely: *Desmodesmus brasiliensis, Desmodesmus subspicatus, Scenedesmus obtusus, Monoraphidium caribeum*, and *Raphidocelis denubiana* var. *elegans*. The occurrence of *Desmodesmus* in nutrient-rich environments, as mentioned earlier, is due to it being a genus adaptable to diverse environmental conditions, being common at all trophic levels (Phinyo et al. 2017). The genus Scenedesmus has already been associated with sites with mild to moderate organic pollution, warning for environments with tendencies to elevate their trophy (Sabkie et al. 2020). We can also mention *Monoraphidium caribeum*, which, although cosmopolitan, is found mostly in eutrophic aquatic environments. The other occurrences were in oligo to mesotrophic conditions, which is in accordance with what is expected for the group (Comas 1996).

The Cascavel River showed the highest species richness (21 taxa). This environment was related to one of the lowest values of turbidity (8.29 NTU) in the area sampled. The Jacutinga River had the highest value for this variable (23.35 NTU), represented by only two species. The correlation of turbidity with Chlorophyceae representatives has been reported previously, where this variable negatively influenced the diversity of the group (Gogoi et al. 2019; Kumar et al. 2020). This relationship is due to the limitation of light penetration into the water, which reduces phytoplankton photosynthesis, in addition to altering the other limnological conditions (Nunes et al. 2022).

In our study, 94% of the taxa found had a rare frequency of occurrence, and only 6% were classified as sporadic. The rarity of the species in lotic environments may be related to the local hydrodynamics, where the greater flow of water prevents the establishment and development of potamoplankton, promoting a constant transport of taxa downstream (Medeiros et al. 2020). Phytoplankton is considered a group of key organisms to indicate changes in aquatic environments due to changes in the structure of biota, in response to physical and chemical factors in water bodies (Wojciechowski et al. 2017).

Since 2007, 91 taxa have been recorded for the Chlorophyceae class in the State of Paraná, and according to the flora of Brazil (2020), 362 species were recorded throughout the country, with publications in São Paulo and Rio de Janeiro predominating, thus showing the need for further studies in Paraná. In conclusion, we can emphasize the importance of taxonomic studies, which serve as a basis for knowledge of biodiversity, providing essential information for ecological, bioindicator and conservation approaches.

Acknowledgments

The authors would like to thank Fundação Araucária/Companhia de Saneamento do Paraná for the scientific initiation grant to Maria Clara Pilatti, Fundação Araucária/Companhia de Saneamento do Paraná for a master's grant to Thais Tagliati da Silva, the Coordination for the Improvement of Higher Education Personnel for the doctoral scholarship of Dra. Gabriela Medeiros, to Fundação Araucária/Coordination for the Improvement of Higher Education Personnel for the master's scholarship to Mailor Wellinton Wedig Amaral,) Dra. Norma Catarina Bueno thanks Fundação Araucária/Companhia de Saneamento do Paraná for funding the research through the call public 26/2018 – Paraná Environmental Sanitation Research Program (PPPSA).

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Maria Clara Pilatti: contributed to the data collections; data analysis and interpretation; critical revision and manuscript preparation, all adding intellectual content.

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Conflicts of Interest

The authors declare that they have no conflict of interest related to the publication of this manuscript.

Data Availability

The data used in our analysis is available at https://collectory.sibbr.gov.br/collectory/public/show/co468?lang=pt_B

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Received: 17/11/2022
Accepted: 29/05/2023
Published online: 10/07/2023

Biota Neotropica 23(2): e20221458, 2023
www.scielo.br/bn

biotaneotropica
ISSN 1676-0611 (online edition)

Point-of-View

A decade of Zoology Summer Course: impressions and impacts of the first university extension course on Zoology in Brazil

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SOARES, K.D.A., BRANDÃO, I., PEREIRA, J., GOMYDE, E., PESSOA-SILVA, M., RIBEIRO, G., ZANINI, F., GROSSEL, L.A. **A decade of Zoology Summer Course: impressions and impacts of the first university extension course on Zoology in Brazil**. Biota Neotropica 23(2): e20221458. https://doi.org/10.1590/1676-0611-BN-2022-1458

Abstract: Although the diversity of animal groups distributed in Brazil provides countless research opportunities, the current scenario does not follow this demand. The reasons for the disconnections range from inequality in the availability of resources for teaching and research to the focus of researchers on specific groups of animals, while others remain neglected. Training new potential Brazilian researchers interested in Zoology is essential for a greater understanding of this diversity, as well as exposing those potential new researchers to new groups and different work possibilities. Thus, the Summer Course in Zoology (in Portuguese, CVZoo) promoted by the Graduate Program in Zoology at the University of São Paulo, over the last ten years, has been seeking to contribute to this training of new researchers in the field of Zoology, as well as in updating teachers through university extension activities. In order to assess the impacts caused by CVZoo on the academic and professional training of the participants, Google forms were sent to participants in the ten editions of the course, as well as compiled information available on the Lattes Platform. Qualitative and quantitative analyses showed the profile of graduates, their expectations, and perceptions about the course. Based on these data, we demonstrate the CVZoo's efficiency in popularizing Zoology throughout the country in contributing to the decentralization of knowledge as well as in meeting the urgent concerns of making access to knowledge more egalitarian and socially fair.
Keywords: *Biodiversity; University extension; Student training; Graduation.*

Uma década de Curso de Verão em Zoologia: impressões e impactos do primeiro curso de extensão universitária sobre Zoologia no Brasil

Resumo: Embora a diversidade de grupos de animais existentes no Brasil ofereça inúmeras oportunidades de estudo, o cenário atual não acompanha essa demanda. Os motivos para essa desconexão vão desde a desigualdade na disponibilidade de recursos para ensino e pesquisa até o foco de pesquisadores em grupos específicos de animais, enquanto outros permanecem negligenciados. O treinamento de novos pesquisadores interessados em Zoologia é essencial para um maior entendimento da diversidade brasileira, assim como a exposição de tais pesquisadores a novos grupos e diferentes possibilidades de trabalho. O Curso de Verão em Zoologia (CVZoo) promovido pelo Programa de Pós-graduação em Zoologia da Universidade de São Paulo, ao longo de dez anos vem buscando contribuir para a formação de novos(as) pesquisadores(as) na área da Zoologia, bem como na atualização de docentes do Ensino Básico por meio de atividades de extensão universitária. Para avaliar os impactos causados pelo CVZoo na formação acadêmica e profissionalizante dos participantes, foram enviados formulários aos participantes das dez edições do curso, bem como compiladas informações disponíveis na Plataforma Lattes. Análises qualitativas e quantitativas evidenciaram o perfil das pessoas egressas, suas expectativas e percepções acerca do curso oferecido. Com base nesses dados, é apontada a eficiência do CVZoo na popularização da Zoologia por todo o país, contribuindo para a descentralização do conhecimento, bem como atendendo às preocupações prementes de tornar o acesso ao conhecimento mais igualitário e socialmente justo.
Palavras-chave: *Biodiversidade; Extensão universitária; Formação discente; Pós-graduação.*

Introduction

1. Research and teaching in Zoology in Brazil

Brazil is a megadiverse country that concentrates in its territory a unique diversity of several animal groups (Mittermeier et al. 1997). Lewinsohn & Prado (2002) estimated that there are between 170 and 210 thousand known species in our country, a number that has been increasing significantly in the last twenty years. However, there is still a long way to go, since estimates suggest the existence of a number seven times greater than the currently described species (Lewinsohn & Prado 2005). In addition to the species that remain without proposed names, an extensive body of knowledge still awaits to be revealed.

Given the potential load of knowledge that this diversity represents, Zoology emerges as an area of knowledge with the purpose of cataloging and understanding both current and extinct animal diversity. The area can be subdivided into several subareas, one of which is Systematic Zoology, whose objectives are to understand the evolutionary history of species and propose hypotheses to name and classify them. However, although more than 500 Brazilian researchers call themselves "systematists" and "taxonomists", they are unevenly distributed, mostly concentrated in the Southeast (about 50%) and South (20%) regions, with emphasis on the states of São Paulo, Rio de Janeiro, Paraná and Rio Grande do Sul (Marques & Lamas 2006). This is quite inconsistent with the diversity of biomes and specialized fauna found in each of the country's regions, and the potential for discovering new species in each of them.

Similar geographic patterns are observed in scientific production in Zoology, with the Southeastern holding the highest part of productivity (70% of papers and 75% of citations) and in graduate programs in the area, in which the South and Southeast regions concentrate most of them (approximately 70% for masters and PhDs; data extracted from Marques & Lamas 2006). However, if we look at federal investment in university projects, we find that the South and Southeast regions once again hold most of the research funds, which include the provision of scholarships for students, and result in greater adherence and academic productivity (Marques & Lamas 2006).

Faced with this unequal scenario of Zoology development in Brazil, the creation and execution of actions that equalize knowledge, teaching and scientific productivity across the country are urgently needed. Among the actions proposed by Marques & Lamas (2006), there are suggestions aimed at training new professionals in different regions of the country, increasing scientific production and disseminating knowledge to different audiences. The offering of specialization courses in meetings and scientific events are also mentioned (Marques & Lamas 2006), but extension projects with the participation of the university community were not considered as one of the possible agents for the expansion and decentralization of Zoology in Brazil.

The Summer Course in Zoology (in Portuguese, CVZoo), created and organized by students of the Graduate Program in Zoology at the University of São Paulo, stands out as an important milestone for university extension in Zoology in Brazil. Below is a brief history of the course.

2. History of CVZoo

The Summer Course in Zoology began in January 2013, organized by students from the Graduate Program in Zoology (PPGZOO) at the University of São Paulo and supervised by Prof. Dr. Alessandra Bizerra. Initially, the course had the following objectives: 1) to disseminate the research lines in Zoology developed by students of the graduate program and 2) to provide teaching practice experiences and thus fill a gap in the professional training of such students (Soares et al. 2020).

The course lasts for two weeks, the first one dedicated to classes on general topics in Zoology, such as Systematics, Philosophy of Science, Animal Behavior and Biogeography, and the second one containing activities with more specific subjects. Despite the fact that the first week of the course has changed little over time (with the exception of the remote editions that occurred in 2021 and 2022), the second week has changed considerably. In the first four editions, participants were divided into three groups, considering their research groups – Vertebrates, Panarthropoda and Non-Panarthropoda - and the activities were carried out jointly. Since the fifth edition, such a division into three groups no longer occurred and students began to assemble their own grid, choosing from several options of workshops and short courses on taxonomic groups (e.g., Annelida, Arthropoda and Chondrichthyes) and research and teaching methods.

As of the third edition, the selection process for participation in the course began to consider the proportion of enrollments coming from the five regions of Brazil (Midwest, Northeast, North, Southeast and South), thus seeking to expand knowledge to more people. From the fifth edition onwards, teachers became part of the course's target audience, participating in workshops in the second week and developing a research project or didactic sequence. Thus, updating knowledge in Zoology for teachers was included as one of the objectives of the course. More detailed information about the participant selection process can be found in the work of Soares et al. (2020).

Since the first edition of the course, members of the organizing committee have sought various ways to raise funds and thus partially or fully defray the cost of accommodation at the Sports Practices Center of the University of São Paulo (in Portuguese, CEPE-USP) and meals at the university restaurant. In this way, the principal aim is to contribute to reducing expenses and facilitating access for students from more distant regions and in less favorable socioeconomic conditions.

As an evaluation criterion, course participants are invited to develop over the two weeks a research project in the format of a master's degree, on a topic within Zoology under the supervision of members of the course organizing committee. On the last day of the course, the projects are presented and evaluated by an examining board, composed of members of the organizing committee not involved in the development of the projects. The participation and frequency of the participants are also considered as an evaluation criterion and make up the final grade.

In ten editions, 460 students from different regions of Brazil and other Latin American countries (e.g., Peru, Colombia) were selected to participate in the course (Table 1), among more than 4,500 enrollments. Over time, adjustments in the number of vacancies were necessary to meet the growing demand for registrations. The offer of vacancies doubled between the first and tenth editions, going from 30 vacancies in 2013 to 60 in 2022, with numbers of people registered above 400 in all editions from the fourth.

Given the already exposed need to provide access to Zoology teaching and equalize the generation of knowledge throughout the country, and considering the ten years of application of an extension course with concerns beyond content, this study had the following objectives: 1) raise and evaluate the profile of the certified participants

Table 1. Number of enrollments and participants selected by course edition.

Edition	N enrollments	N students	N teachers
I	200	30	0
II	364	35	0
III	206	35	0
IV	732	40	10*
V	599	40	10
VI	493	40	15
VII	499	40	15
VIII	400	40	15
IX	624	55	0
X	602	48	7
Total	4.719	403	62

who helped build CVZoo over ten years, 2) investigate their motivations, expectations and evaluations, 3) evaluate the impacts of the course on the academic and professional training of the certified participants.

Material and Methods

To profile the course concluding participants, data on the academic background of them (degrees obtained, universities, region and animal phylum studied) were obtained through the Curriculum Lattes Platform. Only participants who passed the course and received certification were considered.

Two questionnaires were developed, one for participants selected as undergraduate students and the other for participants selected as teachers. Both contained multiple-choice and essay questions and were divided into three parts (Appendix 1). Only the first part had the same content in both questionnaires, being focused on the profile and self-identification of the graduates of the course (e.g., nationality, race, sexual orientation and gender identity) as well as on the research area and current institution. These data allowed us to obtain additional information regarding the profiles of participants. In the second part of the questionnaire addressed to the students, the questions dealt with motivations and expectations related to CVZoo and impressions about workshops and the process of developing a research project. In the second part of the teachers' questionnaire, motivations and expectations were also questioned, as well as the relationship between the topics covered and the school environment. In the third part, students were asked about the influence of CVZoo on academic life (research and extension) while teachers answered questions about teaching and prospects for pursuing an academic career. In order to understand how the target audience has been informed about CVZoo activities and editions, the third part of the questionnaire also included, for both categories, a question about the method by which the participant became aware of the course, involving all means of dissemination incorporated throughout editions (social networks, website, email list and through undergraduate colleagues).

The questions were arranged in Google Forms and sent to students and teachers who concluded in CVZoo on two different occasions. The first research round took place from February 20th to April 19th, 2018 (contemplating certified participants from the first six editions) and the second, from March 4th to June 4th, 2022 (contemplating certified participants from all course editions). For certified participants who answered the forms on both occasions, only the second answer was considered, as it was the most recent, thus excluding the possibility of double entries for the same participant in the quantitative analyses; in the qualitative analyses, both responses were considered. The total (n) of responses for each question on the form was treated independently, so that questions left unanswered by any respondent did not interfere with the calculations for other questions. The publication of the data provided here was authorized by the respondents.

The data obtained through the Curriculum Lattes Platform were compiled in a spreadsheet and standardized (Appendix 2). We categorized the information about the studied phyla following the names of the phyla, when dealing with specialized studies (for example Annelida, Arthropoda and Chordata), and when dealing with less specific studies or involving more than one phylum, we used other three categories: Fauna (for studies with more than one phylum, or communities such as meiofauna or zooplankton); Protists (for studies with unicellular eukaryotes such as foraminifera); and Others (for studies on other topics, not related to metazoans). Similarly, due to the diversity of graduate programs and the number of graduates in each program, we chose to categorize this information by related areas, thus obtaining the following categories of graduate programs: Animal Biology, Biodiversity and Conservation, Biology, Ecology, Teaching, Entomology, Oceanography, Systematics, Zoology and Others (including areas less related to Zoology, such as Botany, Bioinformatics and Genomics, Biochemistry, Ethnobiology, Geology, Museology, among others).

Frequencies of each category and their changing patterns over the ten CVZoo editions were analyzed and described. To compare whether there was a difference in each category (race, gender identity and sexual orientation) over the years, we applied a chi-square test, considering a significance level of 0.05. The answers to the discursive questions were analyzed using content analysis procedures as parameters (Bardin 1977).

Results

We were able to locate Curriculum Lattes data from 371 concluding participants of the course and of these 193 responded to the Google forms.

1. *Profile of participants*

According to data collected from the Lattes Platform, most CVZoo participants came from the Southeast and Northeast regions of the country. While nearly 50% graduated from universities in the Southeast and more than 20% from universities in the Northeast, less than 10% came from each of the other regions of Brazil (Figure 1a).

A similar pattern is observed when we analyze the regions where graduates have completed master's degrees because the Southeastern and Northeastern together account for more than 65% of graduates who attended a master's degree (Figure 1b). However, this pattern changes significantly for the Doctorate course, given that most students (>65%) who continued their studies at the Doctorate level attend or have attended universities in Southeast Brazil (Figure 1c). In the master's degree, some universities concentrate a higher percentage of students. In doctorates, this concentration is even more drastic, with only three universities (UFRJ, UNICAMP and USP) concentrating more than 40% of graduates who are studying or have finished PhD. Altogether,

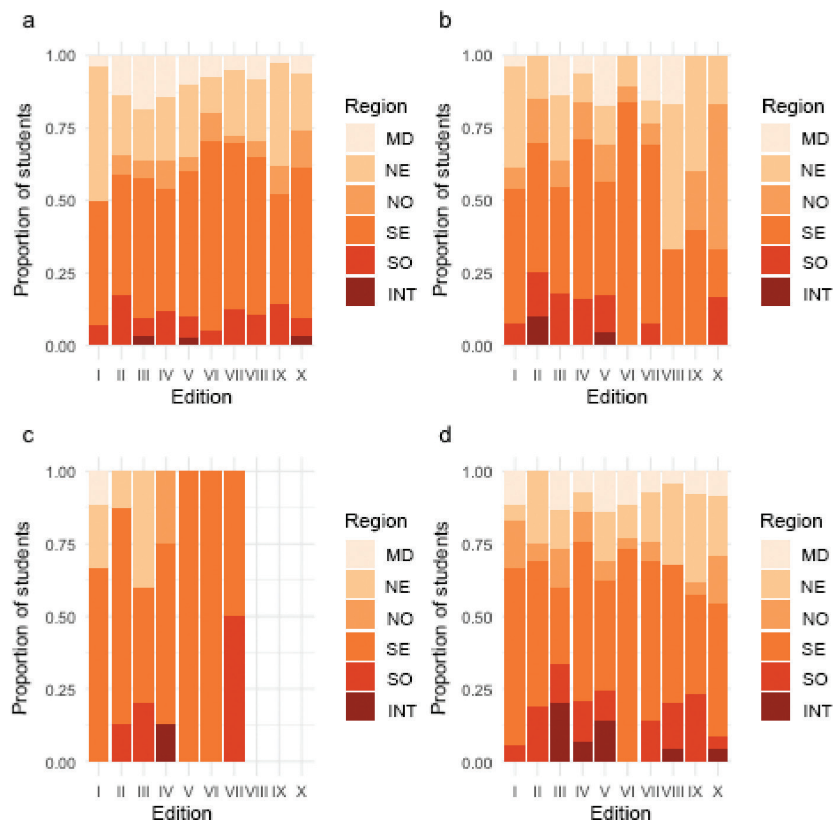

Figure 1. Origin of participants of the 10 editions of the Summer Course in Zoology during the undergraduation period (a), masters (b), doctorate (c), and the current address (d). INT, International; MD, Midwest; N, North; NE, Northeast; SE, Southeast; S, South.

more than 45% (n = 170) of CVZoo concluding participants continued their studies at least at the Master's level, 10% (n = 38) continued at the Doctorate level and two participants at the post-Doctorate level.

Over 30% of CVZoo concluding participants have attended in programs focused on areas related to Zoology (Figure 2) at both levels (master's and doctorate), most of them at the University of São Paulo (USP). Other USP programs also received graduates from CVZoo, such as the graduate programs in Systematics, Animal Taxonomy and Biodiversity (STAB), Ecology, Entomology, Biological Oceanography and Science Teaching. Such results demonstrate that the course has played a decisive role in attracting new students to postgraduate courses at USP. Another interesting fact about the destination of graduates from the course is the diversity of insertion areas. In addition to the postgraduate programs totally focused on the study of animals, such as the Animal Biology, Entomology and Zoology programs, we also observed many graduates, with "zoological" lines of research, but inserted in other programs, such as Ecology and Biodiversity and Conservation. The programs farthest from zoology, such as Genetics and Evolution, Biosystems, Tropical Diseases or Biotechnology, for example, were all compiled in the category "Others". When analyzing the focal phylum, in all CVZoo editions and throughout the various training levels, more than 60% of participants were interested in Chordata or Arthropoda, while only a tiny percentage of graduates dealt with the study of other animal phyla (Figure 3).

According to the answers obtained through the forms on students' race (n = 153), the majority declared themselves as white (62.1%), followed by brown (24.8%), black (11.1%) and other races (2%). These proportions differ substantially from the Brazilian population, of which 54% of the Brazilian population declares itself to be black, including a broad spectrum of skin colors (IBGE 2019). We also note that proportions vary over the editions (Figure 4a). Among teachers (n = 11), most self-declared as brown (63.6%) (Figure 4b).

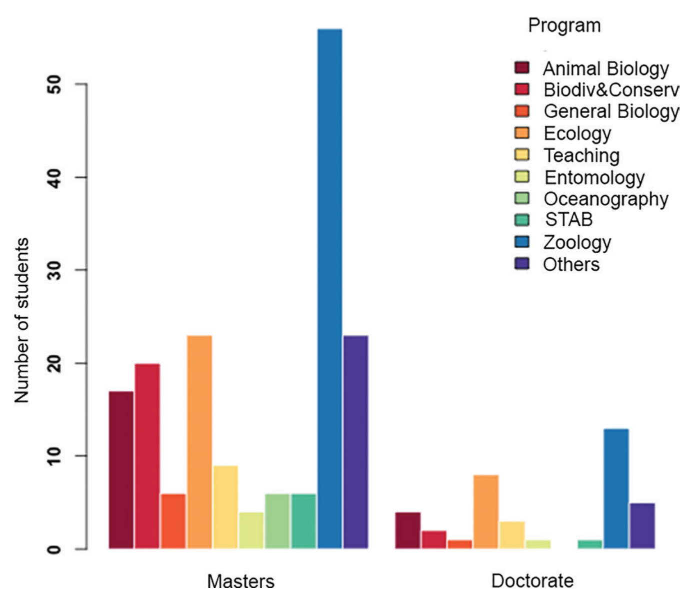

Figure 2. List of focal phyla at different levels of training of course egresses.

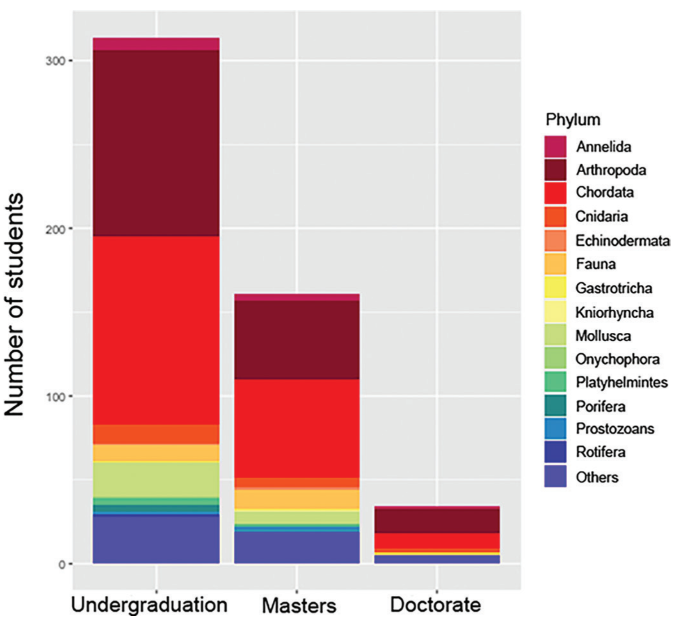

Figure 3. Classes of graduate programs in which CVZoo graduates entered. "Biodiv & Conserv" includes programs focused on Biodiversity and Conservation; "STAB" corresponds to the graduate program in Systematics, Animal Taxonomy and Biodiversity, at the Zoology Museum of USP.

Regarding the gender identity of students (n = 150), the proportion of cisgender men (49.3%) and women (50.7%) varied little over the ten years of CVZoo (Figure 4c) but the representation of transgender students is still low (n = 3). Among teachers (n = 11) women were more numerous (72.7%) than men (27 .3%) (Figure 4d). On the other hand, a great diversity is observed regarding the sexual orientation of those certified participants (n = 153). Heterosexual students make up the majority of those participants (55.5%), followed by bisexuals (22.2%), homosexuals (20.3%) and asexuals (1.3%); only one student did not want to inform his sexual orientation (Figure 4e). Likewise, diversity differs significantly between teachers (n = 12), with 75% declaring themselves to be heterosexual, 16.7% bisexual and 8.3% homosexual (Figure 4f). None of the respondents declared having any disability.

2. *Publicizing the CVZoo*

The most effective ways of publicizing the course have included announcements on social media (49.5%) and referrals to friends and fellow graduates (47.4%). Disclosure through social media has changed over the years. Following the progress and adherence to different forms of virtual communication, especially by the targeted audience, social networks such as Facebook and the course website itself have been more effective in the past (Soares et al. 2018), while Instagram has been the network responsible for greater adherence of subscribers in the last three editions (2020, 2021 and 2022). This highlights the importance of considering the advent of new social communication tools and understanding what content is consumed by users (Soares et al. 2018), as they can increase the reach of the course in future editions. Additionally, many enrollees came to know about CVZoo through the indication of former participants, which provides indications of the satisfaction of these graduates regarding the quality of the course offered throughout all editions, since the indications have remained stable over these ten years.

3. *General impressions about the course*

The course was well rated by the participants (students: n = 192; teachers: n = 19) since 73.5% of the students and 63.2% of the teachers stated that their initial expectations were exceeded, 23.4% of the students and 36.8% of the teachers felt that the initial expectations were met, while for 3.1% of the students the initial expectations were only partially met.

The positive points most mentioned by the respondents were the contact with people from different regions of Brazil (25.3%), the content offered and the quality of the course programming (23.9%), the motivation to enter graduate programs and pursue an academic career (22.5%), and the approximation with students and professors of the graduate programs in Zoology and in Systematics, Animal Taxonomy, and Biodiversity and the lines of research developed at USP (21.1%).

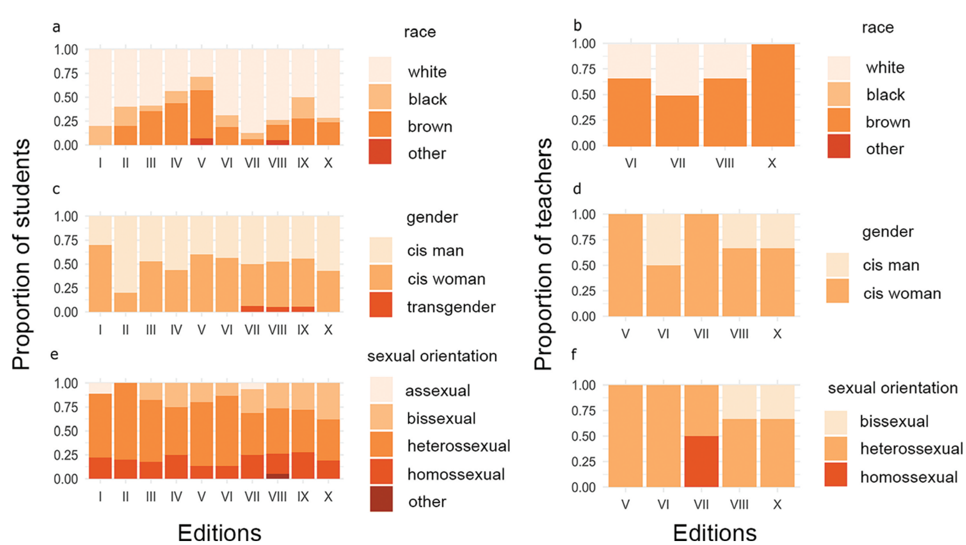

Figure 4. Profile of students (left panel) and teachers (right panel) enrolled over the 10 editions of the Summer Course in Zoology, regarding race (a, b), gender identity (c, d) and sexual orientation (e, f).

These points are in accordance with the extension guidelines established for these activities (FORPROEX 2012).

Regarding the dissemination of research carried out at the university, visits to the IB-USP laboratories and MZUSP collections were cited by 12.8% of the respondents, and their importance is represented in the speech of a participant who claims to have known approaches within the area, which she intends to use in the future when she enters a graduate program. The activity of preparing a research project (5.6%) was pointed out by some respondents as a very positive and challenging point of the course. Even so, it contributed to the development of critical thinking about the work of colleagues and articles already published, in addition to the development of scientific thinking of participants involved in the process. One of the participants commented that she was "fearful" because of the project, "I thought I wouldn't be able to develop it, but I found out that it is an essential part of the entire course process." This denotes how part of teaching still remains dissociated from research and how the gradual insertion of undergraduates in Scientific Initiation (CI) activities can facilitate their understanding of the production of knowledge through the scientific practices (Massi & Queiroz 2010).

Some respondents praised the classes and teaching strategies employed (15.5%) and the dedication of the organizing committee (11.2%). The cost of accommodation and food was cited by 4.2% of the participants, and one of them reported that "the possibility of staying in accommodation and food in the university restaurant were decisive points, because at that time he lived in a state very far from Sao Paulo". This shows that efforts to popularize university extension need to be linked to offering equal conditions for access by all. However, 8.4% of respondents highlighted the need for improvements in CEPE-USP housing facilities.

For some, the environment provided by the course was quite enriching (10%) due to the exchange of experiences and their influence on their academic training. According to one of the respondents, the course would have been a great "watershed". The marked influence of the course is present in the response of another participant, in the following passage: "whenever I teach a short course, I remember how I experienced CVZoo, the enchantment that students have with us, they were the same as I experienced when I was a CVZoo student".

Among the negative aspects of the course, many responses included the appeal for a longer duration of the course (12.6%), suggesting at least one additional week. The remote offering of the last two editions (IX and X), due to the COVID-19 pandemic, was mentioned as a negative point by the participants (5.6%) due to the desire that the activities could have taken place in person. Even so, one of the respondents' comments: "the online format had a positive side because I was able to participate even though I lived far away, but the negative side was the difficulty in concentrating and the tiredness I felt from sitting all day in front of the computer".

4. *Permanence in the academic environment and impacts on research*

Among students, 91.1% stated that CVZoo influenced their permanence or progress in the academic environment (n = 191), and 73.7% confirmed that the knowledge acquired during the two weeks of the course was applied in some way in their research projects developed later (n = 133). Among the acquired knowledge most cited by respondents are procedures and techniques (e.g., statistical analysis, electron microscopy, ecological niche models) and theoretical content such as those related to molecular biology, geometric morphometry, taxonomy, systematics, and scientific writing. For one respondent, the course was important for "creating the habit of studying Philosophy and understanding my research in the Epistemological sense and developing Integrative Taxonomy".

About half of the students (48.4%) highlighted that the project development experience helped in the elaboration of future projects, and 32.3% of the students applied the proposal or part of it later, in activities of CI or even in the selection for graduate studies (i.e., master's and doctorate). One student stated that during the project's elaboration he was introduced to a methodology that he did not yet know, scanning electron microscopy (SEM), and that he later used it in his own master's research project. About 52% of the students claimed that they had not executed the project due to lack of opportunity, change of area or because they had not taken this specific project forward.

For 24 students, the positive results went beyond the practical application, with 17 highlighting the networking developed with members of the course committee and professors at USP and the possibility of getting to know the scientific routine more deeply. Two students mentioned that their advisors at CVZoo were part of their TCC evaluation panel and two others mentioned that their advisors at CVZoo are currently helping with their research projects in graduate school. One of the respondents stated that the course directly influenced the choice of his master's degree and the continuity of his academic career.

Nine students stated that the presentation to an evaluation panel, made up of CVZoo organizers, was an important preparatory experience for similar situations in the future. Terms such as "challenging", "dynamic", "instigating", "enriching", and "profitable" were used to describe the project development experience, demonstrating the good reception of the activity by the participants. Only 5 students claimed that they had not developed a project or did not remember carrying it out. Seven negative responses were observed regarding the development of the research project during the course and among these, two students claimed not to have had a specialist advisor in their animal group or research field.

Among the contents offered in the form of workshops and with the possibility of choice by the participants, those that stood out the most were: Systematics, Taxonomy, workshops of specific taxonomic groups, techniques (Software, MicroCT, Molecular Biology), and scientific writing/methodology. The reasons given by the respondents were learning useful tools, up-to-date information on poorly studied taxonomic groups, discovering new topics of interest, and teaching practices by the lecturers.

5. *Impacts on teaching practice and university extension*

For teachers, 72.2% stated that they had incorporated the knowledge obtained in the course into their teaching practice (n = 12), and 41.7% had implemented the didactic sequences presented at the end of the course (n = 5), which are equivalent to the project developed by undergraduate students. Three teachers preferred to take advantage of the CVZoo opportunity to develop research projects instead of teaching sequences.

Some teachers highlighted the importance of acquiring and updating knowledge in Zoology during the course to improve their classes. One teacher pointed out: "I already did practical classes using collected animals, seeing them with such diverse specimens inspired me to elaborate the classes with greater care. The postgraduate course in management and conservation of wild fauna that I had taken the previous year gained even more meaning." One teacher also commented that she discussed aspects of the research routine, such as collection and animal preservation techniques, with her students. It should be noted that specimens preserved in alcohol were used during practical classes, allowing not only contact with different groups of animals but also a reflection on their use in school spaces.

According to one of the respondents, classes on Biogeography and evolutionary processes were a watershed in her pedagogical practice, giving her greater confidence and autonomy to teach classes on these subjects. Another teacher highlighted that the way in which the contents were addressed in the course encouraged her to explore more teaching possibilities, such as working with drawings, collecting materials in the environment, visiting institutions, using and building objects (e.g., magnifying glasses, microscopes), use of media (e.g., podcast) and games. One teacher commented that she passed on the knowledge acquired during the course to colleagues in the Science area who did not participate in CVZoo, thus expanding the scope of the course and the knowledge that is worked on.

Regarding the engagement towards extension actions, 77.6% (n = 149) of respondents stated that CVZoo would have motivated their participation in other courses and subsequent extension activities. The awakening to university extension can be exemplified by the phrase of one of the respondents about the main motivation for continuing to carry out extension actions: "to perhaps generate the same impact that the course had on me". Among those who answered 'no' to the question (23.4%, n = 44), one of them commented that he already participated in extension activities before the course. Another respondent commented that "if there are more extension activities that show the population, especially young people, the importance of different types of knowledge, from there it is possible to create a new culture, in which the community supports and benefits from the work carried out in the universities". This in fact prevents teaching and research from becoming alienating practices when removed from society, or when exempt from reflections on the knowledge produced within academic walls, but which must be transmitted and discussed with communities (Santos et al. 2016).

The extension activities most cited by the respondents as those of interest and/or already carried out by them were: environmental education actions such as building vegetable gardens, carrying out trails and exchanging knowledge with traditional communities and in schools (23.7%), scientific dissemination by research groups, conservation projects and science museums (20%), and organization and monitoring of events (17%). In addition to CVZoo, respondents reported having participated in other university extension courses (20%) and also mentioned workshops and isolated lectures at their universities or nearby institutions (11.1%). One of the respondents reports that after participating in CVZoo, he began "looking for more extension courses from universities around the world, almost as if he had discovered a new way of interacting with people from other areas".

Discussion

1. CVZoo and the Brazilian scenario

Extension practices are strategic spaces for the implementation of interdisciplinary activities that promote greater contact between the subjects involved, with knowledge of reality being fundamental for the application of efficient methods that allow social transformation. Among the existing actions for the popularization and development of Zoology in Brazil, we present here the experience of the Summer Course in Zoology at USP, which over the course of 10 years has contributed to the training of students and teachers from different regions of the country.

The high proportion of course participants from Southeast and Northeast regions observed here is expected if we consider that these regions concentrate the largest portion of the population (42.1% and 27.8% respectively) (Artes & Unbehaum 2021) and the course has been held in the state of São Paulo. Almost half of concluding participants have continued their studies at Masters or Doctorate levels, a high proportion when compared to the national scenario, which can be justified by the bias of the selection process of course participants, which prioritizes candidates with greater interest in the academic career.

The great interest in Systematics and Taxonomy demonstrated by the participants is quite positive given the urgent need to awaken and train new professionals engaged in the description of biodiversity, including that of lesser-known groups (Marques & Lamas 2006). In addition, due to CVZoo's national coverage, we have increased the incentive to enter this sub-area of Zoology for students from all regions. Thinking of USP as a national reference in both research and teaching (EGIDA 2022), we feel that it is our responsibility to offer, in an extension format, the knowledge of techniques and tools that can be applied by young researchers from other universities spread around the country.

More than a third of Brazilian systematists are dedicated to the taxonomy of fish, mollusks, crustaceans and insects (Diptera, Hymenoptera and Coleoptera). Despite such groups being quite numerous in terms of species, other taxa of extremely rich invertebrates within Arthropoda, or even taxa beyond, such as Nematoda, lack specialists who can dedicate themselves to making their diversity known (Marques & Lamas 2006). CVZoo has actively participated in the popularization of zoological groups that are not numerically diverse (e.g., lophophorates and interstitial pseudocoelomates), and in encouraging research into these relatively understudied groups by including in its thematic grid workshops aimed at presenting the diversity and evolution of groups that are worked on by committee members (e.g., workshops about mammals, flatworms and annelids). In this way, we draw attention to these groups and indirectly fill possible gaps in the academic training of participants from universities without specialists in certain groups. Even groups that are not directly worked on by committee members are often addressed in classes on broad topics (e.g., Metazoa). The University of São Paulo has a privileged didactic collection of zoological material, including specimens of rare groups of non-panarthropod invertebrates that would hardly be seen in another university environment, which is why the promotion of activities involving these animals increases the notion of biological diversity by the course participants.

2. University extension as a path for social transformation

More than half of CVZoo concluding participants have declared themselves as white, which does not reflect the existing racial scenario in the country, in which self-declared brown and black people make up 56.2% of the Brazilian population (IBGE 2019). Considering the Southeast and Northeast regions alone as the most representative of the students enrolled in the course, we have brown and black people constituting 48.9% of the population in the Southeast, and 74.4% in the

Northeast (IBGE 2019). However, when we visualize the national scenario of higher education, we see proportions corresponding to those obtained here, including promising estimates of the decrease in the difference between white and black students over the years. In 1993, black people constituted only 18.2% of the student class, while in 2011 they already represented 37.2% of the total number of students (Picanço 2016). Such an increase can be understood as a reflection of the enactment of the Law of Quotas for Higher Education n° 12.711/2012, in which several universities began to adopt racial quotas and quotas for public school students, thus expanding access for brown, black, and low-income people. However, even though inequality is gradually being reduced, the disadvantages of blacks and browns persist in terms of educational opportunities experienced, a scenario that begins in high school and continues until higher education (Barreto 2015). This highlights the need for affirmative policies that make the access of different ethnic groups to education more equal, including outreach activities.

Women represent the majority of enrollments in higher education (57%), both nationally and in all regions of the country (Barreto 2014). This is a recent situation that began to emerge in the 2000s, but which still cannot be understood as representing equal opportunities for men and women in professional insertion (Barreto 2014). If different graduate courses are analyzed, women make up the majority of those with “lesser prestige” and related to “caring” functions, such as in education and health, while men constitute the majority in exact sciences and technology courses (Artes & Unbehaum 2021), which is exemplified by the higher proportion of female teachers enrolled in CVZoo.

The low representation of transgender participants reflects the national scenario of invisibility and exclusion of transgender people from citizenship, with only 0.02% of the trans population reaching higher education in Brazil, as pointed out by Benevides & Nogueira (2019). Unfortunately, it was not possible to make any comparison with the national scenario in regards to the sexual orientation of people living in Brazil, since data are scarce and were not included in the latest IBGE censuses.

In the interactions promoted between students from different backgrounds and between them and graduate programs, the dialogical interaction between subjects and content was guided by the inseparability between teaching-research-extension. As for the motivation to continue an academic career and enter in a graduate program, CVZoo allows interdisciplinarity between different areas of knowledge, considering the diversity and heterogeneity of existing undergraduate courses in the country and culminating in the expectation of impact on student training, with consequent impact on social transformation (FORPROEX 2012).

Offering the course in a remote format made it possible not only for students and teachers from different Brazilian regions to access it, but also for people whose financial condition would not allow for face-to-face participation. Therefore, in order to expand the scope of the course, it is essential to rethink its format in future editions, considering the possibility of carrying out face-to-face and remote activities together, since simply paying for accommodation and food for participants is not enough to ensure access for everyone.

The data presented here denote the scope of the extension carried out, characterized as an intervention in social reality through the complementation of the academic training of teachers, sometimes quite relegated as secondary importance (Assis & Bonifácio 2011). As discussed by Alarcão (2011), reflection on teaching practice allows students and teachers to exercise their creativity and not only act by reproducing ideas and practices in the same way they were presented. The use of varied didactic strategies by CVZoo lecturers has contributed to reach individuals with different teaching-learning characteristics, in addition to providing the construction of knowledge and production of meanings by its participants.

3. *Final considerations and future perspectives for CVZoo*

Since its inception, the course has prioritized the transdisciplinary approach of zoological groups, the participation of diverse people and the use of varied teaching approaches, based on these precepts that, at each edition, the opportunity to adapt to the academic scenario is recognized and so the social context of the target audience. Thus, affirmative actions, such as the implementation of a quota system for socially vulnerable people (ethnic groups and people with disabilities) are already being implemented in the process of selecting candidates for the eleventh edition. The idea is that the course becomes an increasingly tangible opportunity for people from socially marginalized groups, who are constantly denied access to academic spaces, thus contributing to the reduction of the disparity observed in the representativeness of certain groups in the scenario of Brazilian Zoology.

Another important aspect resides in the need for constant updating of the forms of interaction and dissemination of the course to and with the general public since social networks are always in motion. The effects of the COVID-19 pandemic are known to have reduced the academic productivity of scientists, but the understanding of the impacts on the training and profile of undergraduate students who changed their routine to distance learning is still unknown.

The diversity of workshops offered throughout the course, addressing taxonomic groups or macroecological and macroevolutionary aspects, still needs to be expanded, as well as the interdisciplinary nature of CVZoo activities should be considered a goal, but without losing focus on Zoology and the protagonism of animals. In this way, we aim to make the course grow and renew itself, becoming an integral activity of the academic culture of the student body of USP’s graduate programs and continuing to contribute to the production of human resources in Zoology in the generations to come.

Acknowledgments

The authors would like to thank all the people who organized and participated in the creation and implementation of the Summer Course in Zoology over its ten years. Special thanks to Professor Alessandra Bizerra for supervising and guiding the conduct of the course and to all the people who participated in this study by sending their comments and evaluations. To the graduate programs in Zoology (PPGZOO) and Systematics, Animal Taxonomy and Biodiversity (PPGSATB) at the University of São Paulo, to the Institute of Biosciences and the Museum of Zoology, and to all employees and technicians for the support provided throughout the course activities.

Associate Editor

Carlos Joly

Author Contributions

Karla D.A. Soares: Substantial contribution in the concept and design of the study; Contribution to data collection; Contribution to data analysis and interpretation; Contribution to manuscript preparation; Contribution to critical revision, adding intellectual content.

Ivison Brandão: Contribution to data collection; Contribution to manuscript preparation; Contribution to critical revision, adding intellectual content.

João Pereira: Contribution to data collection; Contribution to data analysis and interpretation.

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Marília Pessoa-Silva: Contribution to data collection; Contribution to data analysis and interpretation.

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Laís A. Grossel: Contribution to data collection; Contribution to data analysis and interpretation; Contribution to manuscript preparation; Contribution to critical revision, adding intellectual content.

Conflicts of Interest

The authors declare that they have no conflict of interest related to the publication of this manuscript.

Ethics

This study does not involve human beings and/or clinical trials and because of this the approval by an Institutional Committee was not required.

Data availability

Supporting data are available at <https://doi.org/10.48331/scielodata.E2K2TQ>.

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Received: 03/01/2023
Accepted: 17/04/2023
Published online: 26/05/2023

Biota Neotropica 23(2): e20231467, 2023
www.scielo.br/bn

biotaneotropica
ISSN 1676-0611 (online edition)

Short Communication

Length-weight relationship and condition factor for *Prochilodus lineatus*, an important commercial fish, in contrasting water-quality environments of the middle Tietê River basin, Southeast Brazil

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URBANSKI, B.Q., BRAMBILLA, E.M., NOGUEIRA, M.G. **Length-weight relationship and condition factor for *Prochilodus lineatus*, an important commercial fish, in contrasting water-quality environments of the middle Tietê River basin, Southeast Brazil**. Biota Neotropica 23(2): e20231467. https://doi.org/10.1590/1676-0611-BN-2023-1467

Abstract: This work was carried out in order to provide the length-weight relationship (LWR) and the allometric condition factor (Ka), as well as its correlation with limnological variables, for *Prochilodus lineatus* from the middle Tietê River basin. Fish were collected using gill nets in two rivers with contrasting environmental conditions, totaling 46 specimens in the highly polluted Tietê River, and 37 in the Peixe River, a relatively well-conserved tributary. Environmental measures were obtained concomitantly to the fish capture. The results showed an isometric growth (b = 3.00) for the fish from Tietê River and a positive allometric growth (b = 3.23) for the fish from Peixe River. The mean value of Ka was unexpectedly higher for the main river (Ka = 2.63) when compared to its tributary (Ka = 2.42), being statistically different from each other. This can be explained by the much higher availability of organic sediments in the main river, resulting from a long-term eutrophication process. Nevertheless, the positive and statistically significant correlations with dissolved oxygen, for both rivers, as well as significant negative correlations with electric conductivity, nitrogen and chlorophyll *a* for Tietê River, indicate the negative effects of the water quality deterioration on the fish condition factor. The work contributes to the expansion of knowledge about *P. lineatus*, the most important commercial fish of the middle Tietê River basin, which is severely impacted by unsustainable human actions.
Keywords: *Animal welfare; Body growth pattern; Curimbatá; Water pollution.*

Relação peso-comprimento e fator de condição para *Prochilodus lineatus*, um importante peixe comercial, em ambientes contrastantes em qualidade da água da bacia do médio rio Tietê, sudeste do Brasil

Resumo: Este trabalho foi realizado com o objetivo de fornecer a relação peso-comprimento (LWR) e o fator de condição alométrico (Ka), bem como sua correlação com variáveis limnológicas, para *Prochilodus lineatus* da bacia do médio rio Tietê. Os peixes foram coletados com redes de espera em dois rios com condições ambientais contrastantes, totalizando 46 espécimes no altamente poluído rio Tietê, e 37 no rio do Peixe, um tributário relativamente bem conservado. Medidas ambientais foram obtidas concomitantemente à captura dos peixes. Os resultados mostraram um crescimento isométrico (b = 3,00) para os peixes do rio Tietê e um crescimento alométrico positivo (b = 3,23) para os peixes do rio do Peixe. O valor médio de Ka foi, inesperadamente, maior para o rio principal (Ka = 2,63) quando comparado ao seu afluente (Ka = 2,42), sendo estatisticamente diferentes entre si. Isso pode ser explicado pela disponibilidade muito maior de sedimentos orgânicos no rio principal, resultante de um longo processo de eutrofização. No entanto, as correlações positivas e estatisticamente significativas com o oxigênio dissolvido, para ambos os rios, bem como correlações significativas negativas com a condutividade elétrica, nitrogênio e clorofila *a* para o rio Tietê, indicam os efeitos negativos da deterioração da qualidade da água sobre o fator condição dos peixes. O trabalho contribui para a expansão do conhecimento sobre *P. lineatus*, o peixe de maior importância comercial da bacia do médio rio Tietê, severamente impactada por ações humanas não-sustentáveis.
Palavras-chaves: *Bem-estar animal; Curimbatá; Padrão de crescimento corpóreo; Poluição das águas.*

Introduction

The length-weight relationships (LWR) are very useful tools in ecological studies. This parameter, associated with the condition factor, is considered an excellent indicator of animal welfare. Its application is particularly important in the management of fisheries resources and in the development and implementation of environmental monitoring programs, especially for fish from degraded ecosystems (Froese 2006, Gubiani et al. 2009, Freitas et al. 2017), allowing comparisons between populations living in diversified environments and feed conditions (Salaro et al. 2015).

In this context, the objective of this study was to provide the LWR, the condition factor, as well as its correlation with limnological variables, for specimens of *Prochilodus lineatus* (Valenciennes 1837) from contrasting environments in terms of water quality in the middle Tietê River basin. This species, popularly named curimbatá, is widely distributed (Langeani et al. 2007) and constitute the most important and abundant fishery resource in this basin (Novaes & Carvalho 2013, Urbanski et al. 2020). Our hypothesis was to find a lower condition factor for individuals from the Tietê River, due to the strong environmental degradation of this river that receives most urban and industrial effluents of the São Paulo metropolis (Buckeridge & Ribeiro 2018, Tundisi 2018).

Material and Methods

The study was carried out in the Tietê River (22°47'31.0" S 48°05'48.8" W) and in its tributary, Peixe River (22°49'42.8" S 48°06'01.5" W). Both sampling areas are located in the municipality of Anhembi, State of São Paulo, Southeast of Brazil, and, despite their proximity, the environments exhibit contrasting water quality conditions.

Fish were collected (IBAMA/SISBIO permanent sampling license to MGN: 13794-1) in four seasonal samplings using gill nets, between December 2016 and October 2019. Morphological identification was made based on specialized bibliography (Britski 1972, Graça & Pavanelli 2007, Ota et al. 2018) and the parameters total weight (WT) and standard length (SL) measured in grams (precision of 1g) and in centimeters (precision of 0.1cm), respectively, immediately after the capture of the specimens. Sex was not discriminated against.

To determine the LWR, linear regression was used (logWT = log a + b logSL) with the removal of outliers using a length-weight graph with a logarithmic scale (Froese 2006). The LWR parameters were compared with the Bayesian predictions according to Froese et al. (2014), available in the FishBase (2022).

The allometric condition factor (Ka) was obtained through the expression Ka = 100*WT/SL^b, where b is estimated by the equation of the length-weight relationship (Froese 2006). Mean Ka values were compared between rivers using a Student's T-test with a 95% confidence interval.

Finally, a normality test was performed and later a Spearman's correlation test was applied to verify the influence of the limnological variables on the obtained condition factor values. The limnological variables used for this analysis were: dissolved oxygen (DO), electrical conductivity, and hydrogenic potential (pH), measured in situ using a Horiba U-5000 multiparameter probe, previously calibrated; and total phosphorus (TP) (Strickland & Parsons 1960), total nitrogen (TN) (Mackereth et al. 1978) and chlorophyll *a* (Talling & Driver 1963), analyzed in the laboratory in rivers surface water samples. All measurements and collection of environmental samples were carried out concomitantly to the capture of fish.

Results

Forty-six specimens from the Tietê River and 37 from the Peixe River were analyzed. The results indicated an isometric growth pattern for fish from the main river (b = 3.00) and a positive allometric growth pattern for those from its tributary (b = 3.23), the latter value being higher than those calculated for *Prochilodus lineatus* in the Bayesian predictions of FishBase (2022) (Table 1, Figure 1).

The specimens from the Tietê River showed mean values of the allometric condition factor (Ka = 2.63) higher when compared to those calculated for the specimens from the Peixe River (Ka = 2.42). The condition factors between the rivers are statistically different (T-test; $p < 0.0002$).

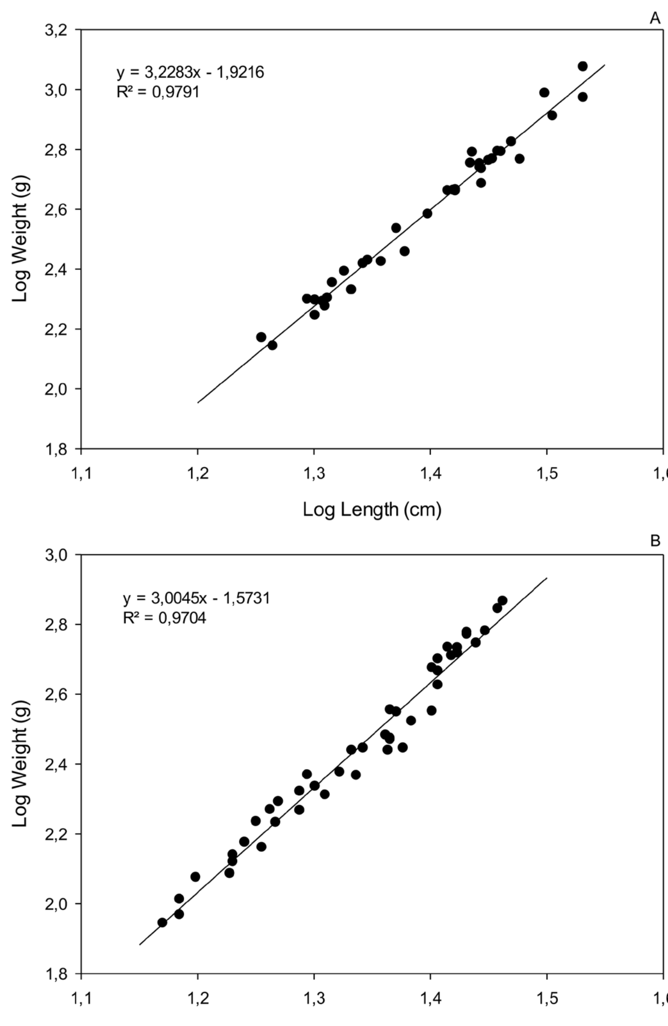

Figure 1. Length–weight relationship for *P. lineatus* in Peixe River (A) and in Tietê River (B).

Table 1. Descriptive statistics and estimated parameters of length–weight relationship for *P. lineatus* in the middle Tietê River basin, Brazil.

Local	Species	n	SL range	WT range	a	Cl a (95%)	b	Cl b (95%)	R²
Peixe River	*Prochilodus lineatus*	37	18,0 – 34,0	139 – 1189	0,0120*	0,0071 – 0,0202	3,2283*	3,0667 – 3,3900	0,979
Tietê River	*Prochilodus lineatus*	46	14,8 – 29,0	88 – 735	0,0267*	0,0163 – 0,0437	3,0045	2,8450 – 3,1639	0,970

*LWR parameter values different from Bayesian LWR predictions in FishBase (2022).

Sample size (n), length and weight range by species; estimated *a* and *b* values with confidence limits (95%), determination coefficient (R²).

Table 2. Mean values and standard derivations of the environmental variables measured at the sampling sites.

Campaign	Date	Local	DO	pH	Conductivity	TN	TP	Chlorophyll *a*
1	DEZ/2016	Peixe River	5.43 (±0.02)	6.77 (±0.22)	86.00 (±0.00)	1.13 (±0.00)	0.11 (±0.00)	20.60 (±0.00)
		Tietê River	1.17 (±0.12)	6.62 (±0.26)	289.33 (±0.47)	7.51 (±0.00)	0.23 (±0.00)	23.20 (±0.00)
2	AGO/2017	Peixe River	4.40 (±0.46)	7.57 (±0.21)	91.50 (±0.50)	0.76 (±0.00)	0.04 (±0.00)	4.00 (±0.00)
		Tietê River	2.25 (±0.09)	7.20 (±0.03)	377.00 (±0.63)	13.00 (±0.00)	0.64 (±0.00)	12.00 (±0.00)
3	ABR/2019	Peixe River	5.47 (±0.10)	6.21 (±0.11)	92.29 (±0.45)	0.85 (±0.02)	0.04 (±0.00)	10.26 (±4.14)
		Tietê River	0.53 (±0.21)	6.45 (±0.16)	409.80 (±0.40)	7.02 (±0.17)	0.26 (±0.01)	50.27 (±0.00)
4	OUT/2019	Peixe River	3.25 (±0.30)	6.61 (±0.23)	124.00 (±0.00)	0.67 (±0.04)	0.03 (±0.00)	1.81 (±0.08)
		Tietê River	0.59 (±0.76)	7.05 (±0.20)	557.71 (±1.28)	14.49 (±0.53)	0.58 (±0.04)	96.37 (±3.83)

Table 3. P value and Spearman's correlation coefficient for Peixe River allometric condition factors and limnological variables.

		DO	pH	Conductivity	TN	TP	Chlorophyll *a*
Ka	Correlation Coefficient	0.476	–0.331	0,207	–0,207	–0.207	–0.207
	P Value	0,00308	0,0458	0,217	0,217	0,217	0,217

Table 4. P value and Spearman's correlation coefficient for Tietê River allometric condition factors and limnological variables.

		OD	pH	Conductivity	TN	TP	Chlorophyll *a*
Ka	Correlation Coefficient	0.512	–0.0314	–0.419	–0.419	–0.0314	–0.512
	P Value	0.000304	0.835	0.00396	0.00396	0.835	0.000304

The means and standard derivations of the limnological variables obtained in the four campaigns are shown in Table 2. Spearman's correlation analysis demonstrated the existence of relations between the Ka values and the variables measured in this study (Tables 3 and 4).

The Ka values for Peixe River show significant positive correlation with the limnological variable dissolved oxygen ($p = 0.0031$) and significant negative correlation with pH ($p = 0.0458$) (Table 3).

The Ka values for the Tietê River show significant positive correlation with the limnological variable dissolved oxygen ($p = 0.0003$) and significant negative correlation with the variables conductivity ($p = 0.0039$), total nitrogen ($p = 0.0039$) and chlorophyll *a* ($p = 0.0003$) (Table 4).

Discussion

The *b* values in the length-weight body ratio for fish vary between 2.50 and 4.00 (Le Cren 1951). For curimbatá, the analysis of different populations distributed in distinct Brazilian river basins, showed a tendency to isometric body growth pattern, with values close to 3.00 for non-sexed animals (Silveira et al. 2015), as observed in this study for specimens of the Tiete River. This was also reported by Nuñer & Zaniboni-Filho (2009) with $b = 3.06$, Batista-Silva et al. (2015) with $b = 3.08$, Nobile et al. (2015) with $b = 2.99$, Silveira et al. (2015) with $b = 3.05$, and Freitas et al. (2017) with $b = 3.06$. Values of b equal to or higher than those calculated for specimens from the Peixe River in this work (3.23) were not found in the literature.

This difference in the types of body growth, comparing Tietê and Peixe Rivers, should be related to the distinctiveness of the environmental conditions. However, other factors such as sample size, seasonality, and sex (Le Cren 1951, Froese 2006), the latter not differentiated in the study, also influence this parameter.

For the condition factor, unexpectedly, the specimens from Peixe River had a lower mean value compared to those from Tietê River. Probably, this can be explained by the huge amount of organic matter deposited in the bottom sediments of the main river. The noticeable accumulation of organic sediments in the Tietê River is due to the long-term huge organic waste discharges (domestic effluents), over more than a century, especially from the São Paulo megalopolis (Tundisi 2018). Therefore, this resource is much more available for consumption by detritivorous and iliophagous fish, such as *Prochilodus lineatus*, in the Tietê River when compared to the tributary.

The target species has a wide displacement capacity, being a long-distance migrator (Castro & Vari 2004). Therefore, considering that fish from the Rio do Peixe analyzed in this study were collected only a few kilometers upstream from the mouth, possibly they also feed in the Tietê River. However, it is important to take into account that the water quality differences between rivers, may influence the fish displacement between both environments. As a result, fish from the Peixe River may spend more time in the tributary, where the sediments are poorer in organic matter and, consequently, would have less accumulation of fat and a lower condition factor than the fish from the Tietê River.

This assumption is supported by statistically significant negative correlation values between conductivity, nitrogen and chlorophyll *a* concentrations and condition factors values for the Tietê River and by positive and significant correlations with dissolved oxygen, for both rivers. This indicates the negative effects of the water quality deterioration on the fish condition factor.

Finally, *Prochilodus lineatus*, proves to be an animal extremely resistant to adverse environmental conditions, including plastic contamination (Urbanski et al. 2020), which is very interesting and needs to be better explored in further studies (e.g. morphological and eco-physiological approaches).

Our results contribute to the expansion of knowledge on *Prochilodus lineatus*, the fish of higher interest for fishermen of the middle Tietê River basin, an environment severely impacted by unsustainable human actions.

Acknowledgments

The authors are grateful to Fundação de Amparo à Pesquisa do Estado de São Paulo and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (BQU, FAPESP N° 2019/00177-4, CAPES Nº 88887.663615/2022-00, CAPES Nº 88887.826892/2023-00) for scholarships, and to Limnetica Consultoria em Recursos Hídricos for logistic support.

Associate Editor

Rosana Mazzoni

Author Contributions

Bruna Q. Urbanski: Substantial contribution in the concept and design of the study; Contribution to data collection; Contribution to data analysis and interpretation; Contribution to manuscript preparation and Contribution to critical revision, adding intellectual content.

Eduardo M. Brambilla: Substantial contribution in the concept and design of the study; Contribution to data collection; Contribution to data analysis and interpretation and Contribution to critical revision, adding intellectual content.

Marcos G. Nogueira: Substantial contribution in the concept and design of the study; Contribution to data collection; Contribution to manuscript preparation and Contribution to critical revision, adding intellectual content.

Conflicts of Interest

The author(s) declare(s) that they have no conflict of interest related to the publication of this manuscript.

Ethics

This study did not involve human beings and/or clinical trials that should be approved by one Institutional Committee.

Data Availability

The datasets generated during and/or analyzed during the current study are available at: https://doi.org/10.48331/scielodata.HIFTCJ

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Received: 16/01/2023
Accepted: 21/06 /2023
Published online: 31/07/2023

Biota Neotropica 23(2): e20221387, 2023
www.scielo.br/bn

biotaneotropica
ISSN 1676-0611 (online edition)

Article

Patterns in composition and occurrence of the fish fauna in shallow areas of the São Francisco River mouth

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BOT NETO, R.L., CATTANI, A.P., SPACH, H.L., CONTENTE, R.F., CARDOSO, O.R., MARION, C., SCHWARZ JÚNIOR, R. **Patterns in composition and occurrence of the fish fauna in shallow areas of the São Francisco River mouth.** Biota Neotropica 23(2): e20221387. https://doi.org/10.1590/1676-0611-BN-2022-1387

Abstract: The construction of dams causes changes in river variables, as a result of direct changes in their hydrological and biogeochemical cycles. One of the most notable changes is the flow regulation, which reduces seasonal events and the hydrostatic pressure exerted by freshwater, increasing the saltwater wedge intrusion into the system. Changing the salinity regime causes modifications in downstream ecosystems as well as in the distribution and composition of the fish fauna. In Brazil, the São Francisco River stands out, which has a system of cascading dams, built between the 70's and 90's. Because of these changes caused in the natural course of the river, this study aimed to analyze the patterns of composition and occurrence of the ichthyofauna at the mouth of the São Francisco River and relate them to the physical and chemical variables of the region. In order to evaluate the patterns of composition and occurrence of the fish fauna at the mouth of the São Francisco River, monthly trawls were conducted along the bank and physical and chemical variables were analyzed in the river channel over a period of one year. The relationship between abundance and species richness with environmental variables was verified using Generalized Linear Models. A total of 101,958 fish belonging to 87 taxa were caught, with emphasis on marine fish, both in number of individuals (99.92%) and in biomass (99.31%). A spatial gradient was detected, in which sites 1 and 2 were under marine influence, sites 3 and 4 represented the transition between the environments and site 5 was under the influence of brackish and freshwater. In general, the effect of the São Francisco River dams on the fish fauna was observed, with a predominance of fauna with more estuarine and less freshwater characteristics.
Keywords: *Salinization; fish assemblage; seine net.*

Padrões de composição e ocorrência da ictiofauna em áreas rasas da foz do rio São Francisco

Resumo: A construção de barragens provoca alterações nas variáveis dos rios, em decorrência de mudanças diretas em seus ciclos hidrológicos e biogeoquímicos. Uma das mudanças mais notáveis é a regulação do fluxo, que reduz os eventos sazonais e a pressão hidrostática exercida pela água doce, aumentando a intrusão das cunhas da água salgada no sistema. Mudar o regime de salinidade causa modificações nos ecossistemas a jusante, bem como na distribuição e composição da ictiofauna. No Brasil, destaca-se o Rio São Francisco, que possui um sistema de barragens em cascata, construído entre as décadas de 70 e 90. Por causa dessas alterações causadas no curso natural do rio, o presente estudo teve por objetivo analisar os padrões de composição e ocorrência da ictiofauna da foz do rio

São Francisco e relacioná-los com as variáveis físicas e químicas da região. Para avaliar os padrões de composição e ocorrência da ictiofauna foram realizados arrastos mensais ao longo da margem e analisadas as variáveis físicas e químicas no canal do rio ao longo de um ano. A relação entre abundância e riqueza de espécies de peixes com as variáveis ambientais foi verificada por meio de Modelos Lineares Generalizados. Foram capturados 101.958 peixes pertencentes a 87 táxons, com destaque para peixes marinhos, tanto em número de indivíduos (99,92%) quanto em biomassa (99,31%). Um gradiente espacial foi detectado, em que os pontos 1 e 2 estavam sob influência marinha, os pontos 3 e 4 representavam a transição entre os ambientes e o ponto 5 estava sob a influência de água doce e salobra. De maneira geral, foi observado o efeito das barragens do rio São Francisco sobre a ictiofauna, com predomínio de fauna com características mais estuarinas e menos dulcícolas.
Palavras-chave: *Salinização; Assembleia de peixes; Picaré.*

Introduction

Several factors have a direct influence on the composition and structure of the ichthyofauna, among them it is possible to highlight factors related to physicochemical characteristics of the environment such as the type of habitat, salinity, temperature and dissolved oxygen (Haedrich & Hall 1976, Blaber & Blaber 1980, Loneragan & Potter 1990, Whitfield 1999). Biological interactions such as competition (intra and interspecific) and predation also play an important role in driving the fish fauna composition (Kennish 1990). In addition to the abiotic and biological factors, the distribution and structure of the ichthyofauna can also be governed by factors such as the climate of the region, the geomorphology of the environment, the slope of the coast, the amplitude of the tide, the cycle of the tide, the tidal currents and the waves (Reise 1985).

The variation of environmental factors changes the primary productivity, causing changes in the fish fauna composition through bottom-up effects (Blaber et al. 1995, Morrison et al. 2002, Oliveira Neto et al. 2004). However, the abundance and specific composition of the ichthyofauna are also closely linked to a group of biological factors such as physiological differences, prey availability, foraging success, competitor density, predation pressure and availability of spawning sites (Baltz et al. 1998, Taylor & Rand 2003).

According to some authors, the distribution and abundance of fish is primarily influenced by physical-chemical factors in the environment, with great emphasis on temperature and salinity, and secondarily by biological interactions (Moyle & Cech 1988, Vieira & Musick 1993). Temperature plays a very important role in the intensity and seasonal variation in the spawning of several species of fish (Ramos & Vieira 2001), however, salinity had a direct influence on the specific composition of the ichthyofauna (Jaureguizar et al. 2003). In this way, the spatial and temporal differences in temperature and salinity characterize the diversity of habitats that exist in ecotones (Matic-Skoko et al. 2005).

The existence of fish with similar niches in ecosystems can occur through the development of strategies that allow the temporal or spatial separation in the use of habitats. In this way, phylogenetically-close species can live in the same area using different habitats (or microhabitats) or being active in different periods (Azevedo et al. 1999). The spatial distribution of species guarantees non-uniformity throughout the environment, however there is also temporal variation that acts on the first. This temporal variation can have both short and long periods. Short-term variations occur mainly as a result of tidal cycles, moon phases and the alternation between day and night. The most common and noticeable long-term variations are seasonal. Most fish fauna found in ecotones have reproductive cycles linked to long-term variations (Oliveira Neto et al. 2004).

Coastal regions are constantly under stress due to various human activities such as overfishing, tourism, urbanization, agriculture and industrial development (Raz-Guzman & Huidobro 2002). Environments located in regions close to urban centers are heavily affected by human activities, leading to a pronounced degradation of these regions (Miranda et al. 2002). In this way, changes arising from anthropic activities may compromise the maintenance of species in the aquatic environment.

The construction of dams causes considerable changes in the physical, chemical (in both water and sediment) and biological variables of the rivers, since their presence conspicuous changes the hydrological and biogeochemical cycles of the river course where they are built (Medeiros et al. 2011). One of the most notable changes is the river flow regulation, which decreases seasonal events (i.e., floods) (Medeiros et al. 2007) and causes a reduction in the hydrostatic pressure exerted by freshwater. The near-coast reduction in hydrostatic pressure exerted by the reduced river flow results in increased penetration of water from the oceans and increase the intrusion of the saline wedge into river systems (Fontes 2002, Coelho 2008), which consequently reduces both the intermediate salinity zones and the estuarine plume (Bennett 1994). Any changes in the inflow of freshwater will cause changes in the structure and functioning of downstream systems and in extreme cases of flow reduction there may be total salinization of this stretch, which will behave like a gulf, with salinities much higher than those found previously in the system (Bate & Adams 2000).

The reduction in freshwater inflow and the alteration in the salinity regime cause several changes in the ecosystems downstream of the dam, which also include changes, both in the distribution and composition of the fish fauna (Chícharo et al. 2006). Under such conditions, fish species with lower tolerance to saline water tend to migrate to upstream areas, while species with higher tolerance tend to increase their abundance in the downstream regions. This can cause direct changes in food webs as a consequence of changes in prey-predator relationships (Baptista et al. 2010). At the same time, there will be a decrease in the estuarine plume, reducing the chemical cues for migration and orientation of species entering the river channel (Bennett 1994), altering migration and spawning patterns in adults and hindering access of larvae and juveniles to nursery areas (Chícharo et al. 2003, 2006). Thus, changes in freshwater inflow caused by dams can impact fisheries in adjacent coastal areas (Chícharo et al. 2003).

In Brazil, we can highlight the case of the São Francisco River, which suffers from impacts to which rivers with dams are subjected, as it has in its course a system of cascading dams, built between the 70's and 90's (Medeiros et al. 2007, Oliveira et al. 2012). However, only after the construction of the Xingó Hydropower Plant (180 km from the coast) in 1994, these impacts intensified, as there was a definitive regulation of the flow of freshwater to the region of the mouth of the São Francisco River (Knoppers et al. 2006, Medeiros et al. 2007, 2011), allowing there greater intrusion of saline (Fontes 2002, Oliveira et al. 2008). In addition, the construction of this plant also generated other impacts in the region, causing changes in other characteristics of this system, due to the retention of nutrients and sediments, causing this stretch of the river to remain in a constantly oligotrophic and highly transparent condition (Medeiros et al. 2007, 2011).

In addition to compromising the permanence of some fish species in these environments, changes in the environment downstream of the dams caused by human activities can facilitate the invasion of allochthonous species. Thus, it is important to identify the structure of the fish fauna, in order to understand how environmental disturbances (natural or anthropogenic) can alter the distribution of resident and transient fish species (Whitfield & Elliot 2002, Vendel et al. 2003). Furthermore, having knowledge about the composition of the fish fauna and how it varies (both temporally and spatially) is fundamental for decision-making and for the sustainable management of species, as well as for preservation actions (Kupschus & Tremain 2001). Understanding and evaluating the impact that these environments are subjected to is of great importance for the maintenance of these regions (Chapman & Wang 2001). Thus, the aim of this study was to describe the spatial and temporal variations in the structure and composition of the fish fauna in shallow areas of the São Francisco River mouth.

Material and Methods

1. Study area

The lower course of the São Francisco River is the easternmost region of the basin (Costa 2003), it has the shortest length when compared to other stretches of the river (274 km), it extends from the Paulo Afonso Hydroelectric complex (state of Bahia) to the mouth into the Atlantic Ocean, between the municipalities of Piaçabuçu (state of Alagoas) and Brejo Grande (state of Sergipe) (Diegues 1994, Sato & Godinho 1999, 2004) and occupies 30,377 km^2 area (5% basin area), which covers the states of Bahia, Pernambuco, Alagoas and Sergipe (CODEVASF 1991, Junqueira 2002). According to Köppen classification, the climate of the Lower São Francisco is AS' (hot and humid, with winter rains) (Bernardes 1951) with an average annual temperature of 25°C (Aguiar Netto et al. 2011) and showing two distinct periods: rainy (between April and August) and dry (between September and March) (Knoppers et al. 2006).

From Paulo Afonso (BA), the vegetation of the Lower São Francisco, although there is a predominance of the formation of Steppe Savannah up to the mouth of the Ipanema river (AL), has areas of ecological tension (Steppe Savannah– Seasonal Forest) with patches of Semideciduous Seasonal Forest from Propriá (SE) and as the São Francisco approaches its mouth, pioneer formations of fluvio-marine influence occur that form the mangroves (MMA 2006).

The coastal region of the Lower São Francisco presents a semi-diurnal mesotide regime (with the spring tide reaching 2.6 m). The wave regime has high energy, with a predominance of NE, E and SE waves throughout the year, with the northeast and east waves being more important during the summer, fall and spring, while the southeast waves occur more markedly in winter (Dominguez 1996). The depth in the region of the São Francisco River mouth is variable, reaching 18 m in the channel located near the municipality of Piaçabuçu (state of Alagoas) and approximately 14 m in the regions close to the mouth (Medeiros et al. 2007).

The modulation or total regulation of flow, aiming at constant water supply, is one of the most notable modifications in dam construction and causes drastic effects by reducing flows and smoothing or interrupting the natural pulsation of the river system (Medeiros et al. 2007). Through the construction of dams, energy generation activity caused major changes in the Lower São Francisco (Medeiros et al. 2007; 2011; 2014). Before the construction of the dams, the flow of the São Francisco River varied according to the natural rainfall pulses in the Upper and Middle São Francisco region, with peaks between 8,000 and 18,000 m^3/s and lows of 600 m^3/s (Santos et al. 2009). After the completion of the last plant (Xingó – 1994) the flow was definitively regulated in 1995 by the Sobradinho dam. Currently, the flow is kept constant at an average volume of 1,850 m3/s, 35% less than in the period prior to the dams (Oliveira 2003; Medeiros et al. 2007; 2011; 2014). The flood peaks that naturally occurred from January to March were eliminated between 1995 and 2001 (Medeiros et al. 2011; 2014). After the construction of the dams, the Lower São Francisco became transparent and oligotrophic (MMA 2006; Medeiros et al. 2007; 2011; Knoppers et al. 2006) and areas that previously had high turbidity became totally transparent (Medeiros et al. 2003; 2007). The lakes and floodplains located on their banks are no longer flooded and seasonally fertilized, which altered their biogeochemical functioning, and due to the lack of nutrients, the areas downstream of the dams had their biological productivity reduced (Santos et al. 2009). Dam-mediated nutrient retention also reduced drastically the local fisheries, resulting in the extinction of species and the reduction of fish stocks (Nascimento, Ribeiro & Aguiar Netto 2013).

2. Data collection

Data were collected monthly, during the daytime, both at high tide and at low tides, and extended over a one-year period (from May 2017 to April 2018) in the region of the Lower São Francisco River.

For fish fauna collection, manual trawls were carried out parallel to the margin, at five sites distributed along the environmental gradient of the Lower São Francisco River between the mouth and the municipality of Brejo Grande (Figure 1) on the river banks. At each site, two trawls were carried out on each tide, totaling 20 monthly trawls (10 at high tide and 10 at low tide). The net used (30 m x 2.8 m; 5 mm mesh) was pulled parallel to the margins for a distance of 50 m to a maximum depth of 3 m.

Concomitantly with the collection of biological material, the physical and chemical variables of the water were also monitored: temperature, pH, dissolved oxygen, salinity and total dissolved solids (both under the water surface and near the bottom), in 13 sampling sites in the channel of the river along the environmental gradient (Figure 1) using a multiparameter probe (Hanna HI9828). Simultaneously, water transparency was measured using a Secchi Disk. In addition to monitoring environmental data, time series (for the study period) and historical series of flow and rainfall for the Lower São Francisco region were obtained from the Hidroweb database of the National Water

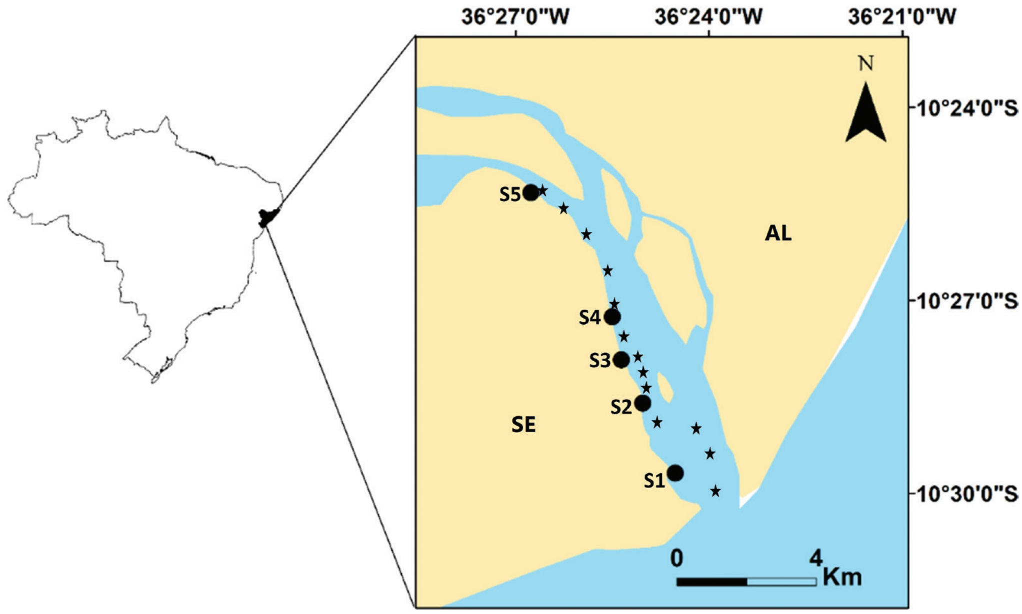

Figure 1. Map of the mouth of the São Francisco River. The circular points marked on the map represent the five sampling sites. And the star-shaped dots represent the thirteen locations where soundings were carried out with the multiparameter probe.

Agency (ANA). These data were important both for the description of seasonal periods and for helping to explain possible differences in the fish fauna associated with the mouth of the São Francisco River dynamics.

3. Data analysis

Statistical analyses were performed in a computational environment R (R DEVELOPMENT CORE TEAM 2019). The representativeness of the fish community samples was evaluated by drawing a species accumulation curve in the Vegan package, using the "specaccum" function (Oksanen 2019), based on all collected samples. A modeled curve was also drawn, based on the species richness estimator presented by Coleman et al. (1982).

In order to reduce the bias caused by samples with very high abundances, those considered outliers were removed, specifically two samples collected at site 3 during the low tide.

The relationships between abundance and species richness with factorial variables (tide, site and month), as well as with environmental variables (temperature, pH, dissolved oxygen, salinity, rainfall, flow and total dissolved solids) were evaluated using Generalized Linear Models (GLM). The use of these models allows the use of data with a probability frequency distribution different from the normal or Gaussian distribution (Zuur et al. 2010). For the richness data, the Poisson distribution was used, through the "glm" function, and for the abundance data, the adopted distribution was the negative binomial through the "glm.nb" function of the Mass package (Venables & Ripley 2002).

The VIF (Variation Inflation Factor) function of the Car package (Fox & Weisberg 2011) was applied to test the multicollinearity of environmental variables (Zuur et al. 2010). Variables with high VIF (>5) were excluded from the model. To select the most explanatory models, the "dredge" function of the MuMin package was used (Barton 2018). The models were selected using the corrected Akaike information criterion (AICc), the delta AIC and the Akaike weights among models. Those with a delta AIC value less than 3 were selected. The greater the Akaike weights, the greater the explanatory power of the models among all those tested.

The graphics were created using the "effect" function of the Effects package (Fox 2003) and the "stripchart" function of the Vegan package (Oksanen 2019). In the elaboration of the abundance graphs, data were transformed into log (n+1) for a better visualization of the patterns.

Finally, the Canonical Correspondence Analysis (CCA) (Ter Braak 1986) was developed with the "cca" function of the Vegan package to assess the influence of environmental variables on the most abundant species. Collinearity between predictive environmental variables was tested using the "ordistep" function also of the Vegan package. Thus, the non-collinear variables that were important for the variability of the most abundant species were plotted on the graph.

Results

A total of 101,958 fish specimens belonging to 87 different taxa were caught. Marine fish accounted for the majority in abundance with 77 different taxa (99.92%) and biomass (99.31%) (Table 1); only 10 taxa were associated with freshwater fish (Table 2). The most abundant taxa were *Rhinosardinia bahiensis* (77.27%), *Atherinella brasiliensis* (7.63%) and *Lycengraulis grossidens* (3.95%). For biomass, the most representative taxa were *R. bahiensis* (36.11%), *A. brasiliensis* (8.42%) and *Sphoeroides testudineus* (13.55%).

The species accumulation curve stabilized from the 200th sample, in line with the modeled curve, and the decrease in variability between samples, demonstrated by the observed values (Figure 2).

Considering the raw values of abundance and species richness over the months, higher abundances were found in April 2018 and lower values in January 2018. As for richness, higher and lower values

Table 1. Species composition, family, number of individuals and percentage of occurrence, total biomass and percentage of biomass of marine and estuarine fish caught at the mouth of the São Francisco River.

Order	Family	*Taxa*	N		Biomass	
			Total	%	Total (g)	%
Elopiformes	Elopidae	*Elops saurus*	13	0.013	460.0	0.178
Albuliformes	Albulidae	*Albula vulpes*	17	0.017	207.6	0.080
Anguilliformes	Ophichthidae	*Mirophis punctatus*	2	0.002	4.00	0.002
Clupeiformes	Engraulidae	*Anchoa spinifer*	6	0.006	96.3	0.037
		Anchoa tricolor	259	0.254	256.2	0.099
		Anchovia clupeoides	45	0.044	543.5	0.210
		Cetengraulis edentulus	614	0.602	10,647.3	4.110
		Lycengraulis grossidens	4,026	3.949	5,941.5	2.294
	Clupeidae	*Harengula clupeola*	27	0.026	51.9	0.020
		Lile piquitinga	281	0.276	1,114.2	0.430
		Opisthonema oglinum	6	0.006	152.8	0.059
		Rhinosardinia bahiensis	78,787	77.274	93,544.4	36.111
Siluriformes	Ariidae	*Cathorops spixii*	14	0.014	502.3	0.194
		Genidens barbus	749	0.735	13,685.1	5.283
Aulopiformes	Synodontidae	*Synodus foetens*	2	0.002	13.9	0.005
Gobiiformes	Eleotridae	*Dormitator maculatus*	13	0.013	19.3	0.007
		Eleotris pisonis	29	0.028	42.7	0.016
		Erotelis smaragdus	2	0.002	5.1	0.002
	Gobiidae	*Bathygobius soporator*	92	0.090	459.6	0.177
		Ctenogobius boleosoma	45	0.044	15.5	0.006
		Gobionellus oceanicus	34	33	294.6	0.114
		Gobionellus stomatus	14	0.014	18.1	0.007
Mugiliforme	Mugilidae	*Mugil curema*	203	0.199	1,799.5	0.695
		Mugil curvidens	286	0.281	2,857.8	1.103
		Mugil liza	27	0.026	348.6	0.135
		Mugil spp	202	0.198	61.7	0.024
	Polynemidae	*Polydactylus virginicus*	409	0.401	2,447.9	0.945
Atheriniformes	Atherinopsidae	*Atherinella brasiliensis*	7,782	7.633	21,808.7	8.419
		Atherinella blackburni	4	0.004	25.3	0.010
Beloniformes	Hemiramphidae	*Hyporhamphus unifasciatus*	909	0.892	9,509.9	3.671
	Belonidae	*Strongylura marina*	167	0.164	2,510.7	0.969
Caragiformes	Carangidae	*Caranx hippos*	1	0.001	37.4	0.014
		Caranx latus	407	399	1,810.0	0.699
		Oligoplites palometa	25	0.025	35.3	0.014
		Oligoplites saliens	22	0.022	326.7	0.126
		Oligoplites saurus	164	0.161	461.6	0.178
		Selene vomer	20	0.020	63.7	0.025
		Trachinotus falcatus	31	0.030	478.6	0.185
Istiophoriformes	Sphyraenidae	*Sphyraena barracuda*	2	0.002	210.4	0.081

Continue...

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...Continuation

Order	Family	*Taxa*	N		Biomass	
			Total	%	Total (g)	%
Pleuronectiformes	Paralichthyidae	*Citharichthys arenaceus*	290	0.284	931.9	0.360
		Citharichthys spilopterus	473	0.464	697.9	0.269
		Paralichthys brasiliensis	1	0.001	148.3	0.057
	Achiridae	*Achirus lineatus*	429	0.421	1,946.6	0.751
		Trinectes microphthalmus	20	0.020	47.5	0.018
		Trinectes paulistanus	3	0.003	16.3	0.006
	Cynoglossidae	*Symphurus tessellatus*	196	0.192	1,189.1	0.459
Syngnathiformes	Syngnathidae	*Cosmocampus elucens*	5	0.005	4.5	0.002
		Microphis lineatus	18	0.018	8.1	0.003
		Pseudophallus mindii	6	0.006	3.0	0.001
Scombriformes	Trichiuridae	*Trichiurus lepturus*	2	0.002	80.3	0.031
	Scombridae	*Scomberomorus brasiliensis*	10	0.010	212.4	0.082
	Centropomidae	*Centropomus ensiferus*	41	0.040	434.1	0.168
		Centropomus parallelus	2	0.002	204.7	0.079
		Centropomus undecimalis	338	0.332	12,846.3	4.959
Perciformes	Gerreidae	*Diapterus auratus*	1,907	1.870	11,353.4	4.383
		Eucinostomus argenteus	147	0.144	744.2	0.287
		Eucinostomus gula	1	0.001	9.2	0.004
		Eucinostomus melanopterus	634	622	8,739.8	3.374
	Serranidae	*Rypticus randalli*	3	0.003	66.2	0.026
	Chaetodontidae	*Chaetodon striatus*	1	0.001	0.4	0.000
	Haemulidae	*Conodon nobilis*	2	0.002	29.2	0.011
		Haemulopsis corvinaeformis	83	0.081	541.0	0.209
		Pomadasys crocro	3	0.003	14.0	0.005
		Pomadasys ramosus	63	0.062	1,239.1	0.478
	Lutjanidae	*Lutjanus griseus*	149	0.146	2,064.7	0.797
		Lutjanus jocu	226	0.222	2,681.8	1.035
		Lutjanus spp	12	0.012	2.0	0.001
		Lutjanus synagris	4	0.004	59.8	0.023
Moroniformes	Ephippidae	*Chaetodipterus faber*	24	0.024	60.6	0.023
Acanthuriformes	Sciaenidae	*Bairdiella ronchus*	23	0.023	279.5	0.108
		Cynoscion leiarchus	1	0.001	566.9	0.219
		Menticirrhus americanus	19	0.019	281.0	0.108
		Stellifer rastrifer	52	0.051	519.1	0.200
Spariformes	Sparidae	*Archosargus probatocephalus*	2	0.002	24.9	0.010
Tetraodontiformes	Tetraodontidae	*Colomesus psittacus*	10	0.010	143.6	0.055
		Lagocephalus laevigatus	8	0.008	601.8	0.232
		Sphoeroides greeleyi	114	0.112	485.5	0.187
		Sphoeroides testudineus	816	0.800	35,099.2	13.549
	Diodontidae	*Chilomycterus spinosus*	1	0.001	0.5	0.000

* Classification of the table according to Nelson et al. (2016).

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Table 2. Species composition, number of individuals and percentage of occurrence, total biomass and percentage of biomass of freshwater fish caught at the mouth of the São Francisco River. With an asterisk the non-native species.

Order	Family	*Taxa*	N		Biomass	
			Total	**%**	**Total (g)**	**%**
Characiformes	Characidae	*Astyanax lacustris*	33	0.032	60.6	0.023
		*Orthospinus franciscensis**	1	0.001	1.2	0.0005
	Iguanodectidae	*Bryconops affinis*	2	0.002	10.8	0.004
	Serrasalmidae	*Metynnis lippincottianus**	8	0.008	230.8	0.089
Cichliformes	Cichlidae	*Cichla kelberi**	5	0.005	915.2	0.353
		Cichlasoma sanctifranciscense	2	0.002	17.5	0.007
		Oreochromis niloticus	10	0.010	530.3	0.205
Gymnotiformes	Gymnotidae	*Gymnotus carapo*	4	0.004	21.4	0.008
Cyprinodontiformes	Poeciliidae	*Poecilia vivipara*	15	0.015	8.9	0.003
Gymnotiformes	Sternopygidae	*Eigenmannia virescens*	1	0.001	0,7	0.0003
		Total	81	0.079	1,797.4	0.694

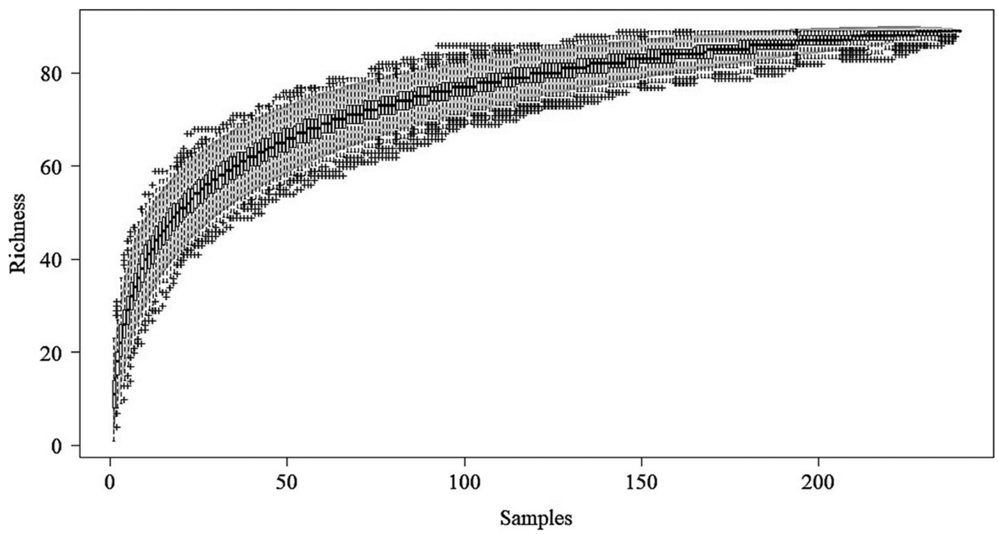

Figure 2. Cumulative curve of species constructed with the fish samples (n = 242) collected in the mouth of the São Francisco River. In gray, the modeled curve based on the Coleman estimator (Coleman et al, 1982). Boxplots were built from mean values. Crosses represent outliers.

Table 3. Descriptive summary of total numeric abundance (n) and richness (s) and mean (μ) ± standard deviation (SD) of fish caught in 2017 and 2018.

Year	Month	n Total	n (μ ± DP)	s Total	s (μ ± DP)
2017	May	13,170	2,947.55 ± 8447.06	247	10.7 ± 4.11
	Jun	4,141	180.5 ± 316.62	243	9.7 ± 3.51
	Jul	2,062	84.95 ± 56.24	223	10.1 ± 3.09
	Aug	3,610	155.85 ± 272.05	194	11.15 ± 4.77
	Sep	2,247	74.4 ± 59.62	214	11.75 ± 3.55
	Oct	2,563	103.1 ± 75.37	225	11.15 ± 3.96
	Nov	2,430	207.05 ± 186.54	220	12.15 ± 5.24
	Dec	1,699	658.5 ± 1612.47	202	12.35 ± 5.16
2018	Jan	1,488	324 ± 628.29	235	12.65 ± 4.97
	Feb	3,117	121.5 ± 131.23	223	11 ± 3.16
	Mar	6,480	128.15 ± 120.4	253	11.25 ± 3.16
	Apr	58,951	112.35 ± 75.07	214	10.7 ± 4.11

occurred respectively in March 2018 and August 2017 (Table 3). As for sites and tides, the high abundances and richness observed in site 3 (P3) at low tide and the low values of these descriptors in site 2 (P2) at high tide are highlighted (Table 4).

In the selection of Generalized Linear Models (GLM) for richness, considering the tide, site and month factors, the selected model (delta < 3; weight = 0.986) listed, in descending order of importance, the tide and the site, with an observed trend of higher values at low tide and in sites S1 and S3 (Figure 3).

In the analysis of abundance, the selected model (delta < 3; weight = 0.932) listed, in descending order of importance, the tide, the month and the site, with an observed trend of higher values at low tide, in

Table 4. Descriptive summary of total abundance (n) and richness (s) and mean (μ) ± standard deviation (SD) of fish caught at five sampling sites at high and low tides.

Site	Tide	n Total	n (μ ± DP)	s Total	s (μ ± DP)
S1	High	2,107	87.79 ± 62.69	267	11.13 ± 3.29
	Low	3,109	129.54 ± 91.81	326	13.58 ± 3.72
S2	High	815	33.96 ± 19.07	150	6.25 ± 2.57
	Low	10,201	425.04 ± 1,402.35	268	11.17 ± 3.38
S3	High	8,807	366.96 ± 924.17	309	12.88 ± 3
	Low	57,594	2,399.75 ± 7,740.79	356	14.83 ± 3.55
S4	High	2,391	99.63 ± 85.8	210	8.75 ± 3.38
	Low	6,542	272.58 ± 546.86	277	11.54 ± 5.17
S5	High	6,233	259.71 ± 380.7	272	11.33 ± 2.9
	Low	4,159	173.29 ± 201.37	258	10.75 ± 3.42

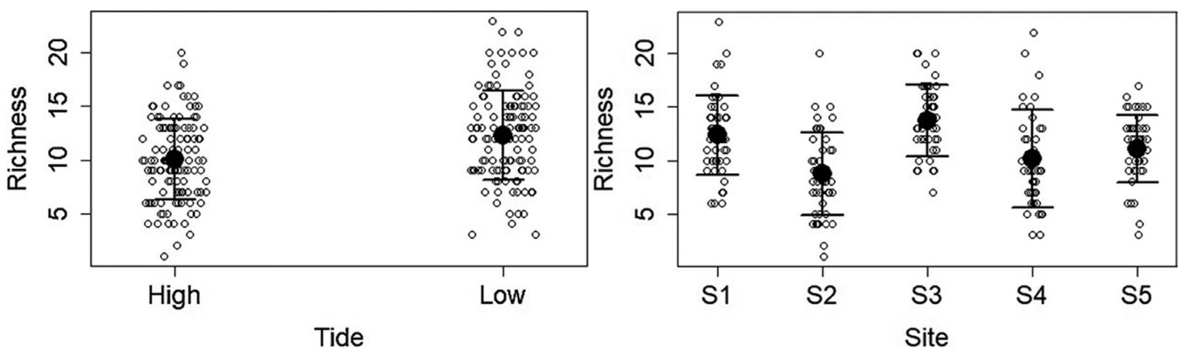

Figure 3. Comparison of mean richness between tides and sampling sites. Black dots represent means and bars represent standard errors.

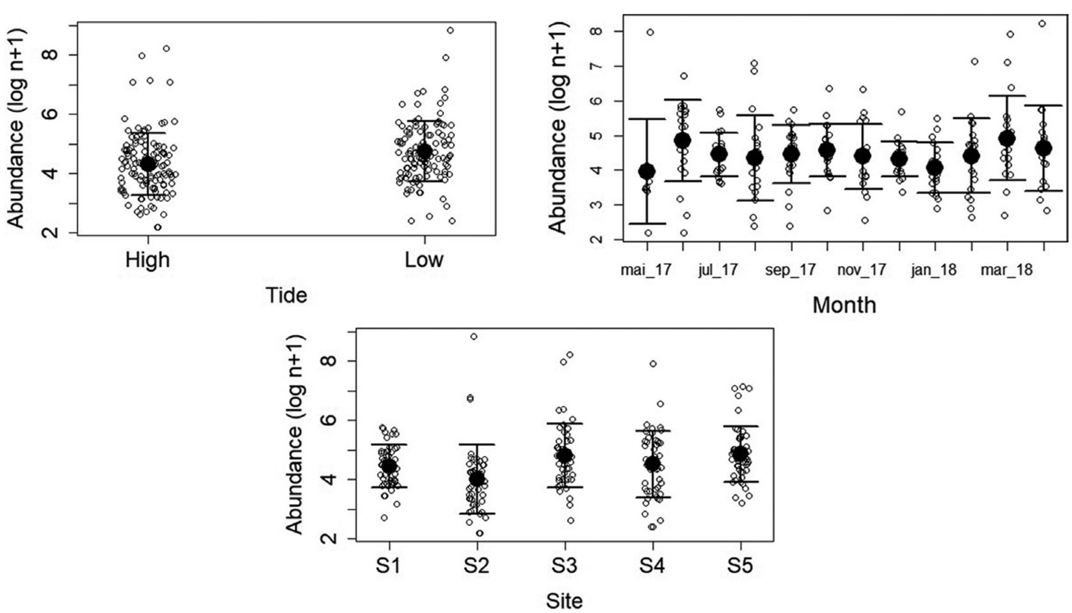

Figure 4. Comparison of mean abundance (log n + 1) of species between tides, months and sampling sites. Black dots represent means and bars represent standard errors.

Table 5. Selection of generalized linear models (GLM) of fish richness according to environmental variables. rich = richness; rain = rainfall; sal = salinity; temp = temperature; flow = flow.

	Richness models	df	logLik	AICc	delta	weight
1	rich ~ pH + rain + temp + flow	5	–671.672	1353.6	0	0.205
2	rich ~ pH + rain + sal + temp + flow	6	–671.851	1354.1	0.46	0.163
3	rich ~ pH + rain + temp +	4	–671.403	1355	1.38	0.103
4	rich ~ pH + temp + flow	4	–671.421	1355	141	0.101
5	rich ~ DO +pH +rain+temp + flow	6	–671.411	1355.2	1.58	0.093
6	rich ~ pH + rain + sal + temp + flow	5	–671.914	1356.1	2.48	0.059
7	rich ~ DO + pH + rain + sal + temp + flow	7	–671.846	1356.2	2.58	0.057
8	rich ~ pH + sal + temp + flow	5	–671.144	1356.5	2.94	0.047

Table 6. Selection of generalized linear models (GLM) of fish abundance according to environmental variables. abu = rabundance; rain = rainfall; sal = salinity; temp = temperature; flow = flow.

	Abundance models	df	logLik	AICc	delta	weight
1	abu ~ DO + rain + sal + temp + flow	7	–1463.208	2940.9	0	0.373
2	abu ~ rain + sal + temp + flow	6	–1464.897	2942.2	1.25	0.199
3	abu ~ DO + rain + sal + temp + flow	8	–1462.939	2942.5	1.60	0.167
4	abu ~ pH + temp + flow	7	–1464.673	2943.8	2.93	0.086

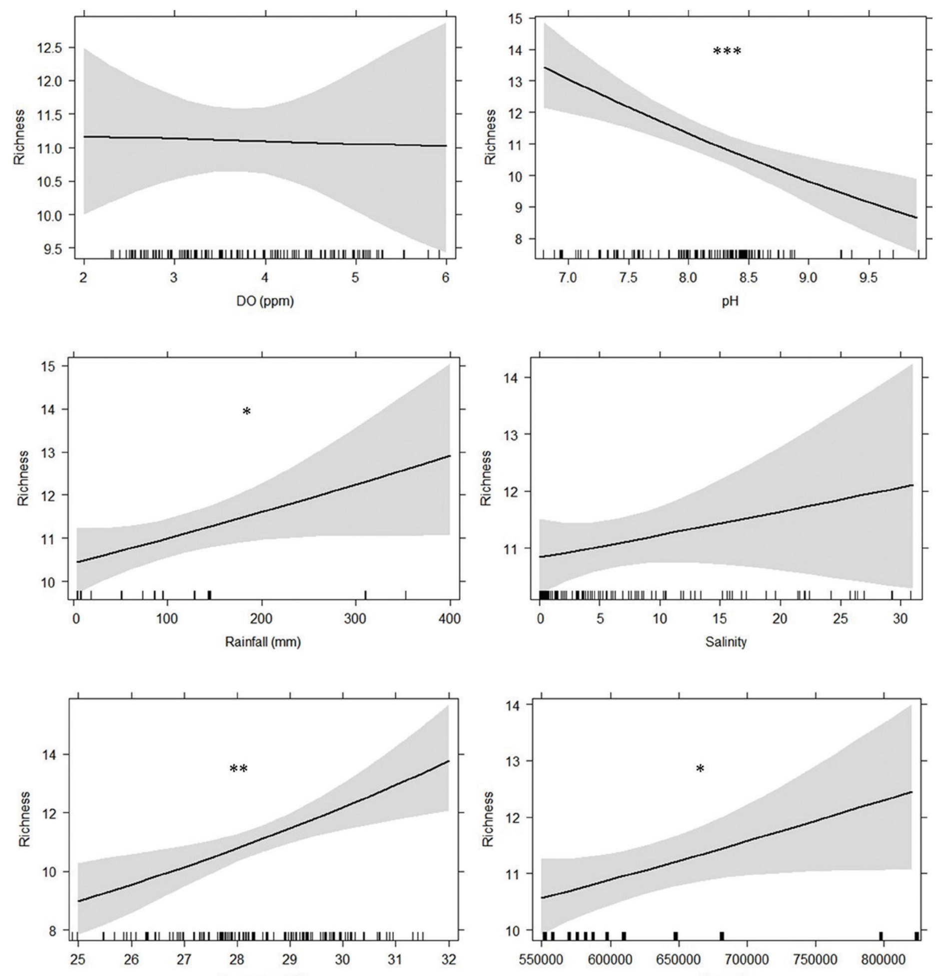

Figure 5. Relationship between richness and selected environmental variables in generalized linear models. The line represents the modeled values and the gray area corresponds to the standard deviation. Asterisks (*) correspond to the significance in the correlation (***p-value < 0.001; **p-value < 0.01; *p-value < 0.05).

May and June 2017 and March and April 2018 and in sites S1, S4 and S3 (Figure 4).

In the model of richness according to environmental variables, the VIF analysis detected collinearity (VIF > 5) of the variable “total dissolved solids”, which was removed from the final model. Eight models were selected (Table 5), and the one with the highest weight (0.205) considered, in decreasing order of importance, the variables pH, rainfall, temperature and flow.

For abundance, the VIF analysis also detected collinearity (VIF > 5) of the variable “total dissolved solids”, which was removed from the final model. Four models were selected (Table 6), in which dissolved oxygen, rainfall, salinity, temperature and flow were selected in order of importance in the model with the highest weight (0.373).

In the model of species richness according to the selected environmental variables, there was a weak correlation with dissolved oxygen (DO), a significant negative correlation with pH and a significant positive correlation with rainfall, temperature and flow. As for salinity, there was a positive correlation, but without statistical significance (Figure 5). It is noteworthy that salinity was not selected in the model with the greatest weight.

In the species abundance model with selected environmental variables, dissolved oxygen and pH were negatively correlated with abundance, while the other variables were positively correlated with abundance (Figure 6). Only for pH, the correlation was not significant.

In the analysis with the most abundant species (n > 0.5%), the variables selected to explain the variability were salinity, pH and temperature, which correlated equally with both axes (Figure 7). However, the variables with the greatest influence on the most abundant fish assemblage were salinity and pH. The total cumulative percentage of explanation of the first two axes corresponded to 94.56%. The first axis represented a spatial gradient, with samples from the sites on the left side of the graph closest to the mouth of the river, positively correlated with the highest values of salinity (sal) and pH (ph) and with the abundance of *Cetengraulis edentulus* (Cede). On the other hand,

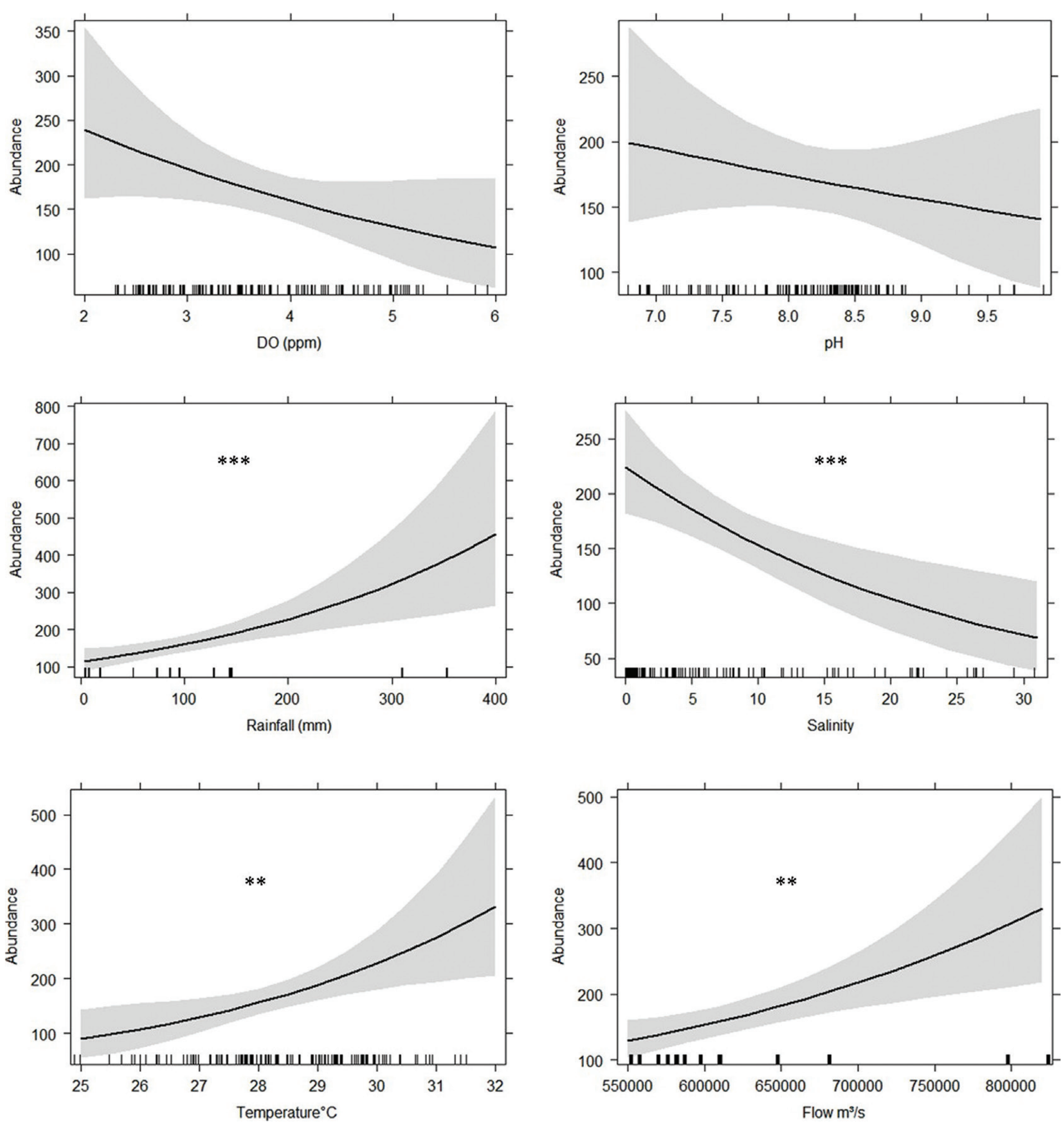

Figure 6. Relationship between abundance and selected environmental variables in generalized linear models The line represents the modeled values and the gray area corresponds to the standard deviation. Asterisks (*) correspond to the significance in the correlation (***p-value < 0.001; **p-value < 0.01; *p-value < 0.05).

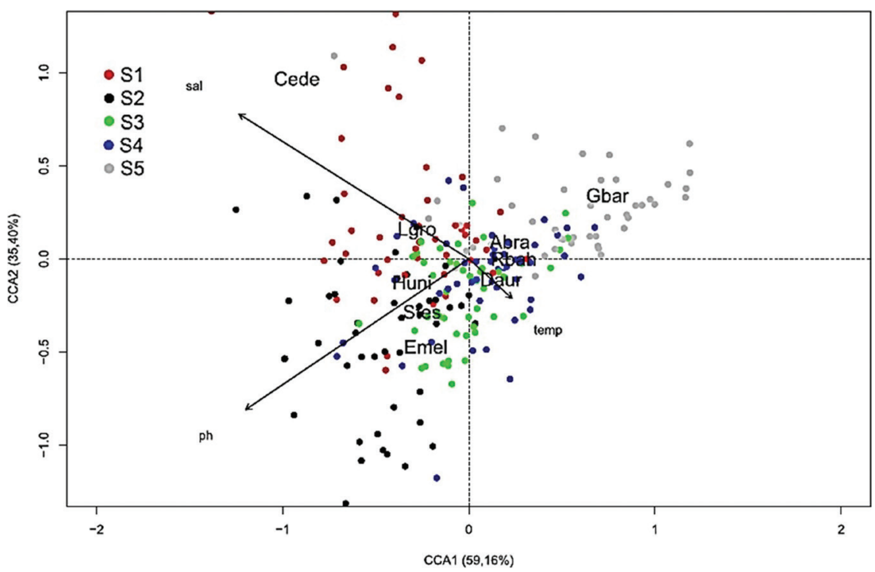

Figure 7. Canonical Correspondence Analysis (CCA) for the most abundant species in relation to the physicochemical parameters of the water. Cede = *Cetengraulis edentulus*; Gbar = *Genidens barbus*; Lgro = *Lycengraulis grossidens*; Huni = *Hyporhamphus unifasciatus*; Stes = *Sphoeroides testudineus*; Emel = *Eucinostomus melanopterus*; Abra = *Atherinella brasiliensis*; Rbah = *Rhinosardinia bahiensis*; Daur = *Diapterus auratus*.

on the right side, the samples collected at site 5 (S5) in the innermost region of the river predominated, correlated with the abundance of *Genidens barbus* (Gbar). The species *Lycengraulis grossidens* (Lgro), *Hyporhamphus unifasciatus* (Huni), *Sphoeroides testudineus* (Stes), *Eucinostomus melanopterus* (Emel) were more associated with external sites and the species *Atherinella brasiliensis* (Abra), *Rhinosardinia bahiensis* (Rbah) and *Diapterus auratus* (Daur) were weakly correlated with sampling sites, which suggests a homogeneous occurrence in the sampling sites.

Discussion

Despite the great economic and cultural importance of the São Francisco River, some areas of the river still lack studies that characterize the distribution of fish species along the environmental gradients (Silva et al. 2006, Barbosa & Soares 2009). This does not mean that the region's ichthyofauna is completely absent from studies, but studies in the region tend to focus on a few species (Assis et al. 2017) or use fishing landing data (D'avilla et al. 2021) which does not always represent the natural spatial distribution of species. A good part of the studies on the Ichthyofauna of the São Francisco River is located in the Alto São Francisco region (Trajano, Secutti & Bichuette 2009, Loures & Pompeu 2012, Dagosta, Marinho & Camalier 2014, Belei et al. 2016). Although there is a lack of ichthyological information referring mainly to the Lower São Francisco, some species associated with brackish/salt water environments have already been cited as visitors to the region, entering the river channel and being caught in freshwater areas (Barbosa & Soares 2009). However, according to these authors, both the number of these species as well as their abundance and participation in fish landings are described as reduced compared to freshwater species. Nevertheless, our results showed a very different situation for the region, since in our samples there was a massive presence of species associated with brackish/saline environments, both in abundance (99.92%) and in biomass (99.31%). According to Barbosa & Soares (2009), only six taxa associated with brackish/saline environments are present in the Lower São Francisco region, namely: *Anchoviella lepidentostole* (Fowler 1911), *Lycengraulis grossidens* (Cuvier 1829), *Eucinostomus melanopterus* (Bleeker 1863), *Eugerres brasilianus* (Cuvier 1830), *Centropomus sp.* and *Bothus sp.* In the present study it was possible to verify in the region the presence of 77 taxa associated with the brackish/saline environment, presenting a species composition similar to other estuarine environments in the northeast region, such as the Parnaíba River estuary in the state of Piauí (De Oliveira 1974) and the Contas River estuary in the state of Bahia (Lima 2010). This discrepancy observed between the study by Barbosa & Soares (2009) and the present study in the ichthyofauna composition of the São Francisco River mouth may be directly linked to the changes caused by the presence of dams in the course of the river and by the constant reduction in the natural flow caused by them, as the reduction of river flow in the system reduces the hydrostatic pressure exerted by the river and allows the penetration of salt water into the river (Coelho 2008) justifying the current expressive presence of the fish fauna in saline/brackish environments.

According to Santos (2009), some species of marine and estuarine fish have always been present in fishing landings in the Lower São Francisco region, but it was only after the installation of the Xingó hydropower plant (in 1994) that the influence of these species became increasingly greater. The presence of dams is also directly linked to the decrease in freshwater fish fauna, as the regulation of the freshwater flow eliminated the effects of floods and consequently extinguished the marginal lakes, which were extremely important grounds for reproduction of fish species native to the São Francisco River (MMA 2006, Nascimento et al. 2013). In addition, studies investigating the action of dams indicate that changes in river flow cause a decrease in native fauna (Granzotti et al. 2018, Pelicice et al. 2018, Ferreira et al. 2020; dos Santos et al. 2022).

Our abundance data denotes the numerical dominance of a few species, since the sum of the percentages of the three most abundant taxa (*R. bahiensis*, *A. brasiliensis* and *L. grossidens*) exceeds 88% total number of individuals caught, largely due to the massive presence of *R. bahiensis* (77.27%). This pattern is very typical for estuarine fish faunas, as few taxa can deal with the highly variable conditions of the estuarine environments and, consequently, reach abundant populations (Kennish 1990, Chaves & Bochereau 1999). This dominance leads to an uneven distribution of the community (Spach et al. 2007). This pattern of dominance of few species is common in other estuaries on the Brazilian coast (Paiva-Filho & Toscano 1987, Teixeira & Falcão 1992, Garcia & Vieira 1997, Vilar et al. 2017, Gurgel-Lourenço et al. 2023) and in various surface saline/brackish environments.

Over the months, the greatest abundance was found in April 2018, mainly due to the large catch of the clupeid *R. bahiensis* during this month, and this increase in abundance may be correlated with the proximity of the beginning of the rainy season in the region, since the period of greatest rainfall for the Lower São Francisco is from May/June to August/September (Knoppers et al. 2006). During periods of greater rainfall, there is an increase in continental drainage and an increase in the availability of nutrients to the aquatic environment, which increases primary productivity and causes a "bottom-up" effect on food webs, increasing the abundance of species. The lowest abundance was observed in January 2018. This month is characterized by lower rainfall for the region, which affects the supply of nutrients. Consequently, there may be a decrease in the abundance of the shallow area community, since the nutrients that would naturally be carried by the river flow to these areas are trapped by the dams in the river course (Bennett 1994, Gillanders & Kingsford 2002, Chícharo et al. 2006, MMA 2006, Silva et al. 2010, Mendes et al. 2021). Thus, the Lower São Francisco region is considered an oligotrophic environment, due to the artificial influence of nutrient retention caused by dams (Medeiros et al. 2007, 2011, Knoppers et al. 2006), considering that the sources are terrestrial, and nutrient input occurs through continental drainage.

The species richness curve reached the asymptote with a smaller number of samples (200) than the total number of samples taken (242) and coincides with the curve modeled according to the estimator present in Coleman et al. (1982). This indicates that the sampling effort used in the present study was sufficient to represent the fish community in shallow areas of the São Francisco River mouth.

Temporally, richness showed the highest values in March 2018, which coincides with the dry period indicated by Knoppers et al. (2006). The decrease in freshwater inflow into the system probably causes greater penetration of salt water and allows for greater occupation of species of marine or estuarine origin, thus increasing local richness. In contrast, the lowest richness was found in August 2017 coinciding with the rainy season in the Lower São Francisco (Knoppers et al. 2006).

Spatially, the highest richness value was observed at site 3, possibly due to structural differences between sampling sites, and this location had finer sediments than the others (personal observation), which probably increased the availability of organic matter and consequently the availability of food for the fish community. It is noteworthy that the greatest amounts of organic matter are usually correlated with sites that have fine sediments (Hedges & Keil 1995, Burone et al. 2003, Oliveira et al. 2014). The lowest richness was verified at site 2 and may be related to the proximity of this site to human occupation, since this sampling site is located in front of the fisherman village of Saramém, a place of constant movement of people and boats. Richness had the highest value during low tide and the lowest value at high tide, as fish caught at high tide tend to be only those that migrate following the tidal wave, as pointed out by Godefroid et al. (2003). However, the difference in water column height between high tide and low tide can also be the cause of this result, since during low tide the fish fauna is condensed in a smaller amount of water, which can facilitate their capture, while in high tide the greater amount of water can facilitate the escape of some species.

Spatiotemporally, the selected models indicated that richness in the sampled region of the Lower São Francisco is mainly controlled by the tide and the sampling site, with the highest values associated with low tide, which may be influencing the fish fauna as mentioned above, and with sites 1 and 3, suggesting that the greatest richness at these sites occurs due to structural differences among sampling sites (type of sediment, environmental complexity, etc.). For abundance, the models indicate the influence of the tidal state (analogously to richness), the month of collection (which probably influences the abundance through the seasonal pattern of rainfall) and the sampling site (which showed greater abundances in the innermost part of the system that may be related to the environmental gradients presented by the system).

Estuarine environments are places of great dynamics, as there is a convergence of terrestrial, oceanic and atmospheric processes that constantly alter their characteristics (Elliot & Mclusky 2002), this makes these environments complex in terms of geomorphology, hydrography, salinity, tidal characteristics, sedimentation and ecosystem energy, which results in a substantially different ichthyofauna (Kennish 2002). Abrupt changes in salinity, temperature, oxygen and turbidity cause rapid variations in its properties, requiring a great energy demand from the existing biota so that it can remain under these stressful conditions (Day et al. 1989). Making these places inhabited by well-adapted and distinct fauna (Odum 2004), however fragile to changes introduced by man (Yanez-Arancibia 1986). The estuarine ichthyofauna has low species richness, since few species are adapted to tolerate the variations in these areas, however the abundance and biomass are high. Most fish are not adapted to carry out their entire life cycle within estuaries. Fish are usually seasonal members of estuarine communities or use the estuary only as a migration route between spawning and feeding areas (Potter et al. 1986; Costa et al. 1994). Estuarine fish assemblages are dominant over other organisms both in abundance and in biomass and therefore play an important role in the energy flow of the estuarine system. The most abundant developmental stage in estuaries are juvenile forms (Kennish 2002). In estuarine systems endemism is low, which raises questions about which species really depend on estuaries and which use these habitats opportunistically (Lenanton & Potter 1987).

Regarding environmental variables, the selected models indicated that the presence of freshwater in the system (through increased rainfall, flow and pH drop) increases the richness in this environment, in line with what was observed by Lazzari et al. (2003), where it is shown that richness decreases in regions dominated by more saline waters. Small changes in freshwater input can generate changes in the fish fauna, with freshwater input being an important factor mainly in the innermost regions of estuarine systems (Greenwood et al. 2007). Moreover, temperature also appears as an important factor for richness, positively correlated with temperature increase, as in other studies carried out in estuaries (Lin & Shao 1999, Lazzari et al. 2003, Spach et al. 2004, Vendel & Chaves 2006, Ignácio & Spach 2010).

As for richness, the models selected for abundance also indicate that the presence of freshwater in the system (through increased rainfall, flow and decreased salinity) promotes an increase in the abundance of fish fauna. The influx of freshwater is directly linked with the transport of nutrients to the estuarine system, generating an increase in local productivity. This source of nutrients is essential for the maintenance of communities in the Lower São Francisco River, since after the construction of dams, this stretch of the river became oligotrophic as previously mentioned (Medeiros et al. 2007, 2011, Knoppers et al. 2006). Furthermore, the models indicate a negative correlation of abundance, both with salinity and with dissolved oxygen (DO), indicating that abundance varies inversely with the longitudinal gradient of these two variables. In estuarine systems, salinity (Valencia & Franco 2004, Cloern et al. 2017) and DO (Macêdo et al. 2000, Valencia & Franco 2004, Favero et al. 2019) decrease towards upstream.

With respect to the most abundant species, as observed for the entire community, the greater abundances at the mouth of the São Francisco River reflect the environmental gradient present in the region, primarily influenced by the tide and secondarily by the influx of freshwater into the system, which cause variations in environmental parameters, such as salinity and pH. *C. edentulus* is classified according to the guild classification of marine environment use proposed by Elliott et al. (2007), as a marine visitor species (MS), as it enters estuarine environments with strong marine influence during its juvenile phase and returns to the ocean in reproductive periods. According CCA, this species is mainly associated with site 1, a place under a strong influence of water from the ocean through tidal waves. In contrast, *G. barbus* was observed at the opposite end of the estuary, mainly associated with site 5, a place with lower salinity and stronger influence of freshwater. This species is classified in the guild of use as anadromous (AN), that is, it is a fish species that frequents the estuarine and marine environments during its growth, but needs to return to places of lower salinity during the reproductive period. During the study period, several juveniles of *G. barbus* were observed in the region of site 5 and on one occasion a large individual was caught performing parental care (mouthbrooding). The CCA also selected groups of species associated both with sites under strong marine influence (sites 1 and 2) and the estuarine environment (site 3). Among these species, there are two marine migrants (MM; *H. unifasciatus* and *E. melanoptarus*), which use the estuarine environment for growth and the marine environment for reproduction, and an anadromous species (AN; *L. grossidens*), most often with immature individuals, and an estuarine species (ES; *S. testudineus*), the latter species carries out its entire cycle within the estuary.

The last group selected by CCA is formed by species with no strong connection with any region of the sampled area, as they were equally distributed throughout the region, probably because they have great tolerance to changes in salinity along the gradient. Within this group there are two resident estuarine species (*A. brasiliensis* and *R. bahiensis*), which can carry out its entire life cycle within the estuarine environment, and an estuarine migrant species (*D. auratus*), which completes its life cycle outside the estuary or has discrete populations in freshwater or marine environments. *A. brasiliensis* were caught at different reproduction stages (immature, developing and mature), indicating that they complete their entire cycle at the site (Bot Neto et al. 2021).

In general, in relation to the factorial variables, there was a high influence of the tide and site for both models. On the other hand, the month influenced only the abundance of species. As for environmental variables, rainfall and temperature were equally important in structuring the fauna. Specifically for richness, pH was highly important in richness, and for abundance, flow and salinity were relevant. As for the most abundant species, the constancy in the occurrence of *A. brasiliensis*, *R. bahiensis* and *D. auratus* in all sampling sites was evident, but with a preference in sites 3, 4 and 5.

A spatial gradient was detected, with sites 1 and 2 under greater marine influence, sites 3 and 4 representing a transition between the environments and site 5 under the influence of brackish and freshwater. This gradient was mainly influenced by short time scale processes, which is the case of the tide, and secondarily by the river flow, which has shown to have a high relevance for the abundance patterns.

Finally, it is evident that this stretch of the Lower São Francisco River presents a longitudinal gradient and a fauna closer to an estuarine environment than to a river mouth. Furthermore, the intrusion of the saline wedge is probably caused by the reduction and regulation of the flow caused by the various dams along the river course, mainly by the Xingó Dam, which is located only 180 km from the mouth.

Acknowledgements

The authors would like to thank the Coordination for the Improvement of Higher Education Personnel (CAPES) for the scholarship granted to Renato L. Bot Neto (PhD Scholarship) in the Graduate Course in Ecology and Conservation at the Federal University of Paraná (PPG-ECO UFPR); to the students of the Fishery Engineering program at the Federal University of Sergipe (UFS), Luane, Kléverton, Paulo, and Priscilla who assisted in sampling and sorting of the material; to our boatman and friend Ronaldinho (Tito) always happy to sail with us through the Velho Chico; to Barbara Carvalho for always being an excellent friend and a great scientific partner in ichthyology.

Associate Editor

Rosana Mazzoni

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Renato Luiz Bot Neto: contribution to data collection; substantial contribution in the concept and design of the study; contribution to writing – original draft; contribution to data analysis and interpretation; contribution to manuscript preparation; contribution to critical revision, adding intelectual content.

André Pereira Cattani: contribution to data analysis and interpretation; contribution to critical revision, adding intelectual content; contribution to manuscript preparation.

Henry Louis Spach: contribution to data collection; substantial contribution in the concept and design of the study; contribution to manuscript preparation; contribution to critical revision, adding intelectual content.

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Olímpio Rafael Cardoso: contribution to writing – review and editing;

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Roberto Schwarz Júnior: contribution to critical revision, adding intelectual content; contribution to manuscript preparation.

Conflicts of Interest

We reiterate have no conflicts of interest to disclose.

Data Availability

Supporting data are available at <https://doi.org/10.48331/scielodata.W0DWQL>.

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Received: 27/06/2022
Accepted: 28/05/2023
Published online: 26/06/2023

Biota Neotropica 23(2): e20221388, 2023
www.scielo.br/bn

biotaneotropica
ISSN 1676-0611 (online edition)

Article

Diet of *Moenkhausia bonita* (Benine, Castro & Sabino 2004) (Characiformes: Characidae) in streams in the basin of rio Formoso, Brazilian Midwest

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CALDATTO, A.M., DIAS, R.M., FERREIRA, A. Diet of *Moenkhausia bonita* (Benine, Castro & Sabino 2004) (Characiformes: Characidae) in streams in the basin of rio Formoso, Brazilian Midwest. Biota Neotropica 23(2): e20221388. https://doi.org/10.1590/1676-0611-BN-2022-1388

Abstract: To characterize the diet composition of *Moenkhausia bonita* and its temporal and ontogenetic variations in streams in the Formoso River basin (MS). The collections were carried out in seven sampling points in two periods throughout the year (dry and rainy). The food items were analyzed according to the volumetric and occurrence frequency methods and the diet was characterized through the Food Index (IAi%). To determine ontogeny, the specimens were divided into five size classes in the dry (D1 to D5) and rainy (R1 to R5) periods. To verify the difference between the species' diet between the size classes and the periods of the year, the Permutational Multivariate Analysis of Variance – PERMANOVA analysis was performed. *Moenkhausia bonita* was classified as an invertivore when it consumed basically both aquatic and terrestrial invertebrates (99.5% of the diet), with higher consumption of aquatic invertebrates. There was a significant difference in the diet of between the dry and rainy periods, and although the species basically consumed the same items in the two studied periods, the proportions were different and there was no difference in the diet between size classes. *M. bonita* diet is based on autochthonous resources regardless of the size class, but that there were different consumption patterns when comparing the different periods of the year. The present study provided the first information on the feeding of *M. bonita* in a lotic environment and diet spectrum in the developmental phases, (ontogeny) and periods of the year, enabling a better understanding of the species, the importance of invertebrates in its diet, and the need for future studies on the biology, autoecology, and behavior of this species.
Keywords: *Feeding; tetra; trophic category; ontogeny.*

Dieta de *Moenkhausia bonita* (Benine, Castro & Sabino 2004) (Characiformes: Characidae) em riachos da bacia do rio Formoso, Centro-Oeste brasileiro

Resumo: Caracterizar a composição alimentar de *Moenkhausia bonita* e as variações temporais e ontogenéticas na dieta desta espécie em riachos da bacia do rio Formoso (MS). As coletas foram realizadas em sete pontos amostrais em dois períodos do ano (seco e chuvoso). Os itens alimentares foram analisados de acordo com os métodos volumétrico e de frequência de ocorrência e a dieta foi caracterizada através do Índice Alimentar (IAi%). Para determinar a ontogenia, os espécimes foram divididos em cinco classes de tamanho nos períodos seco (D1 a D5) e chuvoso (R1 a R5). Para verificar a diferença entre a dieta da espécie entre as classes de tamanho e os períodos do ano foi realizado a Análise de Variância Multivariada Permutacional – PERMANOVA. *M. bonita* foi classificada como invertívora ao consumir basicamente invertebrados tanto aquáticos quanto terrestres (99,5% da dieta), com consumo maior de invertebrados aquáticos. Houve diferença significativa na dieta entre os períodos seco e chuvoso, apesar da espécie consumir basicamente os mesmos itens nos dois períodos estudados, as proporções foram distintas e não houve diferença na dieta entre as classes de tamanho. A dieta de *M. bonita* é baseada em recursos autóctones independente da classe de tamanho, mas que houve consumo diferente entre os períodos do ano. O presente estudo forneceu as primeiras informações sobre a alimentação de *M. bonita* em ambiente lótico e seu espectro alimentar nas fases de desenvolvimento(ontogenia)e períodos do ano, possibilitando melhor conhecimento da espécie, a importância dos invertebrados em sua dieta e a necessidade de estudos futuros sobre a biologia, autoecologia e comportamento desta espécie.

Palavras-chave: *alimentação; lambari; categoria trófica; ontogenia.*

Introduction

The Neotropical region has the most diverse freshwater ichthyofauna in the world, with about 50% of the known fauna (Reis et al. 2016). Brazil is home to great biodiversity of fish (Buckup et al. 2007; Froese et al. 2016). Most of this richness of fish inhabits inland waters, representing about two-thirds of the ichthyofauna that occurs in this region (Nelson et al. 2016).

The state of Mato Grosso do Sul is drained by the Middle Paraguay River and Upper Paraná River basins, where 358 fishes species have been recorded, 257 species of which are recorded in the Paraguay River basin, (Froehlich et al. 2017). The Formoso River basin is a sub-basin of the Miranda River, inserted entirely within the municipality of Bonito, a place that presents tourist trend due to its scenic beauty (Teruya-Júnior 2011). This region is a reference for ecotourism in the country since most of the tourist attractions are linked to water resources (Lelis et al. 2015). Few studies have been conducted on the ichthyofauna in the Formoso River basin, such as the composition and structure of the ichthyofauna in streams comparing conservation gradients (Casatti et al. 2010), the weight-length relationship in stream fishes (Severo-Neto et al. 2018) and studies of the ecological interactions of fishes with habitat characteristics (Nunes et al. 2020).

Eight species of *Moenkhausia* are known in the state of Mato Grosso do Sul (Froehlich et al. 2017). *Moenkhausia bonita* is a small characid species that have been described in the Baía Bonita River, a tributary of the Formoso River (area of this study) (Benine et al. 2004). This species occurs mainly near the water surface, swimming in schools of 10 to 30 individuals (Benine et al. 2004). It is a widely distributed species in the Paraguay River basin but has been recorded in other basins, like La Plata River and Amazon region (Froehlich et al. 2017; Vanegas-Ríos et al. 2019; Fricke et al. 2020). *Moenkhausia bonita* isn't registered on the Red List of endangered species of the Ministry of the Environment (PORTARIA MMA 148/2022) and is classified as Least Concern (LC) according to the International Union for Conservation of Nature (IUCN, 2019).

The differentiation in the diet of a fish species may be due to spatial, temporal, ontogenetic, individual variations, and according to feeding tactics (Abelha et al. 2001). In tropical regions, subject to wide seasonal variations in water level, seasonality is one of the main factors influencing changes in fish diet, since it causes qualitative and quantitative changes in the availability of food items in aquatic ecosystems (Junk et al. 1989; Junk et al. 2021). Seasonal changes in fish diet are especially related to the entry of allochthonous resources into the aquatic environment (Quirino et al. 2017). Ontogenetic variation is an important factor to be verified in the diet of fish, usually accompanied by morphological changes throughout the development of individuals (Hahn et al. 2000; Bozza and Hahn 2010; Alves et al. 2021). Feeding tactics can change as fish grow, due to physical limitations regarding prey and food selectivity (Wainwrigth and Richard, 1995; Arim et al. 2010; Bozza and Hahn 2010; Keppeler et al. 2015; Alves et al. 2021). Dietary ontogenetic changes can reduce intraspecific competition and allow species to successfully establish themselves in environments (Alves et al. 2021). Understanding the relationships between fish fauna and the environment is essential to assist in methods of conservation and environmental restoration (Ferreira & Casatti 2006; Dias et al. 2022). The studies on the trophic ecology of fish are of paramount importance to know both individual and community processes, being important aspects for the conservation of species (Nunn et al. 2012; Tonella et al. 2019). Thus, this study aimed to characterize the diet of *M. bonita* in streams of the Formoso River basin and to verify possible changes in the diet of the species by periods (dry and rainy) and highlight the origin (allochthonous or autochthonous) of the food items most consumed by the species in the respective evaluated periods and to identify ontogenetic diet variations of the species.

Material and Methods

1. *Study area*

The study was carried out in seven points sampled in the streams of the Formoso River basin (MS). The Formoso River basin is located mostly in a limestone region and is situated in the sub-basin of the Miranda River, one of the six sub-basins of the Upper Paraguay basin (Mato Grosso do Sul 2004). The main river names the basin and extends a drainage area of about 136,000 hectares and is within the Serra da Bodoquena (Teruya-Júnior et al. 2009).

The Formoso River basin has an area of 1,334 km², located in the central region of the municipality of Bonito, in the state of Mato Grosso do Sul and is 100 km long (Duarte et al. 2005). The Formoso River is characterized by clear waters, a sandy-clay riverbed, thick litter and dense riparian forest that in some stretches is about 500 m wide from the riverbed (Reys et al. 2005). According to the Köppen classification, the climate of the region is sub-hot tropical, with hot and rainy periods occurring on average between October to April and dry seasons predominating from May to September, with average annual temperatures between 22 °C and 26 °C.

2. *Collecting the fish*

The fish were collected at two times of the year (January/rainy and October/dry 2016) in the seven points sampled in the streams of the Formoso River basin (coordinate 21°02'01"S 56°28'31"W), (coordinate 21°06'34"S 56°28'24"W), (coordinate 21°04'22"S 56°28'26"W), (coordinate 21°04'08"S 56°25'59"W), (coordinate 21°06'24"S 56° 33'42"W) (coordinate 21°02'55"S 56°18'10"W) and (coordinate 21°02'14"S 56°18'39"W) (Figure 1). The fish were sampled using seine net (5 mm mesh) and sieves. The specimens were anesthetized with Eugenol (clove oil; 70 mg/L) and then euthanized and fixed in 10% formalin solution and preserved 70% ethanol. Voucher specimens

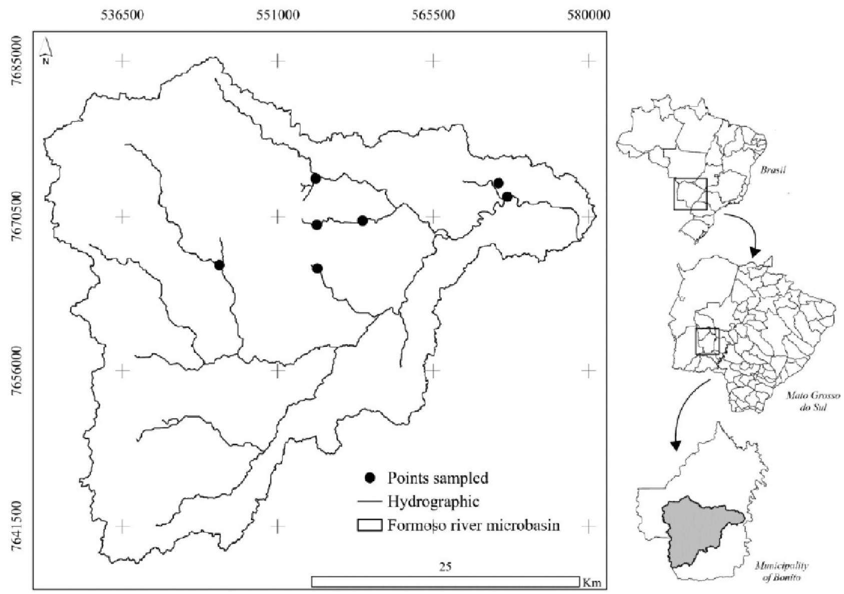

Figure 1. Map showing the location of the study area and the seven points sampled in the streams of the Formoso River basin, Mato Grosso do Sul, Brazil.

were deposited in the Zoological Collection (ZUFMS) of Universidade Federal do Mato Grosso do Sul (ZUFMS-PIS06693).

3. Diet analysis

In the laboratory, the biometry of the individuals of *M. bonita* were measured standard length (SL-mm), and the total weight (g) were taken. The individuals were dissected and the stomachs were removed. Stomach content was analyzed under a stereomicroscope and the food items were identified to the lowest possible taxonomic level with the support of specialized literature (McCafferty 1981; Mugnai et al. 2010). The items were analyzed according to the frequency of occurrence and volumetric methods (Hyslop, 1980). The volume of the items was obtained by compressing the material with a glass slide on a millimeter plate to a known height (1 mm), and the result was converted to milliliters (1 mm^3 = 0.001 ml) (Hellawell & Abel 1971).

4. Data analysis

The food items were grouped according to the following food categories: terrestrial invertebrate, aquatic invertebrate, plant, and other (filamentous algae and fish scale) and according to origin of food items (autochthonous, allochthonous and indeterminate). To characterize the diet the Food Index (IAi%) was calculated *Fi* is the relative frequency of occurrence of item *i* (%) and *Vi* is the relative volume of item *i* (total%) (Kawakami & Vazzoler 1980).

To assess ontogenetic variations in diet, individuals were grouped into five size classes (mm) in the dry (D1 to D5) (D1 = 15,3 – 20,3); (D2 = 20,4 – 25,4); (D3 = 25,5 – 30,5); (D4 = 30,6 – 35,6) and (D5 = 35,7 – 40,7) and rainy (R1 to R5) (R1 = 14,6 – 19,6); (R2 = 19,2 – 24,7); (R3 = 24,8 – 29,8); (R4 = 29,9 – 34,9) and (R5 = 35,0 – 40,0) periods. The groups were separated every five millimeters from the smallest individual for each period. To verify whether the diet of *M. bonita* showed differences in relation to size classes and sampling periods, we performed Permutational Multivariate Analysis of Variance – PERMANOVA (Anderson et al. 2008).

Results

The stomach contents of 240 specimens of *M. bonita* were analyzed during the dry (97) and rainy (143) periods. The diet of *M. bonita* was characterized as invertivorous as it basically consumed both aquatic and terrestrial invertebrates, despite the higher consumption of aquatic invertebrates in both periods (Figure 2). In the diet of *M. bonita*, were identified 30 food items consumed by the species, 27 food items were found in the dry, and 26 in the rainy period (Table 1). The main food items eaten in the dry period were fragments of aquatic insects, Formicidae and larvae, and pupae of Diptera. In the rainy period, the species mainly consumed Formicidae and Aquatic Insect fragments. Resources autochthonous origin were the most consumed in both periods (dry and rainy). The interaction term between period and size classes was not significant. Significant differences were identified in the diet of

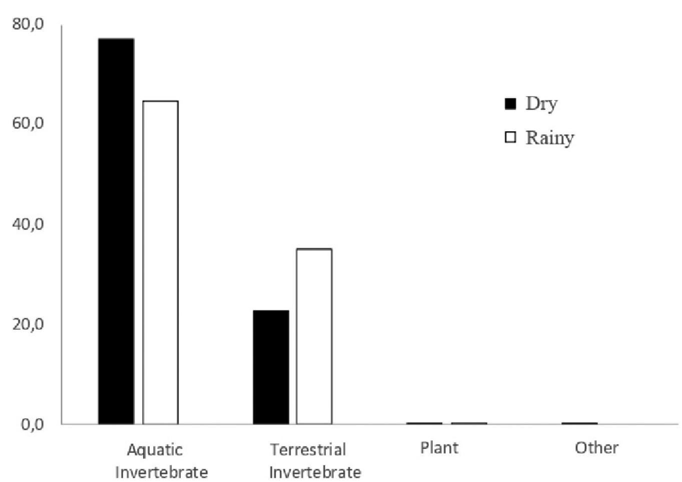

Figure 2. Food categories (IAi%) consumed by *Moenkhausia bonita* in the dry and rainy periods in streams of the Formoso River basin, Mato Grosso do Sul, Brazil.

Table 1. Frequency of occurrence (FO%), volume frequency (VO%) and Food index (IAi%) of the food items and food categories found in the diet of *Moenkhausia bonita* in the dry and rainy periods in streams of Formoso river basin, Mato Grosso do Sul, Brazil. Origin of food items in parentheses. F = Fragments.

Food items	Dry			Rainy		
	FO%	VO%	IAi%	FO%	VO%	IAi%
Aquatic invertebrates (Autochthonous)						
Ephemeroptera	17.5	2.1	1.4	8.2	4.8	1.2
Trichoptera	15.4	3.8	2.2	16.5	3.4	1.7
Trichoptera (pupae)	4.2	2.9	0.5	4.1	0.8	0.1
Plecoptera	6.3	0.6	0.1	1.0	0.1	<0.1
Chironomidae	37.8	3.3	4.6	22.7	2.7	1.8
Chironomidae (pupae)	0.7	0.6	<0.1			
Diptera (larvae)	35.0	10.2	13.3	26.8	3.2	2.6
Diptera (pupae)	32.2	7.8	9.3	20.6	7.4	4.6
Ceratopogonidae	15.4	0.9	0.5	5.2	0.3	0.1
Simuliidae	11.2	0.8	0.3	2.1	0.3	<0.1
Odonata	7.0	0.8	0.2	7.2	1.5	0.3
Coleoptera	6.3	1.6	0.4	8.2	3.9	1.0
Coleoptera (adult)	12.6	6.0	2.8	11.3	5.2	1.8
Hemiptera	4.9	1.9	0.3	1.0	1.2	<0.1
Megaloptera				1.0	0.8	<0.1
Insect exuvia	10.5	5.6	2.2	5.2	1.2	0.2
Aquatic invertebrates (F)	39.2	21.6	31.4	36.1	21.7	23.4
Hydracarina	1.4	<0.1	<0.1	2.1	0.1	<0.1
Nematoda				5.2	0.4	0.1
Oligochaeta				2.1	2.1	0.1
Terrestrial invertebrates (Allochthonous)						
Formicidae	44.1	12.6	20.5	64.9	30.1	58.6
Coleoptera	21.7	6.7	5.4	14.4	5.0	2.2
Hemiptera	3.5	1.1	0.1	1.0	0.6	<0.1
Diptera	4.2	0.6	0.1	1.0	0.3	<0.1
Terrestrial invertebrates (F)	16.1	5.5	3.3	3.1	1.2	0.1
Araneae	11.2	2.5	1.0	7.2	0.9	0.2
Plant (Indeterminate)						
Seeds	1.4	0.1	<0.1			
Plant Fragments	0.7	<0.1	<0.1	3.1	0.6	0.1
Other (Autochthonous)						
Filamentous Algae	2.8	0.2	<0.1			
Fish scale	2.8	0.1	<0.1			

the species between the periods considered (pseudo-F = 5.02; p = 0.02). However, the diet of *M. bonita* did not show ontogenetic variations, which indicates that the species feeds on the same food resources throughout development. The aquatic and terrestrial invertebrates food categories were the most consumed in most size classes (Figure 3). The main food items consumed in the different size classes were aquatic insect fragments, Formicidae, Diptera larvae, and pupae.

Discussion

We classified *Moenkhausia bonita* as invertivorous in the streams of the Formoso River basin, by consuming basically aquatic and terrestrial insects, with a tendency to consume higher proportions of autochthonous invertebrates. In lake environments, the insects were also the main items consumed by *M. bonita* (Carniatto et al. 2014; Carniatto et al. 2016; Quirino et al. 2018) where Chironomidae pupae were the most consumed item in most lakes. Others species of *Moenkhausia* showed a diet based on terrestrial and aquatic insects, such as *M. dichroura* (Toffoli et al. 2010), *M. sanctafilomenae* (Crippa et al. 2009; Toffoli et al. 2010), and *M. intermedia* (Crippa et al. 2009; Vidotto-Magnoni et al. 2009). Several authors emphasize the importance of the insectivorous diet, considering it as an adaptive advantage since the nutritional value of insects is more relevant than other food items present in the environment (Lowe-Mcconnell 1987, Gandini et al. 2012).

In relation to the periods sampled, although the specimens consumed basically the same items, the proportions were unequal, presenting a significant difference in diet according to the two periods sampled. In both periods aquatic invertebrates (mainly fragments of aquatic insects, larvae, and pupae of Diptera) were more consumed. Larvae and pupae of Diptera have different locomotion and dispersal techniques (Backenbury 2000), which often favors the capture of aquatic forms of this insect group by fish (Quirino et al. 2018). In the rainy season, there was a

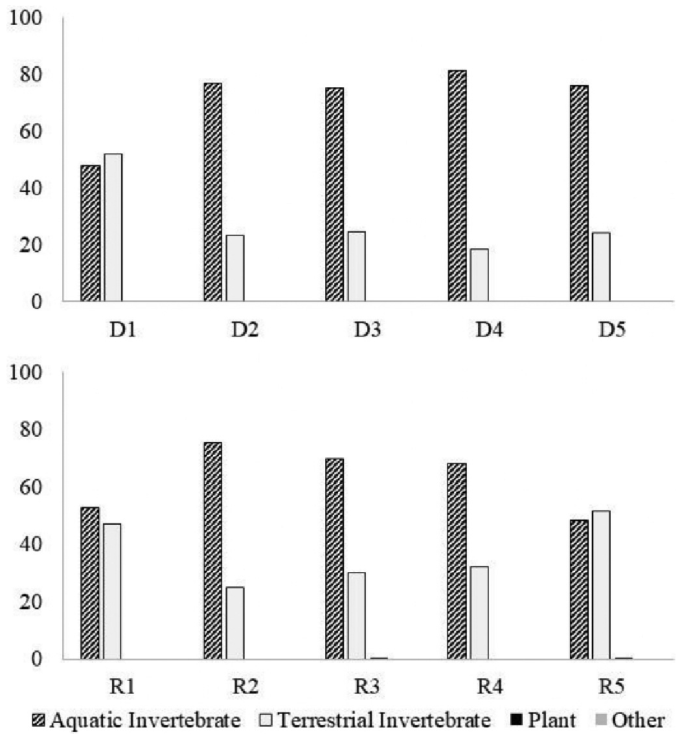

Figure 3. Food categories (IAi%) consumed by *Moenkhausia bonita* in different size classes (A) in the dry (D1 to D5) and (B) rainy (R1 to R5) in streams of the Formoso river basin, Mato Grosso do Sul, Brazil.

higher increment of terrestrial invertebrates (mainly Formicidae). Some studies with tetras of the genus *Astyanax* (Borba et al. 2008; Ferreira et al. 2012a) report an expressive consumption by Formicidae. We assume that the ingestion of this item was possible due to its availability and abundance in the sampled sites and periods. The abundance of Formicidae in the diet of fishes species may be related to the action of rain and wind, which would result in the fall of individuals from the riparian vegetation (Toffoli et al. 2010). With the onset of rainfall, there is increase in water velocity, which provides increase in water volume in the terrestrial environment, which contributes to a greater transport of items into the aquatic environment (Payne 1986).

Regarding size variations, there was no difference in diet among size classes, with aquatic invertebrates being the main food category in most classes for both periods, except for Classes D1 and R5 where the consumption of terrestrial invertebrates was slightly higher. In a study on ontogenetic variations in the diet of *Astyanax janeiroensis*, the authors pointed out that the smallest individuals consumed greater proportions of items of animal origin and the larger ones had a diet based on items of plant origin (Mazzoni et al. 2010). In the process of fish development, it is common for larvae and juveniles to include larger prey items in their diet, modifying their diet (Makrakis et al. 2005; Nunn et al. 2007), that is, as the fish increase in size, they consume wider variety of prey items becoming generalists (Winemiller 1989; Sánchez-Hernández et al. 2012; Keppeler et al. 2015). Morphological changes are factors that instigate fish to seek food resources of various sizes and appropriate nutritional proportions for each developmental stage (Winemiller 1989; Ortiz & Arim 2016). Consumption of small food items by smaller fish individuals is generally associated with mouth opening and position and number of teeth (Dala-Corte et al. 2016; Bonato et al. 2017). In the literature, smaller individuals of characids have a diet based on small aquatic organisms such as microcrustaceans and insect larvae, showing ontogenetic variations in their diets (Araújo et al. 2005; Mazzoni et al. 2010; Lampert et al. 2022). Unlike these studies, we did not find ontogenetic differences in the diet of *M. bonita*. The fact that this species does not present a significant difference between the size classes may be due mainly to the greater consumption of aquatic invertebrates in all stages of developmen generally smaller individuals consume this resource, that making necessary, further studies on the biology, ecology, and behavior of this species. Riparian forests have vast importance in regulating energy flow and nutrient cycling (Vannote et al. 1980). The maintenance of aquatic biodiversity is extremely dependent on the ecological functions performed by forests, mainly in providing abundant terrestrial food of animal and plant origin that falls into the water (Barrela & Petrere Junior 2001). Gregory et al. 1991; Bretschko & Waidbcher 2001; Sabino & Deus e Silva 2004, emphasize the influence of the riparian forest even when fish feed on autochthonous items because the primary source of these food resources has an allochthonous origin, considered the base of the trophic chain in streams. The Formoso River basin is a region with high agricultural and cattle ranching exploitation and with this we have been observing the decline of forest areas, reduction of permanent preservation areas, and increase of urban areas and ecotourism (Teruya-Júnior 2011). Riparian forests can act as an effective barrier against sedimentation and provide resources for stream fauna (Ferreira et al. 2012b), besides hindering the carriage of agrochemicals into the water bodies, particularly in streams that pass through basins subjected to intense agricultural and livestock activity (Sweeney et al. 2004, Martinelli & Filoso 2007).

Taking into account that the streams sampled along the Formoso River basin have forested riparian zones in different degrees of preservation, we can infer that the invertivorous diet of *M. bonita* is favored by food resources coming directly and indirectly from these environments. The results found in this first study with the species in a lotic environment reinforce the importance of resources of autochthonous origin in the food composition of the species. Emphasizing the importance of aquatic invertebrates, mainly immature forms of aquatic insects, which were verified in the diet of *M. bonita*. These resources were important for both times and for all size classes.

Acknowledgements

To the projects of Hydrological Monitoring of the Formoso River and the urban streams of Bonito/MS and the Integrated Monitoring System of the waters of the Hydrographic Basin of the Formoso River; to the Neotropica Foundation of Brazil; to NUPAQ-MS/UFGD for the availability of the laboratory and equipment for analyzing the contents and Drº. Francisco de Paula Severo da Costa Neto and Drª. Karina Keyla Tondato de Carvalho for the availability of the UFMS laboratories, for the explanations about the biology of the species studied and for the discussions.

Associate Editor

Rosana Mazzoni

Author Contributions

Amanda Menegante Caldatto: Substantial contribution to the idea and design of the study, contribution to the analysis and interpretation of data and the writing of the paper.

Anderson Ferreira: Substantial contribution to the idea and design of the study, contribution to data collection, contribution to data analysis and interpretation.

Rosa Maria Dias: Contribution to the analysis and interpretation of data and critical review (adding intellectual content).

Conflicts of Interest

The authors declare no conflict of interest related to the publication of this manuscript.

Data Availability

Supporting data are available at https://doi.org/10.48331/scielodata.BGIQSN

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Received: 27/07/2022
Accepted: 25/04/2023
Published online: 26/05/2023

Biota Neotropica 23(2): e20211260, 2023
www.scielo.br/bn

biotaneotropica
ISSN 1676-0611 (online edition)
Inventory

Phytoplankton composition from Araçá Bay and São Sebastião Channel, São Paulo, Brazil

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TOCCI, B.R.C., MOSER, G.A.O., CIOTTI, A.M. **Phytoplankton composition from Araçá Bay and São Sebastião Channel, São Paulo, Brazil**. Biota Neotropica 23(2): e20211260. https://doi.org/10.1590/1676-0611-BN-2021-1260

Abstract: Despite its small area, Araçá Bay (AB) holds cultural, historical, and economic value and displays great benthic biodiversity. Thus, it is crucial to monitor its environmental health, including knowing the main groups of phytoplankton and their temporal variability. The shallow waters of Araçá Bay are continuously modified by the complex hydrography of the adjacent São Sebastião channel (SSC), challenging standard experimental designs for phytoplankton collection. Here we report changes in phytoplankton composition at intervals of five to six weeks from September 2013 to August 2014 in both Araçá Bay and SSC. Samples were collected twice daily for three consecutive days to increase taxonomic resolution. Our goal was to provide an inventory of species occurrences to aid future public policies and environmental management of the area. Analyses revealed high species richness and 166 different phytoplankton taxa. Diatoms and dinoflagellates were always numerically dominant, but taxa occurrence changed markedly. Diatoms of the genera *Pseudo-nitzschia* were abundant during spring and summer concurrently to signatures of South Atlantic Central Water in the SSC, while *Thalassiosira* occurred when waters displayed relatively lower salinity. The inventory demonstrated several potentially harmful species of microalgae and cyanobacteria, strongly suggesting investments in monitoring programs in this area that currently experience an increase in population.
Keywords: *Biodiversity;* Pseudo-nitzschia; Thalassiosira; *São Paulo coast; coastal marine environments.*

Composição do fitoplâncton da Baía do Araçá e Canal de São Sebastião, São Paulo, Brasil

Resumo: Apesar de sua pequena área, a baía do Araçá (AB) possui grande valor cultural, histórico e econômico, e biodiversidade bentônica. Assim, é fundamental monitorar sua saúde ambiental, que inclui conhecer os principais grupos de fitoplâncton e sua variabilidade temporal. As águas rasas da baía do Araçá são continuamente modificadas pela hidrografia complexa do canal de São Sebastião (SSC), desafiando desenhos experimentais convencionais para coleta de fitoplâncton. Aqui relatamos mudanças sazonais na composição do fitoplâncton, em intervalos de 4 a 6 semanas, de setembro de 2013 a agosto de 2014 na baía do Araçá e no SSC, sendo coletadas duas vezes ao dia por três dias consecutivos em cada campanha de amostragem para aumentar a resolução taxonômica. Nosso objetivo foi fornecer um inventário de ocorrência de espécies para auxiliar futuras políticas públicas e gestão ambiental na área. As análises revelaram alta riqueza de espécies e 166 táxons fitoplanctônicos diferentes. Diatomáceas e dinoflagelados foram numericamente dominantes, mas a ocorrência de táxons mudou acentuadamente entre observações. As diatomáceas do gênero *Pseudo-nitzschia* foram abundantes durante a primavera e o verão concomitantemente às assinaturas da Água Central do Atlântico Sul no CSS, enquanto *Thalassiosira* ocorreu durante períodos de salinidade relativamente mais baixa. O inventário demonstrou várias espécies potencialmente nocivas de microalgas e cianobactérias, sugerindo fortemente investimentos para programas de monitoramento nesta área que vem registrando aumento populacional contínuo.
Palavras-chave: *Biodiversidade; Pseudo-nitzschia; Thalassiosira; litoral paulista; ambientes marinhos.*

Introduction

Phytoplankton communities vary according to the physicochemical conditions of the water (Margalef 1967), but knowledge on the specific composition of these communities remain challenging (Basterretxea et al. 2020). The occurrence and the dominance of a given phytoplankton species reflect its adaptation to the environment (e.g., Anderson et al. 2002, Kremer et al. 2017, Moser et al. 2017, Ryabov et al. 2021). Hence some large-scale generalizations about the taxonomic variability and abundance of phytoplankton can be made in the ocean. Nearshore, however, environmental conditions vary over time scales of hours to days, and the same is true for phytoplankton diversity, for which observations require intense sampling effort. The quantification of species in the world ocean (Sournia, 1991) is a laborious work. Although new instruments and techniques (e.g. Sosik & Olson, 2007) are now available, microscopy analyses remain invaluable for their validation. The availability of phytoplankton species inventories is essential at urbanized coastal sites as they subsidize environmental management actions.

The São Sebastião channel (SSC), located in the north portion of the São Paulo state coast, between the municipalities of São Sebastião and Ilhabela, is partially inserted in the Marine Environmental Protection Area of the North Coast of the State of São Paulo. In the central portion of the channel, a shallow tidal plain (average depth of 1.5 m) limited by rocky flanks (Amaral et al. 2010) is known as Araçá Bay. The bay has an extensive intertidal region, which can be fully exposed to the air and exceeds 300 m in length during low spring tides (Amaral et al. 2018), with a large area of the plain being immersed and submerged within the same tidal cycle (Siegle et al. 2018). Araçá Bay is of esteemed value to the local population because, in addition to harboring high biological diversity (Amaral et al. 2010), it is a stronghold of artisanal fishers who traditionally use small vessels for fishing or leisure (Amaral et al. 2018). In the past years, the north coast of São Paulo experienced increasing population growth and environmental impacts (Xavier et al. 2016), including the discharge of untreated sewage. Indeed, there is evidence that the interaction of the São Sebastião channel with the continent plays an important role in the exchange of nutrients (Gubitoso et al. 2008) on primary productivity (Regaudie et al. 2017).

Available phytoplankton studies in SSC waters consisted of surveys reporting changes in biomass (i.e., chlorophyll concentration) or relative abundance of major taxonomic groups and their relationships with nutrient concentrations (Muller-Melchers 1955, Gianesella et al. 1999, Saldanha-Corrêa & Gianesella 2003). A review of phytoplankton studies carried out along the São Paulo coast provided a comprehensive inventory of the species present from 1913 to 2006 (Villac et al. 2008). However, no further diversity studies are available. More recent analyses of changes in chlorophyll concentration fractionated by size classes (Giannini & Ciotti 2016) and main taxonomic groups (Ciotti et al. 2018a) derived from efforts during the Araçá Thematic FAPESP project (https://biota-araca.org/index.html), conducted from September 2013 to August 2014 and showed the importance of diatoms when phytoplankton biomass increased. The present study is also derived from the Araçá Project phytoplankton dataset (Tocci 2016) and focuses on detailed taxonomic descriptions of phytoplankton in Araçá Bay and SSC, using light microscopy. The main objective is to update the phytoplankton taxa for this region, report their relative occurrence frequencies, and describe differences between the species found in Araçá Bay and the adjacent waters in the São Sebastião channel.

Materials and Methods

The phytoplankton *checklist* is composed of samples derived from three oceanographic stations located in the interior of Araçá Bay (AB)

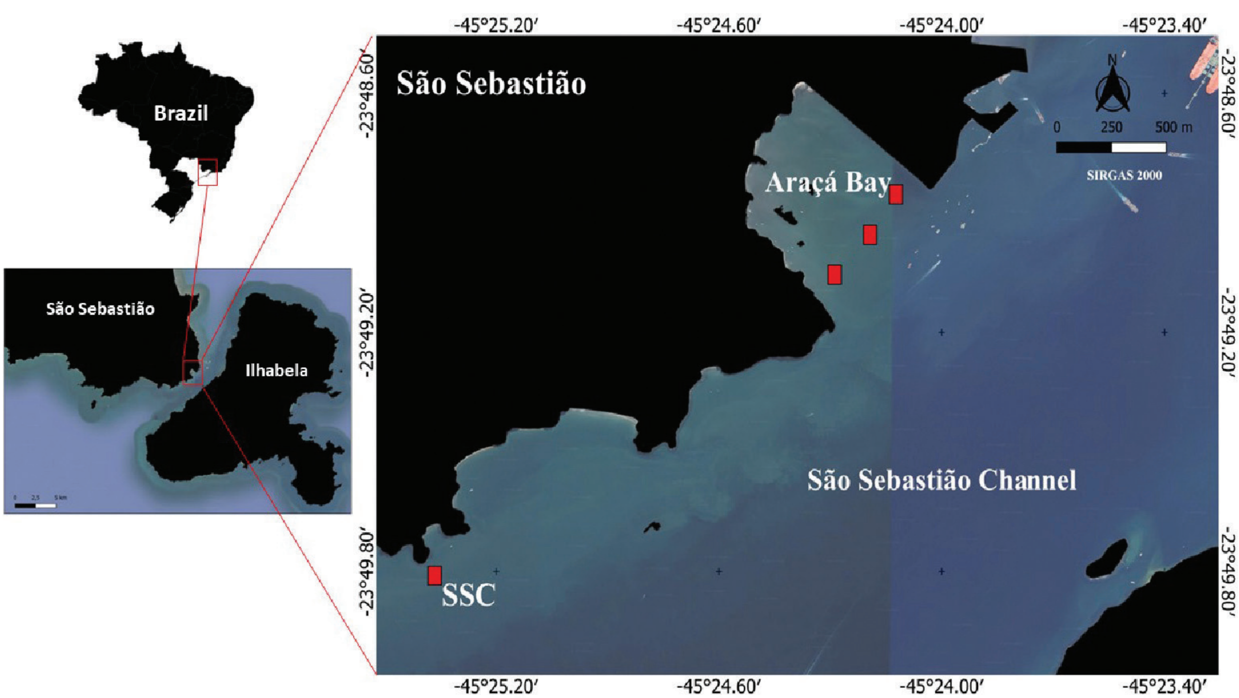

Figure 1. Location of the sampling sites at Araçá Bay (AB) and São Sebastião Channel (SSC) near the oceanographic buoy of SIMCosta Project.

and a single station located in the southern portion of the São Sebastião Channel (SSC) at the 15 m isobath (Figure 1). Nine surveys occurred between September 2013 and August 2014 every five to six weeks, in the morning and afternoon of three consecutive days (Ciotti et al. 2018b) to increase the probability of observing the variable hydrodynamics of SSC and rarer phytoplankton taxa. We used a Sontek Castway CTD to vertically profile the temperature and salinity at each station and a 5 L Van Dorn bottle to collect water for analyses of inorganic nutrients, chlorophyll-a, and phytoplankton cell enumeration. Three water samples were combined to represent AB and SSC to increase the representativeness of the occurring taxa analysis. AB samples refer to the combination of three independent stations located at isobaths between 1.5 and 2 m, while SSC refers to three successive deployments of the Van Dorn bottle (Tocci, 2016). The composite samples were further concentrated (2 L to about 100 mL) by reverse filtration with a 5 µm nylon mesh and preserved with formaldehyde neutralized in hexamethylenetetramine (0.4 %). Climatological data on precipitation rates were consulted on the CPTEC-INPE website (http://clima1.cptec.inpe.br/).

Cell enumeration and taxonomic analysis used Üthermol sedimentation chambers of 5 mL or 10 mL and an inverted optical microscope ZEISS-Axio® Observer D1 equipped with phase contrast and differential interference contrast (DIC). Only cells with maximum linear dimension MLD > 5 µm were counted, and identifications reached the lowest possible taxonomic level (genus and species) only for cells with MLD > 10 µm, with the help of specialized literature (e.g., Tomas 1997, Tenenbaum et al. 2004, Tenenbaum et al. 2006, Garcia & Odebrecht 2009, Haraguchi & Odebrecht 2010). Names and synonyms were checked and updated by queries of the *Algaebase* database (Guiry & Guiry 2021), and the diatom classification followed the work by Medlin & Kaczmarska (2004). The records for *Pseudo-nitzschia* followed the nomenclature of Hasle (1965) that divided the colony-forming species of the genus *Nitzschia* into two complexes: the "delicatissima" – for cells with widths equal to or smaller than 3 µm, and the «seriata» for cells wider than 3 µm. These two complexes were later combined and updated to the genera *Pseudo-nitzshia* (Hasle 1994).

The relative occurrence frequencies of taxa were calculated based on the method described by Matteucci & Colma (1982), which considers the overall number of occurrences of a taxon (65 samples for AB and 65 for SSC samples), following the categories: very frequent (VF) > 70%; frequent (F) ≤ 70% – >40%; infrequent (I) ≤ 40% – > 10%; and sporadic (S) <10%.

Results

Seawater temperature varied from 19.4 to 29.4° C in Araçá Bay (AB) and from 16.4 to 29.7°C in São Sebastião Channel (SSC), while salinity ranged from 30.8 to 36.6 in AB and from 30.9 to 35.7 in SSC (Table 1). A mixture of South Atlantic Central Water (SACW, thermohaline

Table 1. Environmental Variables measured during samplings at **São Sebastião Channel (SSC)** and **Araçá Bay (AB).** Minimum (Min), maximum (Max), average and standard deviation (SD), see Figure 1 for locations.

		SSC				AB			
		Min	**Max**	**Average**	**SD**	**Min**	**Max**	**Average**	**SD**
Temperature	°C	19.4	29.7	24.4	± 2.37	19.4	29.4	24.5	± 2.23
Salinity	–	30.9	35.7	34.3	± 1.21	30.8	36.6	34.4	± 1.13
Ammonia (NH_4)	$\mu mol.L^{-1}$	0.01	1.19	0.33	± 0.25	0.06	6.59	0.82	± 0.82
Nitrate plus Nitrite ($NO_3^- + NO_2^-$)	$\mu mol.L^{-1}$	0.01	0.77	0.27	± 0.14	0.01	2.02	0.52	± 0.33
Phosphate (PO_4)	$\mu mol.L^{-1}$	0.09	0.71	0.32	± 0.15	0.11	0.89	0.41	± 0.17
Silicate ($Si(OH)_4^{-4}$)	$\mu mol.L^{-1}$	0.47	6.25	03.08	± 1.25	0.64	5.62	3.63	± 1.33
Chlorophyll-a (Chla)	$mg.m^{-3}$	0.58	7.18	02.02	± 1.24	01.02	7.56	2.66	± 1.3

Table 2. Percentage of occurrence and summary statistics of the density ($cel.L^{-1}$) of the phytoplanktonic groups (> 5 µm), minimum (Min), maximum (Max), average, and standard deviation (SD) values. Taxonomic groups: centric diatom (CD) including both Coscinodiscophyceae and Mediophyceae, pennate diatom (PD), unarmored dinoflagellate (ND), armored dinoflagellate (TD), silicoflagellate (SI), flagellate (FL), coccolithophorid (CO) and cyanobacteria (CY). São Sebastião Channel (SSC) and Araçá Bay (AB).

Group	SSC					AB				
	%	**Min**	**Max**	**Average**	**SD**	**%**	**Min**	**Max**	**Average**	**SD**
CD	33.6	2.10^3	4.10^5	4.10^4	$\pm 6.10^4$	33.9	4.10^2	3.10^5	5.10^4	$\pm 7.10^4$
PD	67	9.10^2	1.10^6	9.10^4	$\pm 2.10^5$	40	3.10^2	6.10^5	6.10^4	$\pm 8.10^4$
ND	3.9	0	4.10^4	5.10^3	$\pm 8.10^3$	2	0	2.10^4	3.10^3	$\pm 5.10^3$
TD	10	0	8.10^4	1.10^4	$\pm 2.10^4$	3	0	3.10^4	5.10^3	$\pm 4.10^3$
SI	1.1	0	1.10^4	1.10^3	$\pm 2.10^3$	0.3	0	3.10^3	5.10^2	$\pm 7.10^2$
FL	12.7	0	5.10^4	2.10^4	$\pm 1.10^4$	16.7	6.10^2	9.10^4	3.10^4	$\pm 2.10^4$
CO	2.8	0	6.10^4	4.10^3	$\pm 1.10^4$	2.6	0	8.10^4	4.10^3	$\pm 1.10^4$
CY	2.5	0	3.10^4	3.10^3	$\pm 6.10^3$	1.5	0	4.10^4	2.10^3	$\pm 6.10^3$

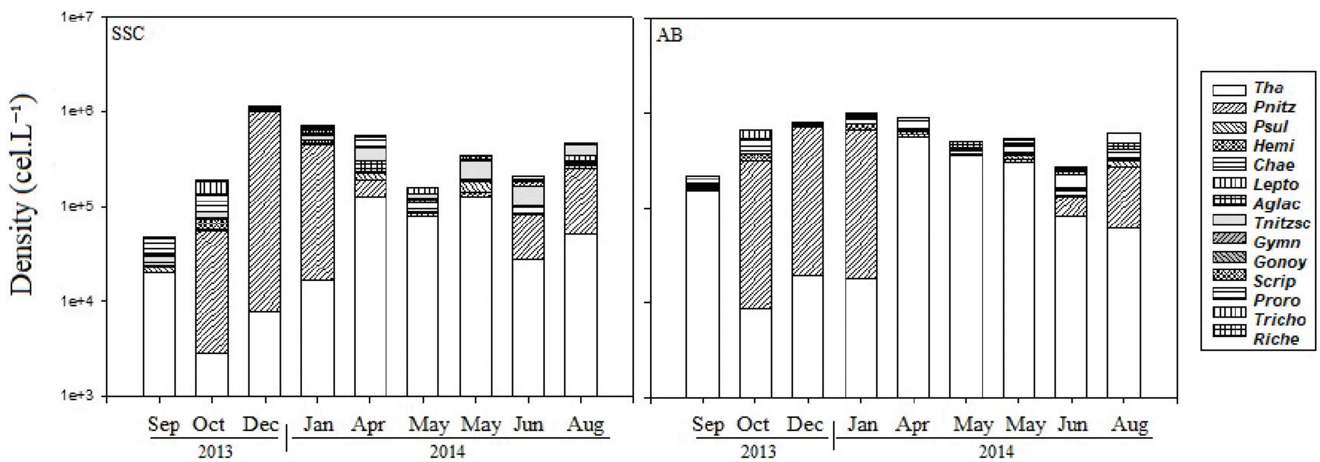

Figure 2. Variation in the density of the predominant phytoplanktonic taxa collected every 5–6 weeks between September 2013 and August 2014. Taxa: *Thalassiosira* sp.1 (Tha), *Pseudo-nitzschia* spp. (Pnitz), *Paralia sulcata* (Psul), *Hemiaulus* spp. (Hemi), *Chaetoceros* spp. (Chae), *Leptocylindrus* spp. (Lepto), *Asterionellopsis glacialis* (Aglac), *Thalassionema nitzschioides* (Tnitzsc), Gymnodiniales (Gymn), *Scrippsiella* spp. (Scrip), *Prorocentrum* spp. (Proro), *Trichodesmium* spp. (Tricho) and Richelia *intracellularis* (Riche). São Sebastião Channel (SSC) and Araçá Bay (AB).

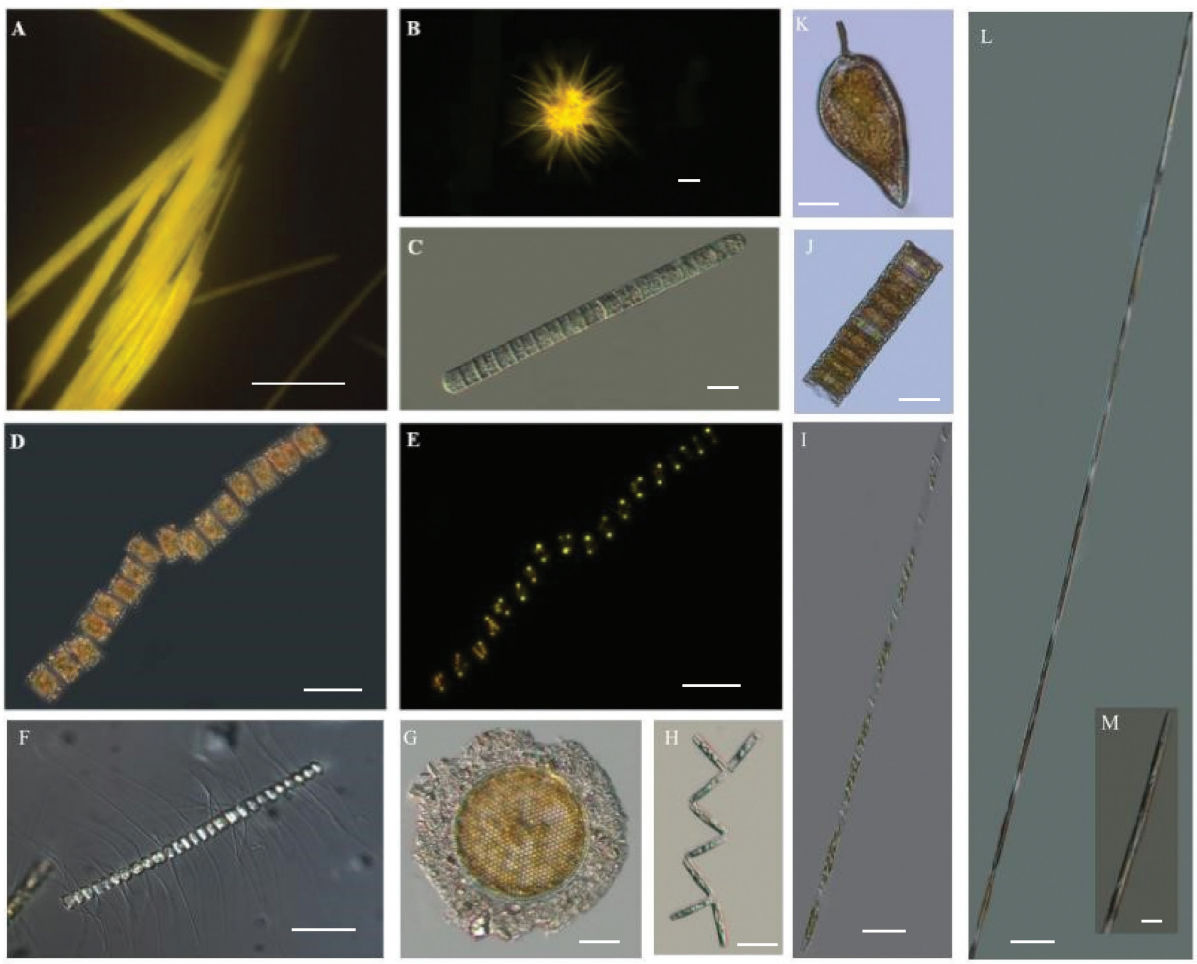

Figure 3. Diazotrophic cyanobacteria of the genus *Trichodesmium,* aggregates in bundles know as "tuffs" (**A**), in spherical "puffs" (**B**) and as a single trichome (**C**). Diatom *Hemiaulus membranaceus* (**D**) and diazotrophic cyanobacteria *Richelia intracellularis* (**E**) within it. Diatoms *Chaetoceros* cf. *debilis* (**F**), *Thalassiosira* sp.1 (**G**), *Leptocylindrus danicus* (**I**) and *Paralia sulcata* (**J**). Diatoms *Thalassionema nitzschioides* (**H**), *Pseudo-nitzschia* "*seriata* complex" sp.1 (see methods for definition) (**L, M**). Armored Dinoflagellate *Prorocentrum micans* (**K**). **A, B** epifluorescence microscopy image at 10x magnification; **E** epifluorescence microscopy image at 20x magnification; **I, L** DIC microscopy image at 200x magnification and **C, F, G, H, M** at 40x magnification; **D, K, J** phase-contrast microscopy image at 400x magnification. Scale bar: **A, B, D, E, F** = 50 µm; **C, G, H, I, J, K, L** = 10 µm; **M** = 05 µm.

Table 3. Taxonomic classification of the phytoplankton community observed in Araçá Bay (AB) and at São Sebastião Channel (SSC), between September 2013 and August 2014, see locations in Figure 1. Relative frequencies at each point: VF = very frequent, F = frequent, I = infrequent, S = sporadic; (MDL > 10 µm for the majority of taxa identified up to genera – species level).

Classification	Relative frequency	
	SSC	AB
Phylum Bacillariophyta		
Class Mediophyceae		
Subclass Biddulphiophycidae		
Order Biddulphiales		
Family Biddulphiaceae		
Biddulphia biddulphiana (J.E. Smith) Boyer	S	I
Family Bellerocheaceae		
Climacodium frauenfeldianum Grunow	I	I
Order Briggerales		
Family Streptothecaceae		
Helicotheca tamesis (Shrubsole) M.Ricard	S	–
Subclass Chaetocerotophycidae		
Order Chaetocerotales		
Family Chaetocerotaceae		
Bacteriastrum cf. *hyalinum* Lauder 1864	S	S
Bacteriastrum delicatulum Cleve	–	S
Chaetoceros aequatorialis Cleve	S	S
Chaetoceros affinis Lauder	S	I
Chaetoceros brevis F.Schütt	S	S
Chaetoceros concavicornis L.A.Mangin	S	S
Chaetoceros compressus Lauder	S	S
Chaetoceros curvisetus Cleve	I	I
Chaetoceros danicus Cleve	S	S
Chaetoceros cf. *debilis* Cleve	F	F
Chaetoceros decipiens Cleve	I	I
Chaetoceros didymus Ehrenberg	I	I
Chaetoceros lorenzianus Grunow	I	I
Chaetoceros peruvianus Brightwell	S	S
Chaetoceros subtilis Cleve	S	S
Order Hemiaulales		
Family Hemiaulaceae		
Cerataulina pelagica (Cleve) Hendey	I	I
Eucampia zodiacus Ehrenberg	S	S
Hemiaulus hauckii Grunow ex Van Heurck	I	I
Hemiaulus membranaceus Cleve	I	I
Hemiaulus sinensis Greville	S	I
Family Isthmiaceae		
Isthmia cf. *nervosa* Kütz	–	S
Subclass Cymatosirophycidae		
Order Cymatosirales		
Family Cymatosiraceae		
Cymatosira lorenziana Grunow	S	S
Subclass Thalassiosirophycidae		
Order Lithodesmiales		
Family Lithodesmiaceae		
Lithodesmium undulatum Ehrenberg	S	S
Ditylum brightwellii (T.West) Grunow	S	S
Order Thalassiosirales		
Family Thalassiosiraceae		
Detonula pumila (Castracane) Gran	–	S
Thalassiosira sp. 1	VF	VF
Thalassiosira sp. 2	F	F
Thalassiosira cf. *decipiens* (Grunow) Jørgensen	S	F
Thalassiosira gravida Cleve	S	S
Thalassiosira punctigera (Castracane) Hasle	F	F
Thalassiosira cf. *minuscula* Krasske	I	I
Family Skeletonemataceae		
Skeletonema cf. *costatum* (Greville) Cleve	I	I
Family Lauderiaceae		
Lauderia annulata Cleve	I	I
Order Stephanodiscales		
Family Stephanodiscaceae		
Cyclotella cf. *litoralis* Lange & Syvertsen	S	I
Cyclotella cf. *striata* (Kützing) Grunow	S	S
Cyclotella cf. *stylorum* Brightwell	I	I
Order Eupodiscales		
Family Odontellaceae		
Odontella aurita (Lyngbye) C.Agardh	S	I
Family Parodontellaceae		
Trieres mobiliensis (Bailey) Ashworth & E.C.Theriot	S	I
Class Coscinodiscophyceae		
Order Asterolamprales		
Family Asterolampraceae		
Asteromphalus flabellatus (Brébisson) Greville	S	S
Order Coscinodiscales		
Family Coscinodiscaceae		
Coscinodiscus asteromphalus Ehrenberg	S	I
Coscinodiscus granii L.F.Gough	S	S
Coscinodiscus wailesii Gran & Angst	S	
Family Heliopeltaceae		
Actinoptychus senarius (Ehrenberg) Ehrenberg	I	I

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Classification	Relative frequency SSC	Relative frequency AB
Family Hemidiscaceae		
Azpeitia sp.1	S	S
Family Leptocylindraceae		
Leptocylindrus danicus Cleve	I	I
Leptocylindrus minimus Gran	I	I
Order Rhizosoleniales		
Family Probosciaceae		
Proboscia alata (Brightwell) Sundström	S	S
Family Rhizosoleniaceae		
Dactyliosolen fragilissimus (Bergon) Hasle	I	I
Dactyliosolen phuketensis (B.G.Sundström) G.R.Hasle	–	S
Guinardia delicatula (Cleve) Hasle	I	I
Guinardia flaccida (Castracane) H.Peragallo	I	I
Guinardia striata (Stolterfoth) Hasle	I	I
Neocalyptrella robusta (G.Norman ex Ralfs) Hernández-Becerril & Castillo	S	S
Rhizosolenia hebetata Bailey	S	S
Rhizosolenia hyalina Ostenfeld	S	S
Rhizosolenia styliformis T.Brightwell	S	S
Sundstroemia setigera Medlin, L.K., Boonprakob, A., Lundholm, N. & Moestrup	I	S
Sundstroemia pungens Medlin, L.K., Boonprakob, A., Lundholm, N. & Moestrup	S	I
Order Triceratiales		
Family Triceratiaceae		
Triceratium favus Ehrenberg	I	I
Order Paraliales		
Family Paraliaceae		
Paralia sulcata (Ehrenberg) Cleve	F	F
Subclass Corethrophycidae		
Order Corethrales		
Family Corethraceae		
Corethron sp.1	I	I
Class Bacillariophyta (incertae sedis)		
Order Bacillariophyta (incertae sedis)		
Family Bacillariophyta (incertae sedis)		
Neomoelleria cornuta (Cleve) S.Blanco & C.E.Wetzel	I	I
Class Bacillariophyceae		
Order Bacillariales		
Family Bacillariaceae		
Bacillaria paxillifera (O.F.Müller) T.Marsson	S	S
Cylindrotheca closterium (Ehrenberg) Reimann & J.C.Lewin	F	VF
Denticula sp.1	S	–
Fragilariopsis doliolus (Wallich) Medlin & P. A. Sims	I	I
Nitzschia sp.1	S	I
Nitzschia longissima (Brébisson) Ralfs	S	I
Nitzschia incurva var. *lorenziana* R. Ross	I	F
Pseudo-nitzschia spp	VF	VF
Pseudo-nitzschia "delicatissima complex" sp.2	I	I
Pseudo-nitzschia "*seriata* complex" sp.1	F	F
Pseudo-nitzschia "*seriata* complex" sp.2	F	F
Tryblionella sp.1	I	F
Order Cocconeidales		
Family Cocconeidaceae		
Cocconeis sp.1	I	I
Order Cymbellales		
Family Cymbellaceae		
Cymbella sp.1	S	S
Order Naviculales		
Family Diploneidaceae		
Diploneis cf. *bombus* (Ehrenberg) Ehrenberg	S	I
Diploneis didymus (Ehrenberg) Ehrenberg	–	S
Diploneis cf. *smithii* (Brébisson) Cleve	–	S
Diploneis weissflogii (A.W.F.Schmidt) Cleve	F	F
Family Naviculaceae		
Haslea wawrikae (Husedt) Simonsen	I	I
Haslea cf. *trompii* (Cleve) Simonsen	I	I
Navicula sp.1	VF	F
Family Plagiotropidaceae		
Meuniera membranacea (Cleve) P.C.Silva	I	I
Family Pleurosigmataceae		
"*Pleurosigma/Gyrosigma*" Complex	F	VF
Family Stauroneidaceae		
Stauroneis sp.1	S	I
Order Fragilariales		
Family Fragilariaceae		
Fragilaria sp.1	S	I
Order Licmophorales		
Family Licmophoraceae		
Licmophora tincta (C.Agardh) Grunow	I	F
Order Thalassiophysales		
Family Catenulaceae		
Amphora sp.1	I	F

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Classification	Relative frequency	
	SSC	AB
Order Surirellales		
Family Surirellaceae		
Stenopterobia sp.1	–	S
Surirella sp.1	–	S
Subclass Fragilariophycidae		
Order Thalassionematales		
Family Thalassionemataceae		
Lioloma pacificum (Cupp) Hasle	I	I
Thalassionema frauenfeldii (Grunow) Tempère & Peragallo	I	S
Thalassionema nitzschioides (Grunow) Mereschkowsky	VF	VF
Thalassiothrix sp.1	S	S
Subclass Urneidophycidae		
Order Plagiogrammales		
Family Plagiogrammaceae		
Plagiogramma sp.1	–	S
Order Rhaphoneidales		
Family Asterionellopsidaceae		
Asterionellopsis glacialis (Castracane) Round	I	I
Family Rhaphoneidaceae		
Delphineis sp.1	F	F
Rhaphoneis sp.1	I	F
Phylum Miozoa		
Superclass Dinoflagellata		
Class Dinophyceae		
Order Dinophysiales		
Family Dinophysaceae		
Dinophysis “acuminata/sacculus” complex	I	I
Dinophysis cf. *caudata* Kent	S	–
Dinophysis microstrigiliformis Abé	S	–
Dinophysis cf. *ovum* F.Schütt	S	–
Dynophysis tripos	I	I
Ornithocercus cf. *magnificus* Stein	–	S
Order Gonyaulacales		
Family Ceratiaceae		
Tripos cf. *declinatus* (G.Karsten) F.Gómez	I	I
Tripos azoricus (Cleve) F. Gómez	S	–
Tripos furca (Ehrenberg) F. Gómez	I	I
Tripos fusus (Ehrenberg) F. Gómez	I	I
Tripos hircus (Schröder) F. Gómez	I	I
Tripos longirostrum (Gourret) Hallegraeff & Huisman	S	S

Classification	Relative frequency	
	SSC	AB
Tripos macroceros (Ehrenberg) Hallegraeff & Huisman	S	–
Tripos cf. *massiliense* (Gourret) F.Gómez	I	I
Tripos muelleri Bory	I	I
Tripos trichoceros (Ehrenberg) Gómez	S	S
Family Gonyaulacaceae		
Gonyaulax cf. *spinifera* (Claparède & Lachmann) Diesing	F	I
Family Pyrocystaceae		
Alexandrium cf. *tamarense* (Lebour) Balech	S	S
Pyrophacus horologium F.Stein	S	S
Order Gymnodiniales		
Family Gymnodiniaceae		
cf. *Gymnodinium* sp.1	VF	F
cf. *Gymnodinium* sp.2	S	S
cf. *Gymnodinium* sp.3	S	S
cf. *Gymnodinium* sp.4	S	S
Family Gyrodiniaceae		
Gyrodinium sp.1	S	S
Order Peridiniales		
Family Heterocapsaceae		
Heterocapsa rotundata (Lohmann) Gert Hansen	F	I
Heterocapsa sp.1	I	I
Family Oxytoxaceae		
Oxytoxum scolopax F.Stein	–	S
Oxytoxum crassum J.Schiller	S	–
Corythodinium tesselatum (F.Stein) Loeblich Jr. & Loeblich III	S	–
Corythodinium constrictum (F.Stein) F.J.R.Taylor	S	–
Family Podolampadaceae		
Podolampas palmipes Stein	S	S
Family Protoperidiniaceae		
Protoperidinium crassum (Balech) Balech	S	S
Protoperidinium curtipes (E.G.Jørgensen) Balech	S	–
Protoperidinium divergens (Ehrenberg) Balech	S	S
Protoperidinium leonis (Pavillard) Balech	S	–
Protoperidinium mariebouriae (Paulsen) Balech	S	–
Protoperidinium parviventer Balech	S	S
Protoperidinium pentagonum (Gran) Balech	S	S
Protoperidinium steinii (E.G.Jørgensen) Balech	I	I

Continue...

...Continuation

Classification	Relative frequency	
	SSC	AB
Family Pyrocystaceae		
Pyrocystis lunula (F.Schütt) F.Schütt	I	–
Order Prorocentrales		
Family Prorocentraceae		
Prorocentrum balticum (Lohmann) Loeblich III	F	F
Prorocentrum gracile F.Schütt	F	F
Prorocentrum micans Ehrenberg	F	F
Prorocentrum cordatum (Ostenfeld) J.D.Dodge	F	F
Prorocentrum scutellum B.Schröder	F	F
Order Thoracosphaerales		
Family Thoracosphaeraceae		
cf. *Scrippsiella*	S	S
Scrippsiella acuminata (Ehrenberg) Kretschmann, Elbrächter, Zinssmeister, S.Soehner, Kirsch, Kusber & Gottschling	VF	F
Scrippsiella spinifera G.Honsell & M.Cabrini	I	I
Phylum Ochrophyta		
Class Dictyochophyceae		
Order Dictyochales		
Family Dictyochaceae		
Dictyocha fibula Ehrenberg	F	I
Octactis octonaria (Ehrenberg) Hovasse	I	I
Phylum Haptophyta		
Class Coccolithophyceae		
Order Coccolithales		
Family Calcidiscaceae		

Classification	Relative frequency	
	SSC	AB
Umbilicosphaera cf. *sibogae* (Weber Bosse) Gaarder	F	F
Order Syracosphaerales		
Family Calciosoleniaceae		
Calciosolenia brasiliensis (Lohmann) J.R.Young	S	S
Calciosolenia murrayi Gran	S	–
Family Rhabdosphaeraceae		
Discosphaera sp.1	I	S
Discosphaera tubifera (Murray & Blackman) Ostenfeld	S	–
Rhabdosphaera sp.1	I	S
Family Syracosphaeraceae		
Calciopappus sp.1	S	–
Syracosphaera sp.1	I	S
Syracosphaera pirus Halldal & Markali	S	–
Syracosphaera prolongata Gran ex Lohmann	S	S
Phylum Cyanobacteria		
Class Cyanophyceae		
Order Oscillatoriales		
Family Microcoleaceae		
Trichodesmium erythraeum Ehrenberg ex Gomont	I	F
Trichodesmium thiebautii Gomont ex Gomont	I	F
Order Synechococcales		
Family Pseudanabaenaceae		
Pseudanabaena sp.1	I	S
Order Nostocales		
Family Nostocaceae		
Richelia intracellularis J.A.W.F.Schmidt	I	I

index 20.0 °C; 36.36, according to Miranda, 1985) and Coastal Water (CW, salinity below 35 and temperature higher than 20.0 °C) was observed during December 2013 in both AB and SSC. The CW was a mixture of oceanic water masses and continental outflows and dominated SSC in all samplings with temporally variable thermohaline characteristics (see Ciotti et al. 2018a same dataset). Maximum concentrations of ammonia, nitrate + nitrite, phosphate, silicate, and chlorophyll, were generally observed at AB, but their average values were similar at both sampling sites (Table 1). During all surveys, we observed smaller volumes of accumulated precipitation compared to the regional climatology.

Phytoplankton densities were as high as 10^6 cel L^{-1} (Table 2). Despite the significance of picoplankton and nanoplankton for Brazilian coastal waters, it is worth mentioning that this inventory covered organisms greater than 5 µm. Diatoms were the predominant taxonomic group at both AB and SSC, with pennate diatoms representing 67% of phytoplankton species in the latter during the sampling period. Comparatively larger abundances of flagellates and armored dinoflagellates were noticeable at AB and SSC, respectively (Table 2).

The diatom genus *Thalassiosira* predominated during fall and winter (Figure 2), while *Pseudo-nitzschia* spp. prevailed during October and December 2013 (spring events) and January 2014 (summer) (Figure 2), after periods of high precipitation rates and when surface waters were warm and showed high phosphate concentrations. The highest densities of *Pseudo-nitzschia* spp. in October 2013 (10^5 10^5 cel.L^{-1}) were concurrent with the presence of cold waters (19.4°C, 35.5) near the SSC bottom (data presented in Ciotti et al. 2018a).

A total of 166 taxa were identified, with 86 genera, 129 species, 33 morphotypes, and 4 complexes, distributed in eight classes: Mediophyceae (42), Coscinodiscophyceae (23), Bacillariophyceae (37), Dinophyceae (48), Dictyochophyceae (02), Coccolithophyceae (10) and Cyanophyceae (04) (Figure 3 – frequent taxa). Of these, 148 taxa were in samples from within AB and 155 from SSC. *Trichodesmium* was frequently observed at AB, with the occurrence of *T. erythraeum* and *T. thiebautii* in the form of free trichomes, tufts, and puffs

 http://dx.doi.org/10.1590/1676-0611-BN-2021-1260

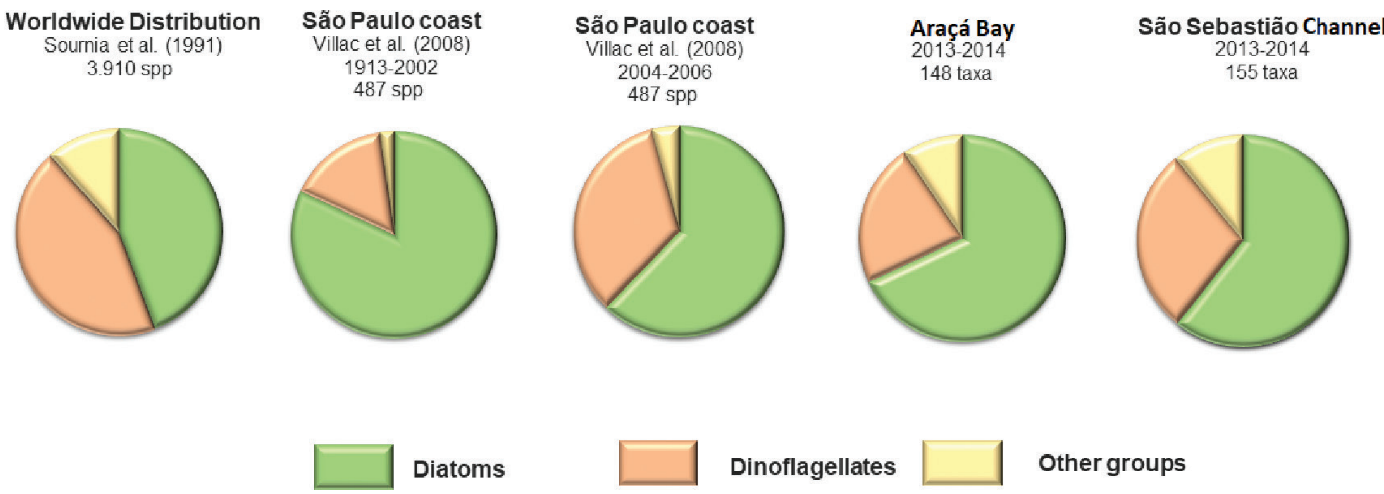

Figure 4. Relative contributions of the main taxonomic groups (diatoms, dinoflagellates and others – coccolithophorids, silicoflagellates, cyanobacteria, among others) at different levels: worldwide distribution (Sournia et al. 1991), data from 1913–2002 for the state of São Paulo (Villac et al. 2008), data from 2004–2006 for the state of São Paulo (Villac et al. 2008), data from 2013–2014 for Araçá Bay (AB) and sampling site at São Sebastião Channel (CSS). (Figure adapted from Villac et al. 2008).

(Figure 3) The cyanobacterium *Richelia intracellularis* was observed in association with diatoms of the genus *Hemiaulus* only, for the species *H. hauckii, H. sinensis*, and *H. membranaceus* predominantly and with up to 4 trichomes of *R. intracellularis* (Figura 3).

The richness of the classes differed slightly between the two sites, as did the relative frequency of each taxon (Table 3). Diatoms were the most frequent (AB = 68%; SSC = 60%), with the class Mediophyceae having the highest percentage in AB (28%; 41 taxa) and SSC (25%; 39 taxa), with the genus *Thalassiosira* being very frequent (AB = 100%; SSC = 98%). Although the frequencies of classes Coscinodiscophyceae (AB = 15%; SSC = 14%), Dictyochophyceae (AB = 1%; SSC = 1%), Coccolithophyceae (AB = 4%; SSC = 6%) and Cyanophyceae (AB = 3%; SSC = 3%) did not vary notably between sites, the taxa from these classes displayed infrequent or sporadic occurrences, with the exceptions for the frequent diatom *Paralia sulcata* and the coccolithophorid of the genus *Umbilicosphaera* at both sites, *Dictyocha fibula* at CSS and cyanobacteria of the genus *Trichodesmium* at AB. The class Dinophyceae (AB = 24%; SSC = 30%) showed a larger percentage contribution and numerical richness at SSC (46 taxa) than at AB (36 taxa), with the species *Scrippsiella acuminata* showing the highest frequency. The species *Cylindrotheca closterium* and *Thalassionema nitzschioides*, of the class Bacillariophyceae (AB = 25%; SSC = 21%), had higher frequencies in AB and the genus *Pseudo-nitzschia* in both locations (AB = 82%; SSC = 70%).

Overall, our inventory showed that diatoms and dinoflagellates represented together, over 80% of the total (Figure 4), similar to what was presented by Villac et al. (2008) for the coast of São Paulo state (diatoms 62%, dinoflagellate 34%).

Discussion

Our results are analogous to those presented by Villac et al., (2008), who reported 193 distinct taxa over a longer extension of the São Paulo coast (between Cananéia and Ubatuba) from 2004 to 2006. Their inventory included 120 diatoms, 65 dinoflagellates, and 3 silicoflagellates. In the present study, however, we observed larger contributions of the diatom genera *Pseudo-nitzschia, Thalassiosira, Chaetoceros, Hemiaulus, Cyclotella, Coscinodiscus, Guinardia, Rhizosolenia, Thalassionema, Cylindrotheca,* and *Leptocylindrus*, and the dinoflagellate genera *Prorocentrum, Scrippsiella, Tripos, Gymnodinium, Dinophysis,* and *Heterocapsa*. One addition to Villac et al. (2008) inventory was the diazotrophic cyanobacteria *Richelia intracellularis* (unfrequent taxa), either free or in symbiosis with diatoms at both AB and SSC. Although this result can be partially related to our sampling design, differences in environmental conditions between the two studies cannot be discarded, reinforcing the importance of frequent assessments of phytoplankton genera or species.

The 5 to 6-week interval observations revealed some temporal distinctions in the taxonomic composition of the phytoplankton. For example, the diatom genera *Thalassiosira* and *Pseudo-nitzschia* were consistently frequent (Table 3, Figure 2). However, their abundances tended to alternate. In addition, *Thalassiosira* (class Mediophyceae) was frequent when taxa richness was high, while when *Pseudo-nitzschia* (class Bacillariophyceae) was predominant, the richness of taxa was low, and their highest abundances occurred synchronically to intrusions of South Atlantic Central Water in SSC.

In temperate marine ecosystems, the succession between dominant phytoplankton taxa tends to be seasonal, leading to blooms (e.g., Cui et al. 2018, Fragoso et al. 2021). The genus *Pseudo-nitzschia*, with about 55 species (Guiry & Guiry 2021), can form blooms in coastal regions globally (e.g., Trainer et al. 2012), and some species are known to be potentially harmful by producing the neurotoxin domoic acid (Hasle 2002). The genus *Thalassiosira* contains more than 100 species (Round et al. 1990), but as for *Pseudo-nitzschia* and other genera of the class Bacillariophyceae, such as *Navicula*, *Pleurosigma,* and *Gyrosigma*, species-level identification requires scanning electron microscopy.

Our results suggest not only the establishment of urgent monitoring programs for harmful algal blooms (HABs) given the frequent potential species year-round at both sites but also that these programs need to encompass proper techniques for distinguishing taxa, as species identification by optical microscopy alone is incomplete (e.g., Hoppenrath et al. 2007, Hamsher et al. 2011, Fernandes et al. 2014, Sterrenburg et al. 2015).

In our study area, the typical physical accumulations of phytoplankton cells nearshore can episodically include organisms that advect from the open ocean guided by winds (Lugomela et al. 2002), which may be the case for the diazotrophic cyanobacteria *Trichodesmium* spp. and *Richelia intracellularis* at both sampling sites. Slicks of the genus *Trichodesmium* are commonly observed in surface waters of the Brazilian Current (Detoni et al. 2016) or in inner shelf waters (< 50 m) during the summer (Brandini et al. 1989). The occurrences could be linked to the relatively low nitrogen input from the continent, as the observations took place during a dry period (Tocci 2016), favoring the growth of diazotrophic cyanobacteria. However, at least for *Trichodesmium*, the advection of waters from offshore by mesoscale winds (Castro Filho & Mirada 1998) could be a source of these organisms for the coast. Moreover, favorable upwelling winds will favor intrusions of the South Atlantic Central Water in the SSC, not only enhancing the local concentration of nutrients and primary production rates (Regaudie et al. 2017) but also transporting diatoms, such as *Pseudo-nitzschia*, that impacted the overall taxa richness. These results indicate the need for future phytoplankton monitoring programs assessing the offshore contribution of water masses to SSC.

The observation of unfrequent taxa of tycopelagic diatoms (Table 1) at AB and SSC included the predominance of *Cylindrotheca closterium, Diploneis weissflogii*, and *Thalassionema nitzschioides.* The Bacillariophyceae *Cocconeis* sp.1, *D. didymus, D.* cf. *smithii*, *Licmophora tincta, Delphineis* sp.1*, Rhaphoneis* sp.1, and *Surirella* sp.1 showed larger densities at AB than SSC, and some species only observed at AB, such as *Diploneis didymus, Diploneis* cf. *smithii, Stenopterobia* sp.1, *Surirella* sp.1, and *Plagiogramma* sp.1, probably a result from the bay hydrodynamics that due to its shallower depth (Siegle et al. 2018) allows organisms to resuspend to the water column during each tidal cycle. This continuous exchange of phytoplankton organisms between the sediments of the bay and SSC water needs further evaluation for a better description of this ecologically important system.

Note that some of the identified taxa are mentioned in the literature as non-toxin-producing bloom formers (Odebrecht et al. 2001, Hallegraeff et al. 2003, Moestrup 2004, Villac et al. 2008), such as the diatoms *Asterionellopsis glacialis, Cerataulina pelagica, Cylindrotheca closterium, Guinardia delicatula, Leptocylindrus minimus*, and *Chaetoceros* spp.; the dinoflagellates *Tripos fusus* and *Tripus hircus*; and the silicoflagellate *Dictyocha fibula, may alternatively bloom*. However, accumulations of these species may result in many other ecologic and economic impacts (Castro et al. 2016). Additionally, results also reveal the lower diatom diversity when the genus *Pseudo-nitzschia* was abundant, which occurred during SACW intrusions in the channel.

Our results stress the demand for the urgent implementation of monitoring programs that aid public policies for environmental safety. The occurrences of taxa are known to be potentially harmful, highlighting the dinoflagellates of the genera *Alexandrium*, cf. *Gymnodinium*, *Dinophysis*, *Gonoyaulux*, and *Prorocentrum* are unsettling. Although some initiatives are in place, our results demonstrate the need for a comprehensive monitoring program that includes modernized methodologies and hydrodynamical modeling. If conditions for blooming are favored with nutrients input by sewage and warming of seawater temperatures, they may cause fish death, mollusk poisoning, and several public health problems (Hallegraeff et al. 2003).

Acknowledgments

The authors thank colleagues from Laboratório Aquarela and technician staff from CEBIMar/USP for their assistance during field work and data acquisition. This work was funded by Fundação de Apoio à Pesquisa do Estado de São Paulo (FAPESP– 2011/50317-5). A.M.C. received a CNPq fellowship (PQ 312422/2019-9), G.A.O.M. received a FAPERJ/UERJ fellowship (Prociência/2019), B.R.C.T. received a CNPq scholarship (process number 133002/2013-6). This is a contribution of the NP-Biomar/USP. We also thank the detailed revisions provided by two anonymous reviewers.

Author Contributions

B.R.C. Tocci: Contribution to data collection; Contribution to data analysis and interpretation; Contribution to manuscript preparation.

G.A.O. Moser: Contribution to manuscript preparation; Contribution to critical revision, adding intelectual content.

A.M. Ciotti: Substantial contribution in the concept and design of the study; Contribution to data collection; Contribution to data analysis and interpretation; Contribution to manuscript preparation; Contribution to critical revision, adding intelectual content.

Conflicts of Interest

The authors declare that they have no conflict of interest related to the publication of this manuscript.

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Received: 02/09/2021
Accepted: 04/04/2023
Published online: 19/05/2023

Biota Neotropica 23(2): e20221439, 2023
www.scielo.br/bn

biotaneotropica
ISSN 1676-0611 (online edition)

Point-of-View

Fisheries monitoring in Brazil: How can the 2030 agenda be met without fisheries statistics?

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Abstract: Every activity that involves exploitation of natural resources, such as fishing, needs to be organized and conducted based on information from monitoring programs to allow continuous evaluation. With the increasing fishing pressure in Brazil, the understanding of the importance of fisheries monitoring programs and how they can inform and assist in conservation decision-making remains limited. Based on the literature on fisheries and participatory conservation, we call attention to the need to generate information on the national fisheries sector in order to improve fisheries in the country. Given the context of the need to generate information on fishing stocks under exploitation, as well as to identify potential alternative fisheries and carry out various sectoral analyses in compliance with the 2030 Agenda for Sustainable Development, we present and discuss in the present paper the lack of a system of continuous fishing monitoring in Brazil and its effects on the fisheries sustainability in the country.
Keywords: *sustainable development; fishing resources; conservation.*

Monitoramento da pesca no Brasil: como cumprir a agenda 2030 sem estatísticas da pesca?

Resumo: Toda atividade que atua envolvendo a exploração de recursos naturais, como a pesca, precisa ser organizada e conduzida com base nas informações dos programas de monitoramento para permitir uma avaliação contínua. Com o aumento da pressão pesqueira no Brasil, o entendimento da importância dos programas de monitoramento da pesca e como eles podem informar e auxiliar na tomada de decisões de conservação permanece limitado. Com base na literatura sobre pesca e conservação participativa, chamamos a atenção para a necessidade de gerar informações sobre o setor pesqueiro nacional para melhorar a pesca no país. Dado o contexto da necessidade de gerar informações sobre os estoques pesqueiros em exploração, bem como identificar potenciais alternativas de pesca e realizar diversas análises setoriais em conformidade com a Agenda 2030 para o Desenvolvimento Sustentável, é apresentada e discutida no presente trabalho a falta de um sistema de monitoramento contínuo da pesca no Brasil e seus efeitos na sustentabilidade da pesca no país.
Palavras-chave: *desenvolvimento sustentável; recursos pesqueiros; conservação.*

Introduction

Every activity that involves the exploitation of natural resources, such as fishing, needs to be organized and conducted based on information from monitoring programs to allow continuous reassessment of the activity in order to adjust procedures and support management actions, which ensure the protection of stocks, and biodiversity (Pereira et al. 2013; Mendonça, 2018). The periodic monitoring of this information system makes it possible to identify and correct knowledge gaps and to guarantee access and transparency to the actors involved such that maximum efficiency is achieved and the resource is harvested in a sustainable way.

The fragility of the fisheries policy laws and the lack or inefficiency of monitoring and management have been the main drivers of the depletion of fisheries resources on a planetary scale, where various fisheries have capture levels above the natural replacement capacity of the exploited stocks, compromising the sustainability of the activity and the health of the fish, seas and oceans (OCEANA, 2016), as well as inland aquatic ecosystems (Allan et al. 2005). To face this reality and the need to maintain a balance between human population growth and social, economic and environmental demands, representatives of heads of state and government met in September 2015 at the headquarters of the United Nations (UN) in New York and launched the “2030 Sustainable Development Agenda” with 17 Sustainable Development Goals, or SDGs (UN, 2015). In 2017, Brazil presented its Voluntary National Report on the SDGs at an event to support SDG 14 “Conserve and ensure the sustainable use and development of the oceans, seas, freshwater bodies and marine resources” (Brazil, 2017). In this report, the country described actions linked to the elaboration of plans for the management of fisheries resources, including monitoring with coverage of species relevant to the fisheries sector and for the conservation of biodiversity.

Decision makers need updated data on exploitation in order to control and promote the activities without reaching the overexploitation threshold (FAO, 2020). Given the context of the need to generate information on fishing stocks under exploitation, as well as to identify potential alternative fisheries and carry out various sectoral analyses in compliance with the 2030 Agenda for Sustainable Development, we present and discuss in the present paper the lack of a system of continuous fishing monitoring in Brazil and its effects on the fisheries sustainability in the country.

Results and Discussion

1. *Fisheries in Brazil and the precariousness of monitoring*

South America has the greatest diversity of fish on the planet, considering marine, estuarine and freshwater species, corresponding to about 30% of all fish species in the world, about one-third of the world’s freshwater fish species, and one-fourth of the planet’s marine fish species (Buckup et al. 2007, Reis et al. 2016, Cassemiro et al. 2023). Brazil has the largest hydrographic network in the region and more than 8500 km of coastline, making this country the continent’s leader in diversity of species of fish (Buckup et al. 2007, Reis et al. 2016).

Fishing is the extraction of aquatic organisms from their natural environment for the purpose of consumption, recreation and commercialization as food or hobby (aquarium) (Frédou et al. 2021). The large territorial extent, combined with the enormous diversity of native fish species, gives the Brazil a huge potential for both marine and freshwater fisheries. The lack of monitoring hampers properly responses to the multiple threats facing aquatic ecosystems in Brazil, which include expansion of agricultural and urban areas, overfishing, pollution, river damming and construction of hydroelectric power plants, aquaculture, few river regulations, soil erosion and silting of the freshwater environments, deforestation, ghost fishing, modification and diversion of the river channels, species introductions, irregular water abstraction for different urban, industrial and agricultural uses, release of domestic and industrial effluents and chemical products from agricultural activities, and others (Azevedo-Santos et al. 2011, Azevedo-Santos et al. 2021, Bergmann et al. 2020, Castro 1999, Castro & Polaz 2020, Doria et al. 2021, Figueredo & Giani 2005, Fearnside et al. 2021, Giacomini et al. 2011, Pereira et al. 2016, Pelicice et al. 2017, Pelicice et al. 2021, Rocha et al. 2023, Vitule et al. 2015, Vitorino et al. 2022, Vieria et al. 2023, Zeni et al. 2019). In Brazilian marine ecosystems, the activity is practiced along the entire coast and is related to a territorial strip that houses about 2/3 of the Brazilian population (Araújo & Maia 2011).

Despite providing the livelihood of many riverine and coastal populations, information on the socioeconomic importance and sustainability of fishing activity is fragmented, limited or even non-existent, especially those related to artisanal or small-scale fishing (Silva 2014, FAO 2022), which is precisely the most widespread modality in Brazil. There is a notorious lack of an integrated fisheries monitoring system that generates essential information on the socioeconomic situation of fishermen, in addition to biological, economic, environmental and technological data linked to fishing activities (EMBRAPA 2021) for the development of fisheries management programs, and, consequently, the organization of the activity and maintenance of fish stocks in exploitation (Silva 2014). Historically, there is enormous inefficiency in the census and maintenance of records obtained by fishing colonies and the federal government on fishing activities and catches, that generates an inaccurate and underestimated census of the fishing production chain (Rodrigues, 2022). The information available on continental fisheries in Brazil has already been classified as extremely poor, in quality and quantity (Welcomme 1990, Agostinho et al. 2007).

The first fish landing monitoring in Brazil records were published by the Brazilian Institute of Geography and Statistics (IBGE) for the years from 1946 to 1953 (IBGE 1955). Subsequently, responsibility for monitoring the activity was transferred to the Fisheries Development Superintendency (SUDEPE), an agency under the Ministry of Agriculture, Livestock and Supply (MAPA) from 1950 to 1988 (MAPA 1962). The structuring of fisheries monitoring in Brazil gained more tools with the creation, in the mid-1990s, of the Fishing Statistical Data Generation System (ESTATPESCA) (Aragão 2008). In 1989 SUDEPE was abolished and incorporated into the newly created Brazilian Institute of the Environment and Renewable Resources (IBAMA), and from 1989 to 2007 monitoring was the responsibility of IBAMA's Center for Research and Fisheries Extension in the Northeast (CEPENE) (IBAMA 1995). Isolated and discontinued initiatives took place in several parts of the Paraná-Paraguay basin, such as those of Embrapa for the Paraguai river basin, and Iguaçu river basin (Petrere & Agostinho 1993, Okada et al. 2005), Paraná Tietê e Grande basins (AES-Tietê 2007). Even with these tools and structural mobilization, many limitations regarding the methods used made the data insufficient to elucidate the real scenario of fishing in Brazil, a deficiency that persists to the present.

In 2008, despite the progress made with the creation of the Ministry of Fisheries and Aquaculture (MAPA), the values began to be estimated using statistical imputation models (Zamboni et al. 2020); fisheries statistics were published until 2011, and the transfer of information on fishing activity to FAO were completely suspended beginning in 2014 (FAO, 2018). Since then, Brazilian fisheries began to face deeper difficulties, without specific public policies for the sector, such as the establishment of quotas for fishing, strengthening the local socio-economy, access to lines of credit and support for the governance of fishing communities (EMBRAPA 2021).

One of the main obstacles to the maintenance of a fisheries monitoring program is the cost of technical personnel and the maintenance of monitoring activities, which require the responsible institutions to adjust their collections and methodologies to include as much information as possible to help in the planning and operationalization of financial resources (Mendonça 2018). Establishing operational fisheries monitoring in Brazil represents a great logistical, human, and financial challenge, considering the territorial extent of the country, the difficulty access to certain locations, the diversity of ecosystems, fish and fishing methods, and the diffuse character of fishing activity. One of the promising strategies launched by specialists and researchers around the world is self-reporting, such as the model proposed by the São Paulo Fisheries Institute (IP/SP) and the actions used by the Chico Mendes Institute for Biodiversity (ICMBio) under the Global Socioeconomic Monitoring Initiative for Coastal Management (SocMon), which is used in more than 30 countries to generate information for coastal management (Gomes & Barros 2017, Mendonça 2018, Dias & Seixas 2019), a strategy linked to the obtaining of information in a participatory manner with community involvement.

It is essential that Brazilian authorities resume fisheries monitoring programs. The relevance of monitoring fish landings for sustainable fisheries management is highlighted by Fredou et al. (2021), who emphasize the importance of government involvement in continuous programs for the collection and analysis of data in a systematic way, encompassing technical, socioeconomic, and ecological information and ensuring the application of the Fisheries Ecosystem Approach. In fact, monitoring represents the main challenge for national fisheries management. From this perspective, public managers, researchers, local leaders and other actors will be able to devise more effective and participatory strategies for each region, fishing fleet, fishing gear, target and fish species (including non-target species). This would significantly reduce the risk of generating perfunctory data. It is also very important that authorities seek to improve methodologies, including the consideration of new variables, such as: i) morphometric and reproductive data on target and non-target species, aiming at stock assessment analysis, minimum capture size, size at first maturity (L50), natural mortality and fishing mortality; ii) social and economic data, including prices of fish sales at each step from the producer to the final consumer and the costs of labor, boats and other operating expenses, in addition to relevant information on family income and on satisfaction with the profession. With this volume of information, it will be possible to generate ecosystem models encompassing all of the variables necessary for efficient fisheries management, not just production estimates.

In order to meet the needs of generating information on the national fishing sector and help in complying with the SDGs, there is an urgent need to create a federal institute destined exclusively for the management of fisheries in the country, which can actually develop actions that strengthen discussions in the sector, in line with the conduct of scientific research and the need to guide public policies to promote sustainable fishing in Brazil. Attention should be paid to the following guidelines:

I. Work with existing data and metadata reporting systems and create online systems for exchanging information, including reporting on key indicators, and providing opportunities for horizontal and vertical coordination.
II. Create a national network that allows the compilation of data collected punctually by state and municipal governments through their secretariats.
III. Expand and strengthen the Permanent Management Committees, which enable the effective participation of civil society in building an efficient fisheries system.
IV. Create a National Fisheries and Aquaculture Database similar to the Brazilian navy's National Bank of Oceanographic Data (BNDO).
V. Create digital tools (apps and statistical programs applied to fisheries and aquaculture) to obtain and process national fisheries data.
VI. Use self-reporting to carry out fishing monitoring, as it enables the recording of accurate data on fishing, enabling better conditions for collecting information, reducing costs, logistics and the need for technical personnel to visit fishermen.
VII. Resumption of strategic programs for the assessment of marine and estuarine fish stocks similar to the Program for the Sustainable Assessment of Living Resources in the Exclusive Economic Zone of Brazil (REVIZEE).
VIII. Create certification or quality seals backed by fish tracking mechanisms, for both species of commercial and ecological importance and for non-target species.
IX. Modernization and expansion of the Floating Teaching Laboratories Project funded by the Ministry of Education.
X. Popularization of ocean science with a view to democratizing scientific knowledge and promoting the right to information and social participation.

Conclusion

In contrast to increasing fishing pressure in Brazil, the understanding of the importance of fisheries monitoring programs and how they can inform and assist in conservation decision-making remains limited. Based on the literature on fisheries and participatory conservation, we call attention to the need to generate information on the national fisheries sector in order to improve fisheries in the country. Fishing monitoring data and information is critical for decision-making on conservation and to guide public policies that promote sustainable fishing in Brazil.

Acknowledgments

We thank Instituto Maranhense de Estudos Socioeconômicos e Cartográficos (IMESC) for sharing part of the data analyzed in this study; Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES – Finance Code 001) and Fundação de Amparo à Pesquisa e ao Desenvolvimento Científico e Tecnológico do Maranhão (FAPEMA); Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq; grants PDCTR-08797/22 to PSB and 307974/2021-9 to FPO); and Fundação Amazônia de Amparo a Estudos e Pesquisas (FAPESPA; grant 028/2021 to ECG).

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Conflicts of Interest

The authors declares that they have no conflict of interest related to the publication of this manuscript.

Ethics

This study did not involve human beings and/or clinical trials that should be approved by one Institutional Committee.

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Received: 31/10/2022
Accepted: 26/04/2023
Published online: 05/06/2023

Biota Neotropica 23(2): e20231471, 2023
www.scielo.br/bn

biotaneotropica
ISSN 1676-0611 (online edition)

Article

Changes in sperm motility of amazonian fish Tambaqui *Colossoma macropomum* (Cuvier 1816) (Characiformes: Serrasalmidae) exposed to two pesticides

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Abstract: The great biodiversity of neotropical fish species that have external fertilization as a reproductive strategy, like the tambaqui, requires more careful analyzes in toxicological tests of the various pesticides implemented in Brazilian agriculture over the last few years. In this context, the objective of the present work was to evaluate possible sperm alterations in tambaqui (*Colossoma macropomum*) semen exposed to two different pesticide residues. Seminal samples of sexually mature tambaqui males from a local fish farm were used. Semen was collected eight hours after hormone induction into graduated glass tubes. After initial assessment of the lack of prior activation, the experiment was carried out in a factorial scheme, testing two pesticides widely used in agricultural systems (glyphosate and fenitrothion). For each pesticide, five concentrations were tested (6, 12, 24, 120 and 240 mg/L), with motility analysis at times 0, 30 and 60 seconds after activation. As a control, activation with 0.9% NaCl solution and motility analysis at the same times described for pesticides were used. Results indicate that in natura samples exhibited initial motility of 89.2 ± 4.9% and mean duration of 100 seconds (up to 10% sperm motility). The reduction in sperm motility occurred significantly ($p < 0.05$) after 30 seconds in all concentrations tested, except for the concentration of 240 mg/L because no activation was observed. The tests described here demonstrate that tambaqui semen was sensitive to the process of exposure to pesticide residues, and can be used in biomonitoring analyzes of the aforementioned agricultural pesticides.
Keywords: *Agriculture; biomonitoring; ecotoxicology; amazonian fish; aquatic pollution; seminal quality.*

Alterações na motilidade espermática do peixe amazônico Tambaqui *Colossoma macropomum* (Cuvier 1816) (Characiformes: Serrasalmidae) exposto a dois pesticidas

Resumo: A grande biodiversidade das espécies de peixes neotropicais que possuem a fertilização externa como estratégia reprodutiva, a exemplo do tambaqui, exige análises mais criteriosas em testes toxicológicos dos diversos defensivos agrícolas implementados na agricultura brasileira ao longo dos últimos anos. Nesse contexto, o objetivo do presente trabalho foi avaliar possíveis alterações espermáticas no sêmen de tambaqui (*Colossoma macropomum*) exposto a resíduos de dois diferentes pesticidas. Foram utilizadas amostras seminais de machos de tambaqui sexualmente maduros provenientes de uma piscicultura local. O sêmen foi coletado oito horas pós indução hormonal em tubos de vidro graduados. Após avaliação inicial de inexistência de ativação prévia, foi realizado o experimento em esquema fatorial, sendo testados dois pesticidas muito utilizados em sistemas agrícolas (glifosato e fenitrotiona). Para cada pesticida foram testadas cinco concentrações (6, 12, 24, 120 e 240 mg/L), com análise da motilidade nos tempos 0, 30 e 60 segundos pós ativação. Como controle, foi utilizada a ativação com solução de NaCl a 0,9% e análise da motilidade nos mesmos tempos descritos para os pesticidas. Resultados indicam que as amostras *in natura* exibiram motilidade inicial de 89,2 ± 4,9% e tempo de duração médio de 100 segundos (até 10% de motilidade espermática). A redução da motilidade espermática ocorreu de forma significativa ($p < 0,05$) após 30 segundos em todas as concentrações testadas, exceto na concentração de 240 mg/L por não ter sido observada ativação. Os testes aqui descritos demonstram que o sêmen de tambaqui se mostrou sensível ao processo de exposição aos resíduos de pesticidas, podendo ser utilizado em análises de biomonitoramento dos referidos defensivos agrícolas.

Palavras-chave: *Agricultura; biomonitoramento; ecotoxicologia; peixe amazônico; poluição aquática; qualidade seminal.*

Introduction

In recent decades, the world has been facing a serious problem: the “Biodiversity Crisis”. As the human population grows exponentially, increasing demand for natural resources, species are becoming extinct both locally and globally, especially in tropical zones of the world, at rates much higher than natural extinction rates. This is caused directly due to human actions, such as pollution, destruction of natural habitats, modification of natural habitats, deforestation, agricultural expansion, overfishing and overhunting, introduction of exotic species, fragmentation of habitats, among others (Wilson 1985, Savage 1995, Primack & Rodrigues 2001, Brooks et al. 2022, Singh 2002, Brook et al. 2006, Pimm et al. 2006, Laurance 2006, Wheeler 2008, Costa et al. 2012, Pimm et al. 2014, Ceballos et al. 2015). When we compare biodiversity and health of freshwater environments with terrestrial or marine, the scenario is even worse: the so-called “Freshwater Biodiversity Crisis”. Although the threats are the same as those already mentioned, the proportional area of freshwater environments is much smaller when compared to terrestrial or marine environments, representing less than 1% of the planet’s surface, but comprising a very rich biodiversity. In addition, several human activities are dependent on freshwater, and humanity directly depends on this resource as well (Dudgeon et al. 2006, Darwall et al. 2018, Harrison et al. 2018, Latrubesse et al. 2019, Reid et al. 2019).

Even though the “Biodiversity Crisis” has become an increasingly serious problem, especially the “Freshwater Biodiversity Crisis”, due to agricultural expansion, Brazil has arisen as one of the countries that most employ pesticides in the past decades, more expressively from 2002 onwards, showing that we are failing to face and deal appropriately with the “Biodiversity Crisis”. This fact raises concerns about the increased use of these substances, mainly due to the possibility of contaminating man and animals (Rembischevsk & Caldas 2018). A fact that hinders the conservation of Brazilian fauna. In this context, the determination of lethal and sublethal doses of pesticides in living organisms should be analyzed in toxicity tests (Ragassi et al. 2017).

In an attempt to monitor the environmental changes caused by the indiscriminate discharge of toxic substances with xenobiotic potential, researchers report the need for ecotoxicological studies as a way to assess the possible aggressions of these substances that are released into the natural environment, such as agricultural pesticides, and their interaction with ecosystems and their biodiversity (Montanha & Pimpão 2012). According to Torres et al. (2017), such indiscriminate release of polluting agents in aquatic environments has become a limiting factor for the continued supply of fish consumer markets, whether from fishing or even aquaculture. In addition to the direct risks to human health, it is possible that fish are being contaminated by toxic products that reach aquatic environments, which may represent an additional risk for their consumers (Waichman 2008).

The tambaqui *Colossoma macropomum* (Cuvier 1816) is the most farmed native fish species in the country (PEIXEBR 2022), as it presents a good adaptation to climate conditions, which are considered ideal for round fish species. It is important to emphasize that the cultivation of native species to supply the market is important to reduce the negative pressures that fishing can exert on the populations of these species, and to prevent the local extinction of species as well. *Colossoma macropomum* presents external fecundation and fertilization, as well as annual reproduction and total spawn, with the river flooding period being the main spawning season for this fish (Vieira et al. 1999). In teleosts with external fertilization such as round fish, when spawning occurs, the gametes are released into the environment for fertilization to occur (Witeck et al. 2011). At that moment, gametes are exposed to various contaminants present in the water, including heavy metals mercury, zinc, lead, copper, and cadmium (Kime & Nash 1999), as well as pesticides leached from the soil by the rain or even by the inadequate disposal of containers and waste (Ferreira 2016), which end up acting as endocrine disruptors in fish (Uren-Webster et al. 2014). Furthermore, these trace elements, at certain levels, can impair sperm motility and oocyte fertilization (Kime 1995), thus causing often irreversible damage to the maintenance of natural stocks renewal and reproductive cycles, and, consequently, to the maintenance of the variability and diversity of fish.

In the past few years, several studies involving the characterization of sperm and embryos from a number of native fish species have generated successful sperm analysis protocols in conjunction with the cryopreservation technique, conservation of cells at low temperatures. This allows the availability throughout the year of biological samples for many species of environmental interest (Viveiros et al. 2009, Carneiro et al. 2012, Salmito-Vanderley et al. 2016), whose usefulness in ecotoxicological tests can now be analyzed. In this context, the objective of the present work was to evaluate possible sperm alterations in the semen of tambaqui *C. macropomum* exposed to two different pesticides, to assess whether these pesticides cause any negative effects that interfere with the reproduction of the species, and, consequently, affecting the maintenance of natural stocks renewal.

Material and Methods

Our study was conducted during the period between January and May of 2021, in the facilities of a fish farm located in the municipality of Santa Inês, State of Maranhão, northeastern Brazil (latitude 03°40'00" south and longitude 45°22'48" west), at approximately 250 km from the capital city of São Luís. Experiments were conducted with the approval of the Ethics Committee for the Use of Animals at the Universidade Estadual do Maranhão (CEUA/UEMA) under license 01200.002200/2015-06(449).

Seminal samples were collected from six males of the tambaqui species *C. macropomum*, selected from the breeding stock of the fish farm and which presented semen release when submitted to gentle pressure in the abdominal region. Then, the fish were placed in masonry tanks, with constant water circulation for subsequent hormonal induction, using 2.0 mg of raw carp pituitary extract – CPE per kg of live fish (Pinheiro et al. 2016).

After 8 hours of hormonal induction, the fish were restrained and their urogenital region was cleaned and dried with a paper towel to avoid contamination (by water, mucus, or urine). Then, semen collection was performed in graduated glass test tubes. Samples were kept in an isothermal box at a temperature ranging from 4 to 6 °C.

Samples were identified and analyzed individually at the fish farm breeding laboratory for absence of sperm motility by observation under an light microscope at 400× magnification. After confirmation that sperm were immobile, 2 µL aliquots of semen from each sample were activated with 100 µL of 0.9% NaCl saline solution for initial characterization, observing the staining, viscosity, initial subjective sperm motility rate and motility duration time, also under an optical microscope with 400× magnification. Samples with subjective motility above 80% were included in the experiment (Santos 2013).

Milt samples of each male were exposed to two pesticides (glyphosate 480 g/L – ISORGAN; and fenitrothion, SUMITHION 500 g/L) at five concentrations (6, 12, 24, 120 e 240 mg/L), with dilution in 0.9% NaCl saline solution. Exposition began with the direct activation of 2 µL aliquots of semen with 100 µL of each of the pesticide dilutions. There was an immediate subjective analysis of sperm motility at times 0 (after homogenization), 30 and 60 seconds after activation/exposure, with the aid of a light microscope with a magnification of 400×, by the same evaluator. As a control treatment, we conducted the analysis of sperm motility after activation with a 0.9% NaCl solution (Santos 2013) with the same analysis times described for pesticide residues.

The chosen experimental design was a factorial test. A statistical analysis was performed through the assessment of means and standard deviations, from which the analysis of variance test (ANOVA) was obtained. When there was observable difference between treatments, the Skot-knott test was applied, at a significance level of 5% in the statistical program SISVAR 5.7.

Results

During the initial observation period, semen samples from all individuals presented white color and high viscosity. *In natura* samples exhibited an initial motility of 89.2 ± 4.9% and a mean duration of 100 seconds (up to 10% of sperm motility). The activations were carried out at environmental temperature (27–29 °C) and the solutions containing pesticide residues showed a ph ranging from 5.2 to 5.5 for Glyphosate, and from 5 to 5.7 for fenitrothion, based on the value of 5.9 for NaCl.

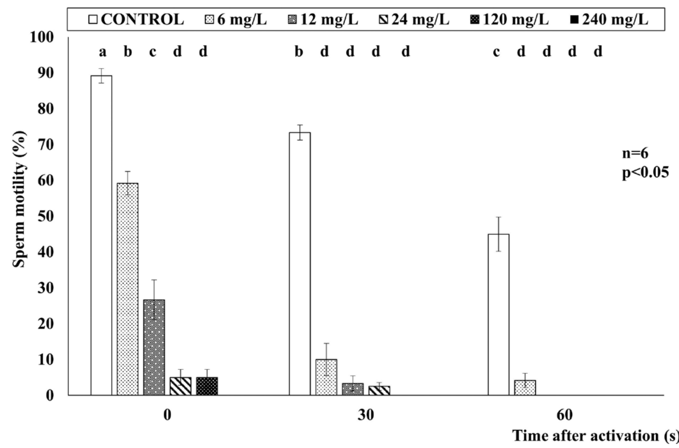

Figure 1. Percent sperm motility (mean ± standard error) of Tambaqui semen exposed to different concentrations of glyphosate.

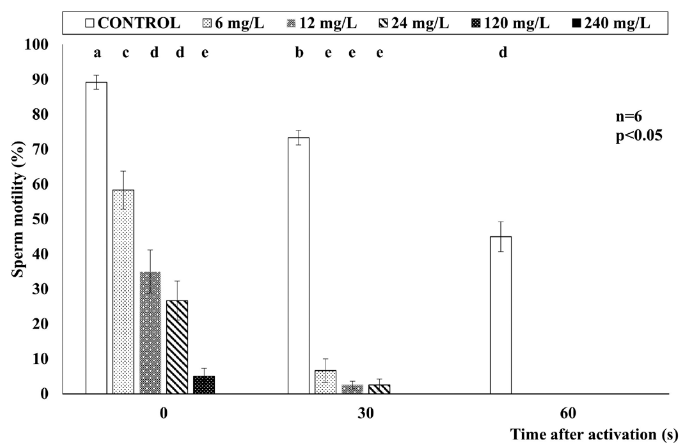

Figure 2. Percent sperm motility (mean ± standard error) of Tambaqui semen exposed to different concentrations of fenitrothion.

From the tested treatments with direct exposure of semen to pesticide residues, it was possible to observe the effect of exposing tambaqui semen to residues of glyphosate (Figure 1) and fenitrothion (Figure 2) soon after exposure. All treatments showed a significant reduction ($p < 0.05$) when compared to sperm activated only with 0.9% NaCl, with more deleterious effects at concentrations above 24 mg/L glyphosate, and sperm agglutination at a concentration of 240 mg/L, it is not possible to attribute sperm motility rate subjectively.

Discussion

Glyphosate is an herbicide belonging to the organochlorine group and is classified as an extremely toxic product (Class I) (ANVISA 2021), widely used mainly in plant cultures, such as rice, potatoes, bananas, onions, corn, pastures, soybeans, ornamental shrubs, and flowers, at different stages of development. Changes in body structure, early hatching of eggs, embryo mortality and larval depigmentation are indicative of the toxic effects of these substances (Sanchez 2015).

Lopes et al. (2014), in an experiment conducted with fish *Danio rerio* (Hamilton 1822), observed that sperm exposed for 24 and

96 hours to 5 and 10 mg/L of glyphosate exhibited a decrease in motility and in its duration. Moreover, according to these authors, individuals that were subjected to higher concentrations for a prolonged period showed functional and membrane changes in sperm mitochondria, as well as a reduction in DNA integrity, indicating that glyphosate is a highly dangerous agent for the reproduction of this species, and even harming others.

The tambaqui species is considered a biological model due to its resilience to environmental changes (Val & Oliveira 2021). It has been pointed out as a bioindicator of environmental pollution and used in several toxicological studies, such as the assessment of genotoxic and hematological parameters (Carvalho-Neta et al. 2015), and branchial lesions and erythrocytic abnormalities (Castro et al. 2019), both using individuals from an environmental protection area from the Upaon-Açu island, State of Maranhão, northeastern Brazil. Regarding agricultural defensives specifically, studies carried out with tambaqui by Cunha et al. (2018) indicated alteration and damage of nuclear erythrocytes in the gills and liver of tambaqui exposed to pesticides such as Deltamethrin.

As observed for glyphosate, sperm motility rates were significantly reduced ($p < 0.05$) after direct activation in all tested concentrations of fenitrothion (Figure 2), with no sperm motility being verified at a concentration of 240 mg/L right after direct activation (time 0). Fenitrothion, also belonging to the group of organophosphates, is a class II insecticide (moderate toxicity) widely used in pest control (Milanez et al. 2007). In Brazil, it has been used in agriculture since 1959, authorized in cotton, onion, chrysanthemum, apple, and soy crops, to control ants (ANVISA 2021). In aquaculture, it is used to control insect larvae, just as already reported in Bangladesh, India, to combat the tiger beetle (Rahma et al. 2020). Even considering their history of use in Brazilian agriculture and livestock, there are few organophosphates and pesticides in general that are authorized by the national legislation for fish farming (Tavechio et al. 2009), with no formal authorization or indication of the use of fenitrothion in aquaculture being recorded.

Agricultural pesticides, mainly pyrethroids and organophosphates, have been largely used in Brazil since the 1990s, with the main objective of providing increases in agricultural production by combating pests that, if not controlled, can exterminate the entire crop in a short period of time (Moraes 2019). However, while these substances bring benefits to agriculture, the number of studies that prove the ecotoxicological effects of pesticides on human and animal health, especially those living in aquatic environments, is undeniable (Montanha & Pimpão 2012, Santana & Cavalcante 2016, Ribeiro & Américo-Pinheiro 2018).

Several species of Neotropical fish employ external fecundation and fertilization as a reproductive strategy, with total discharge of male and female gametes in the water (Witeck et al. 2011), a fact that provides a large exposure of gametes to the contaminated environment (Rodrigues et al. 2019). As a result, the entire fertilization process, from sperm motility to embryonic development, can be directly affected by excess contaminants, such as pesticide residues leached from the soil by rain, or even by inadequate disposal of containers and residues (Kime & Nash 1999, Ferreira 2016). These contaminants greatly influence the reproduction and renewal of fish species stocks, which can lead to an environmental imbalance and a reduction in species diversity (Mondal et al. 2015).

Amazonian fish known as round fish, such as the tambaqui *Colossoma macropomum*, and the pirapitinga *Piaractus brachypomus* (Cuvier 1818), as well as their hybrids, have become the most important native species of Brazilian fish farming, especially in the North and Northeast regions of the country (Muniz et al. 2008, PEIXEBR 2021), due to their good adaptation to the climatic conditions found in those regions, which are considered ideal for these species. They are species that have external fecundation and fertilization, with annual and total reproduction, with the river flooding period being the main spawning season for this group of fish (Vieira et al. 1999). In captivity, they are reproduced through the hormonal induction technique, using raw Carp Pituitary Extract – CPE (Maria et al. 2011), in addition to synthetic hormones.

Normally, the action of organophosphates occurs through the irreversible inhibition of enzyme acetylcholinesterase (AChE), responsible for the degradation of acetylcholine, the main neurotransmitter in the central nervous system of insects (Barboza et al. 2018). In this sense, organophosphates are widely used in fish farms to control fish parasites, as well as to combat insect larvae of order Odonata, which have the habit of preying on fish larvae and causing financial damage to producers (Fortunato et al. 2020). Despite being efficient in combating and chemically controlling dragonfly nymphs and other insects, organophosphates have been proven to be considerably toxic (Queiroz 2017).

Organophosphates combined with pyrethroids are already used both in agriculture and in livestock, as the mixture of both promotes synergism in their actions (Trevis et al. 2010). In a study to assess the toxic effect of the mixture between organophosphates and pyrethroids in *Pimephales promelas* larvae, it was observed that the combination resulted in high toxicity (Wheelock et al. 2005), demonstrating the importance of conducting studies aimed at evaluating the application of the mixture of these two pesticides in aquatic environments.

In a recent study published by Santos et al. (2021), when evaluating the toxic effects of pesticides on the reproductive processes of freshwater fish based on articles published from 2000 to 2019, they observed that insecticides were present in 78% of the studies, mainly Endosulfan (35%) and Cypermethrin (13%), which are classified respectively as organochlorine and pyrethroid. The authors also highlighted that the most reported routes of action in the studies (57.5%) were reproductive endocrine disorders, with changes ranging such as decreased fertility due to histological damage to testicles and ovaries; impairment of the vitellogenesis process and interruption in the steroidogenesis process; delay in gonad maturation evidenced by alterations in the Gonadosomatic Index; alteration in reproductive and parental behavior; compromised olfactory response and consequent disorder in reproductive migrations; as well as disturbances in the coordination of courtship behavior of male and female fish and spawning time (Jaensson et al. 2007, Singh & Singh 2008, Marcon et al 2015, Sumon et al. 2019).

Our study is the first one reporting the use of sperm cells from native Neotropical fish (tambaqui) directly exposed to pesticides in ecotoxicological tests. Tests demonstrated that these organisms are highly susceptible to changes that can be caused by contact with pesticide residues, such as glyphosate and fenitrothion. Therefore, the presence of pesticides in freshwater environments can interfere in the reproduction of tambaqui fishes, and consequently, in the renewal of this fish species stocks. However, the determination of a protocol for analysis must be conducted, as to standardize the techniques and to express a result that can be taken into consideration by competent government bodies that mediate requests for the authorization of use of new chemical substances as agricultural pesticides in Brazil. In addition, we believe that the results here obtained would be extrapolated to other native species that have a similar reproductive cycle or biology, helping us to understand how contaminants generated by agricultural production can affect the reproduction of these species, and how can we adopt conservation actions to prevent this.

Acknowledgment

We thank the Institutional Program of Scientific Scholarships at UEMA, the AQUAPESC fish farm, and FAPEMA for financing our project.

Associate Editor

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Simone de Jesus Melo Almeida: Contribution to data collection and analysis; Contribution to manuscript preparation.

Claryce Cunha Costa: Contribution to data collection and analysis; Contribution to manuscript preparation.

Achilles Nina Santos Ferreira: Contribution to data collection and analysis; Contribution to manuscript preparation.

Erivânia Gomes Teixeira: Contribution in the concept and design of the study; contribution to manuscript preparation; Contribution to critical revision, adding intellectual content.

Erick Cristofore Guimarães: Contribution to data analysis and interpretation; Contribution to manuscript preparation; Contribution to critical revision, adding intellectual content.

Pâmella Silva de Brito: Contribution to manuscript preparation; Contribution to critical revision, adding intellectual content.

Felipe Polivanov Ottoni: Contribution to manuscript preparation; Contribution to critical revision, adding intellectual content.

Raimunda Nonata Fortes Carvalho-Neta: Substantial contribution in the concept and design of the study; Contribution to critical revision, adding intellectual content.

Conflicts of Interest

The authors declare that they have no conflict of interest related to the publication of this manuscript.

Data Availability

The data used in our analysis is available at Biota Neotropica Dataverse https://doi.org/10.48331/scielodata.PIL6JB

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Received: 19/01/2023
Accepted: 06/04/2023
Published online: 16/06/2023

Biota Neotropica 23(2): e20221453, 2023
www.scielo.br/bn

biotaneotropica
ISSN 1676-0611 (online edition)

Inventory

The butterflies (Lepidoptera, Papilionoidea) of the Parque Estadual Intervales and surroundings, São Paulo, Brazil

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SHIRAI, L.T., SILVA, R.O., DIAS, F.M.S., ROCHELLE, A.L.C., FREITAS, A.V.L. **The butterflies (Lepidoptera, Papilionoidea) of the Parque Estadual Intervales and surroundings, São Paulo, Brazil.** Biota Neotropica 23(2): e20221453. https://doi.org/10.1590/1676-0611-BN-2022-1453

Abstract: The Global South has witnessed increasing sampling of its immense biological diversity during the past century. However, the diversity of many regions remains unknown, even at pristine and highly threatened places, such as in the Atlantic Forest; and for bioindicator, umbrella, and flagship groups. The present study reports the first butterfly list of the *Parque Estadual Intervales*, São Paulo, Brazil and surroundings, a key protected area in the last massive continuous of the Atlantic Forest. We compiled data from museums and four years of field work, under three sampling methods. We also aimed at providing resources to support conservation efforts by analyzing 27 years of climatic data (detailed in the Supplementary Material, in English and in Portuguese), discussing our results also for non-academics, and producing scientific outreach and educational material. A companion article dealt with the experiences of science outreach and capacity development, and illustrated the butterfly catalogue of the species sampled in the park. We found 312 species that sum to 2,139 records. The museum had 229 species (432 records), and we sampled 142 species (1,682 individuals), in a total effort of 36,679 sampling hours (36,432 trap and 247 net and observation hours). The richest families were Nymphalidae (148 species) and Hesperiidae (100 species). Most species were sampled exclusively by active methods (79.8%), but other sources (passive sampling, citizen science, etc.) also found unique records. We found the highest diversity metrics from January to May, and we demonstrated that winter months had less richness and abundance. We illustrated the 20 species common to all regions, and listed those that were found more than seven months in the year, as well as the most abundant species in trap sampling, with forest dwellers as well as species common to open and fragmented areas. The dominant species in our trap datasets was the iridescent white morpho, *Morpho epistrophus* (Fabricius, 1796), and we suggest it to become the park butterfly mascot.
Keywords: *Species list; Paranapiacaba continuum; fish carrion bait; biodiversity knowledge shortfalls.*

As borboletas (Lepidoptera, Papilionoidea) do Parque Estadual Intervales e arredores, São Paulo, Brasil

Resumo: O Sul Global testemunhou crescente amostragem de sua imensa diversidade biológica durante o século passado. Entretanto, a diversidade de muitas regiões permanece desconhecida, mesmo em locais pristinos e altamente ameaçados, como na Mata Atlântica; e para grupos bioindicadores, guarda-chuva e emblemáticos. O presente estudo reporta a primeira lista de borboletas do Parque Estadual Intervales, São Paulo, Brasil e arredores, uma unidade de conservação chave no último maciço contínuo de Mata Atlântica. Compilamos dados de museus e quatro anos de campo, sob três métodos de amostragem. Também visamos oferecer recursos para apoiar os esforços de conservação, analisando 27 anos de dados climáticos (detalhados no Material Suplementar, em inglês e em português), discutindo nossos resultados numa linguagem também para não acadêmicos, e produzindo material de divulgação científica e educativos. Um artigo irmão tratou das experiências de divulgação científica e capacitação, e ilustrou o catálogo de borboletas das espécies amostradas no parque. Encontramos 312 espécies em 2.139 registros. O museu tinha 229 espécies (432 registros), e amostramos 142 espécies (1.682 indivíduos), em um esforço total de 36.679 horas de amostragem (36.432 armadilhas e 247 horas de rede e observação). As famílias mais ricas foram Nymphalidae (148 espécies) e Hesperiidae (100 espécies). A maioria das espécies foi amostrada exclusivamente por métodos ativos (79,8%), mas outras fontes (passiva, ciência cidadã, etc.) também encontraram registros únicos. Encontramos as maiores métricas de diversidade de janeiro a maio, e demonstramos que os meses de inverno tiveram menos riqueza e abundância. Ilustramos as 20 espécies comuns a todas as regiões, e listamos aquelas que foram encontradas em mais de sete meses no ano, bem como as espécies mais abundantes em armadilhas, com espécies florestais e também comuns em áreas abertas e fragmentadas. A espécie dominante em nossas armadilhas foi a morfo branca iridescente, *Morpho epistrophus* (Fabricius, 1796), e sugerimos que se torne a borboleta mascote do parque.

Palavras-chave: *Lista de espécies; contínuo de Paranapiacaba; isca de peixe; deficit de conhecimento da biodiversidade.*

Introduction

Biodiversity studies are of central importance for the future, and inventories are one of their building blocks. Sampling distribution is, however, far from homogeneous, with most knowledge concentrated in the wealthiest, temperate, countries of the world (*e.g.* Girardello *et al*. 2019). The unequal sampling effort increases the relevance of carrying out inventories in the richest and megadiverse regions on Earth, usually found in the tropics, especially in those countries threatened by governments openly against science and the preservation of its environment (Myers *et al.* 2000, Alves *et al*. 2018, Andrade 2019, Angelo 2019, Tollefson 2019, Ferrante & Fearnside 2021, Hipólito *et al*. 2021). Despite an increase in sampling in the Global South during the past century (Girardello *et al*. 2019), it is remarkable that some regions with pristine natural environments remain unsampled, even at highly diverse and threatened regions, such as in the Atlantic Forest (Myers *et al*. 2000); and for biological groups that are relevant for conservation, such as bioindicator, umbrella, and flagship groups.

Bioindicator and umbrella groups are particularly useful for conservation planning and future attempts to understand what has been lost because they provide snapshots of the plant, animal or fungal species at a given time. The information they provide is more useful when inventories are performed according to best practices and standards. For example, to assess the fauna of a given location, it is important to use different sampling methods, to collect periodically (*e.g.* monthly) and for many years, to span different environments and altitudes, and to be careful with specimen and data curation (Santos *et al*. 2008, Shirai *et al*. 2019). That is rarely the case for several reasons, namely the financial and temporal availability of trained people, aggravated in

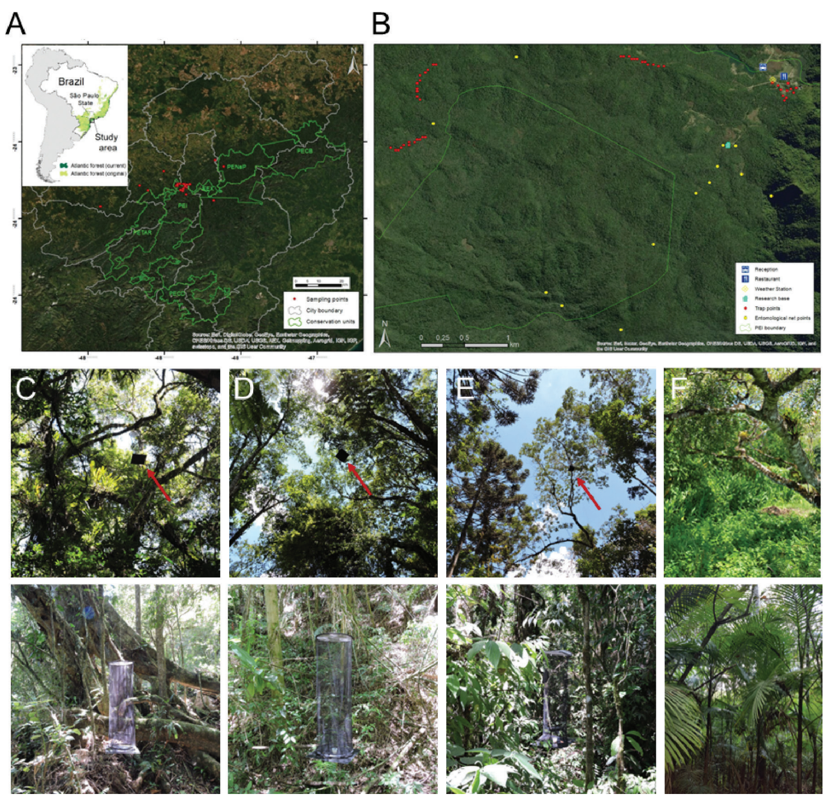

Figure 1. Study area: (a) map of the Paranapiacaba Continuum: Intervales (PEI), *Parque Estadual Carlos Botelho* (PECB), *Parque Estadual Turístico do Alto da Ribeira* (PETAR), and *Parque Estadual Nascentes do Paranapanema* (PENaP), and *Estação Ecológica Xitué* (EEX); together with nearby *Parque Estadual Cavernas do Diabo* (PECD). The inset map shows the original distribution of the Atlantic Forest in light green and in dark green the remaining fragments, highlighting the importance of this Continuum, the largest continuous forest remanent; (b) map of sampling points by traps and net inside the PEI, showing some of its infrastructure; and representative images of canopy (above) and understory (below) traps of the PEI dataset: (c) "Minotauro" primary forest, (d) "Cidreira" secondary forest, and (e) "Guapiara" open road, with edge effect. (f) strategic traps set at PEI's infrastructure for the PEI 1y dataset.

places like South America (Elliot *et al*. 2018). However, in taxa that are easy to collect and identify even by non-specialists, approaches like citizen science and capacity development can be powerful allies to complement collection by scientists (Uehara-Prado *et al.* 2007, Davis 2015, Santos *et al*. 2016, Elliot *et al*. 2018, Mota *et al*. 2022, Shirai *et al*. 2022). It also helps when the bioindicator or umbrella group is aesthetically appealing, which is the case of most butterflies (Lepidoptera, Papilionoidea: Hesperiidae, Lycaenidae, Nymphalidae, Papilionidae, Pieridae, and Riodinidae).

Here we provide the first butterfly list of Intervales state park (*Parque Estadual Intervales* or PEI), a protected area located in the last massive continuous of the Atlantic Forest (Figure 1a). Despite the importance of this area, and associated environmental and social threats (see Methods), it is remarkable that we did not find any study sampling this bioindicator group, butterflies, although some works report the collection of focal taxa at the region (listed in Methods). The PEI boundaries were not determined for biological reasons, so we searched for inventories in the surrounding municipalities, all of which are in the state of São Paulo, Brazil (Figure 1a). The only published inventories near or at the PEI either collected all butterflies and did not report the species list (Brown & Freitas 1999) or had a species list but of a single tribe (Ithomiini, Shirai *et al*. 2017) or of a single guild on specific flowers (nectar-feeding butterflies on *Lantana* L. (Verbenaceae), Santos *et al*. 2015).

We report the butterflies of the PEI and surroundings with data compiled from museums and four years of field work by us, under three sampling methods. Our aims were to contribute to the scientific community, but also to the local community, supporting conservation efforts in different ways. Here, we provide the butterfly list, discussing it academically but also in a language accessible for non-academics. We also analyzed 27 years of climatic data collected at the park but so far without any formal synthesis. This climatic profile can be reported by the protected area to support decision-makers to conserve the area with robust data, so we present it in the Supplementary Material both in English and in Portuguese. Some of the spread individuals returned to the park and are in display for visitors, along with informative posters with the results of this study (http://doi.org/10.5281/zenodo.5893810, press release available at https://www.infraestruturameioambiente.sp.gov.br/fundacaoflorestal/2022/02/parque-estadual-intervales-realiza-primeiro-plantio-coletivo-de-mudas/). Also, the "Butterflies of Intervales" outreach booklet is publicly available (http://doi.org/10.5281/zenodo.5068939). Finally, the experiences of science outreach and capacity development with the PEI staff, and an illustrated butterfly catalogue of species sampled here can be found in the companion publication (Shirai *et al*. 2022).

Material and Methods

1. Study area

The *Parque Estadual Intervales* (hereafter PEI, 41,704 ha) is part of the largest continuous of the Atlantic Forest at the *Serra do Mar* (1,109,546 ha, Ribeiro *et al.* 2009, inset of Figure 1a). The PEI is located within the Paranapiacaba Continuum (*ca*. 140,000 ha *c.f.* decrees) which, along with the contiguous ecological station Xitué and *Parque Estadual Carlos Botelho* (PECB), *Parque Estadual Turístico do Alto da Ribeira* (PETAR), and *Parque Estadual Nascentes do Paranapanema* (PENaP) (Figure 1a), is a UNESCO Biosphere Reserve and a World Heritage Site. This Continuum plays a central role in conserving one of the five hottest world hotspots (*cf.* Myers *et al.* 2000), due to its unique extension of continuous forest. For example, the Continuum is one of the last three areas capable of hosting viable populations of the biome's top predator, the jaguar (*Panthera onca* (Linnaeus, 1758)), which is vulnerable to extinction (ICMBio 2018) due to hunting and habitat loss (Beisiegel & Nakano-Oliveira 2020)

It is estimated that 85% of the PEI still has well preserved old growth forest with very low levels of disturbance (Nisi 2006), which is predominantly dense montane ombrophilous forest (Leonel 2010). Intervales means "between valleys," in reference to the hills that range from 140 m to 1,200 m. The complex landscape with vast intact forest hosts important remnants of wild life, for instances, 9.2% of the PEI fauna of 532 species preliminarily inventoried in the 1990's was threatened (Nisi 2006). Particularly, several arthropods in the Red List (ICMBio 2018) were found at the calcareous caves of the region, some exclusively at the PEI. The pristine forest, the caves and the almost 400 species of birds (an international hotspot for birdwatching) are among the main attractions of the park. Illegal hunting, mining, and overexploitation of juçara palm (*Euterpe edulis* Mart.) are among the main threats to the life it preserves (Shirai *et al*. 2022).

The climate of the region can be classified as dry-winter subtropical highland, or Cwb in the Köppen-Geiger system (*c.f.* Beck *et al*. 2018), with rainy summers (December to March) and a noticeable, dry, winter (June to September). The PEI has, however, different stations with distinct climatic profiles. At the PEI main station (ca. 800 m.a.s.l), the annual precipitation is of 1,400 mm, with the summer having an average precipitation of 187 ± 30 mm, and the wettest month (January) having 208 mm (WorldClim 2.5' data from 1970–2000, for 24°15'56" S 48°24'48" W, 841 m a.s.l., which is the coordinate of the "reception" in Figure 1b; Fick & Hijmans 2017, www.worldclim.org/data/worldclim21.html, see also Figure 2 of Leonel 2010). During winter, the average precipitation is 66 ± 21 mm, with the driest month (August) having 49 mm (WorldClim *op. cit*.). At another PEI station in the lowland (*ca*. 150 m a.s.l.), at Saibadela, the picture can be very different as its nonseasonal tropical rainy climate reaches 4,000 mm annual precipitation, with the rainy season always with > 200 mm/month, frequently > 400 mm/month, and the driest period (May to August) with > 100 mm/month (Morellato *et al.* 2000).

We had access to 27 years of maximum and minimum temperature and relative humidity data from the weather station located near the PEI reception (Figure 1b). Data collection began in April 1992 and, although it is collected until the present time, we analyzed it until June 2019. The weather station was visited three times a day (08h, 14h, and 20h), every day, resulting in 28,990 measurements (9,663 at 08h, 9,676 at 14h, 9,651 at 20h), spread in 9,722 days of 320 months of 27 years (Tables S2–S4). More details of the data and its analyses are provided in the Supplementary Material. This data showed high correlation with the temperature and humidity data we measured at the PEI trap sampling (Table S6).

2. Butterfly data sampling

This study joins datasets from museums (*Museu de Diversidade Biológica* of the University of Campinas, ZUEC-LEP and the Zoology Museum of the University of São Paulo, MZUSP, having also consulted the *Coleção Entomológica Padre Jesus Santiago Moure*, Zoology Department of the Federal University of Paraná, DZUP; and the National Museum at Rio de Janeiro, MNUFRJ) and sampling by the authors (referred by our initials) from different locations, under different methods.

In the Supplementary Material we describe the material and methods of each dataset (summarized in Table 1), including coordinates, method(s), collectors, specialists who identified the material, aim, sampling effort, and design. We organized them as follows: 1. museum data, and 2. sampling data by us (with PEI species fully illustrated in Shirai *et al*. 2022). We divided the museum data in: **1.1**. PEI ZUEC dataset, and **1.2.** PENaP dataset (*Parque Estadual Nascentes do Paranapanema*) – both datasets used the entomological net as the sampling method (communicated by the collectors). We divided the sampling data in: **2.1.** PEI dataset; **2.2.** PEI fish dataset (carrion bait test in the Atlantic Forest); **2.3.** PEI 1y dataset (one-year monitoring for capacity development and science outreach); **2.4.** AG dataset (Apiaí and Guapiara sampling); and **2.5.** Other.

Our sampling was carried out in the following periods: **2.1.** PEI dataset (illustrated in Shirai *et al*. 2022) at 14 to 18.III.2016 (pilot), 06 to 13.III.2017, 07 to 12.IV.2017, 11 to 16.VI.2017, 21 to 23.VII.2017, 10 to 14.IV.2018, 26 to 29.VII.2018 with sampling effort of 5,760 trap hours (16 days * 30 traps * 12 trap hours/day), and ca. 76 net hours; **2.2.** PEI fish dataset (07 to 12.III.2018 and 09 to 15.XII.2018) with sampling effort of 3,456 trap hours (8 days * 36 traps * 12 trap hours/day), and ca. 15 net hours; **2.3.** PEI 1y dataset 14 to 17.I.2019, 18 to 21.II.2019, 18 to 21.III.2019, 15 to 18.IV.2019, 27 to 30.V.2019, 17 to 20.VI.2019, 16 to 19.VII.2019, 20 to 23.VIII.2019, 17 to 20.IX.2019, 14 to 17.X.2019, 25 to 28.XI.2019, 09 to 12.XII.2019 with sampling effort of 4,752 trap hours (36 days * 11 traps * 12 trap hours/day); and **2.4.** AG dataset XII.2014, 06 to 19.VIII.2017, 18 to 27.XI.2017, 17 to 26.II.2018, 17 to 25.V.2018, 25.VIII to 03.IX.2018, 14 to 23.XII.2018, 08 to 17.III.2019, 02 to 12.VII.2019, 19 to 29.IX.2019 with sampling effort of 22,464 trap hours (39 days * 48 traps * 12 trap hours/day) and 156 hours of active search.

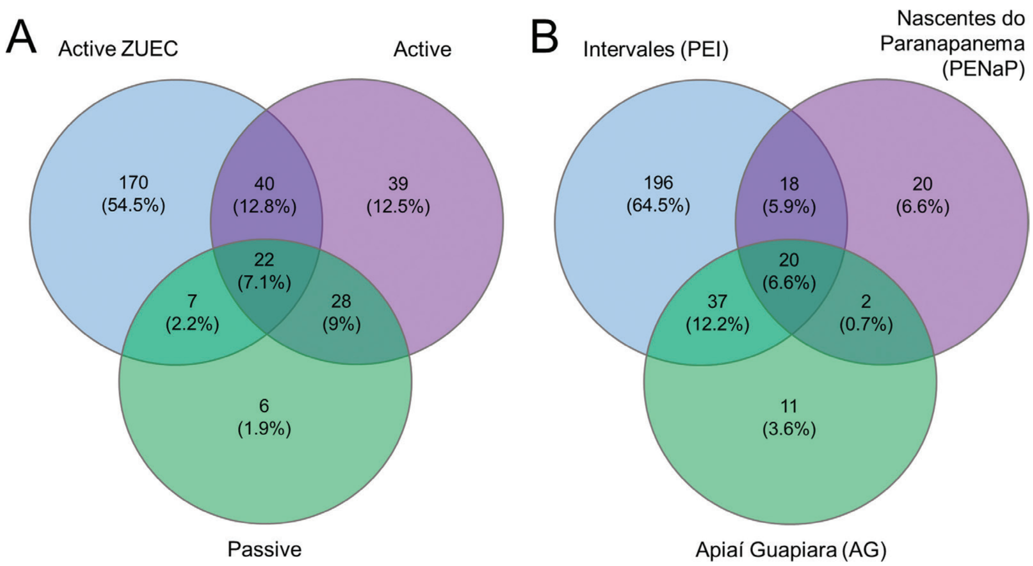

Figure 2. Venn diagrams of (a) sampling methods (active *versus* passive), separating the sampling from the ZUEC museum and our study for the active search method; and (b) different regions (PEI, PENaP, AG) – the 20 species common to all regions illustrated in Figure 3.

Table 1. Summary of each dataset, with information of when they were sampled (month and year), how (method and details of trap studies), and sampling effort. Museum data: PEI ZUEC (*Parque Estadual Intervales* in the ZUEC collection) and PENaP (*Parque Estadual Nascentes do Paranapanema*). Sampled in this study: PEI, PEI fish (carrion bait test in the Atlantic Forest), PEI 1y (one-year monitoring for capacity development and science outreach), and AG (Apiaí and Guapiara). Full sampling data is in the Supplementary Material.

Dataset	Months	Year(s)	Sampling effort	Method(s)	No. Traps	Bait	Stratum(a)
PEI ZUEC	I, II, IV, VIII, XI, XII	1992, 2000–2003, 2007–2010, 2013	NA	net	NA	NA	NA
PENAP	III, IV	2012	NA	net	NA	NA	NA
PEI	III, IV, VI, VII	2016–2018	5,760 trap h; 76 net h	net, trap	30	banana	understory, canopy
PEI fish	III, XII	2018	3,456 trap h; 15 net h	trap	36	banana x fish	understory, canopy
PEI 1y	I to XII	2019	4,752 trap h	trap	11	banana	understory
AG	all but I, IV, VI, X	2014, 2017–2019	22,464 trap h; 156 net/obs h	net, trap, obs	48	banana	understory

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We classified species in subfamilies and used the phylogenetic hypotheses of Warren *et al*. (2008, 2009), Wahlberg *et al*. (2009, 2014), Seraphim *et al*. (2018), Li *et al*. (2019), and Dias *et al*. (2019). We based the identification of specimens on the specialized literature (*e.g.* Brown 1992, Uehara-Prado *et al*. 2004, Warren *et al*. 2016, Lamas 2022), and on the collections of the ZUEC-LEP and MZUSP. These are also the entomological collections where we housed the specimens collected by us, with material from LTS at Unicamp (ZUEC–LEP 10.018–10.070, 10.253–10.262, 11.312–11.445) and ROS at MZUSP. Part of the Unicamp material (~10%) has a pair of legs stored in EtOH and is available to the scientific community upon request.

We searched for information about the butterflies of the region by looking at the literature, asking specialists, and consulting a database with the state of the art of species lists in Brazil (Shirai *et al*. 2019). We found only three studies (Brown & Freitas 1999, Santos *et al*. 2015, Shirai *et al*. 2017), none of which had a species list for more than a tribe. Other studies reported the collection of focal taxa at the PEI (*e.g*. *Forsterinaria pronophila* (A. Butler, 1867) in Freitas & Peña 2006, *Blepolenis bassus* (C. Felder & R. Felder, 1867) in Penz *et al.* 2013, *Taygetis ypthima* (Hübner, [1821]) and *T. rectifascia* (Weymer, 1907) in Siewert *et al.* 2013, *Godartiana muscosa* (A. Butler, 1870) in Zacca *et al*. 2016, *T. acuta* Weymer, 1910 in Freitas 2017, *T. yphtima* in Uehara-Prado & Freitas 2019), and the referred specimens belong to ZUEC-LEP and ZUEC-AVLF private collection.

Except from ZUEC-LEP, other large butterfly collections in the country did not have material from the PEI: MZUSP (ROS pers. obs.); DZUP (O. H. H. Mielke, pers. comm.); the MNUFRJ probably did not have material from the region but even if it had, it would be reduced to ashes due to the fire in September 2018. In the 30 years and 300 research projects in the park, no foreign institution has had an official project that collected butterflies (T. B. Conforti, pers. comm.). Therefore, the museum data reported below belonged solely to ZUEC-LEP (now MZUSP has sampled material by ROS, and DZUP and MNRJ entomological collections also house PEI specimens donated by us).

We consulted ZUEC-LEP by the online database available at the SpeciesLink website (www.splink.org.br) at the very beginning and end of this study, filtering by the collection (ZUEC-LEP) and the municipalities of and around PEI (Capão Bonito, Eldorado, Guapiara, Iporanga, Ribeirão Grande, Sete Barras), returning results only for Ribeirão Grande and Sete Barras. We searched the Nymphalidae specimens with missing IDs in the database and checked every drawer of the collection to update the online database by including their identity.

3. *Data treatment*

We counted the number of individuals found at the ZUEC-LEP, those sampled by us under active searches and other sources (here referred as records), as well as under baited traps designs (here referred as abundance). The number of records and abundance are not comparable because specimens deposited in a museum, sampled with the net by different observers, or observational records (such as citizen science) do not reflect biological abundance. We also counted the number of species (richness), excluding genera not identified to the species level due to taxonomic issues (*e.g., Hermeuptychia* sp. Forster, 1964, Tan *et al*. 2021) or museum specimens identified as such (*e.g*., *Actinote* sp. Hübner, [1819], *Adelpha* sp. Hübner, [1819], etc); except from *Autochton* sp. Hübner, 1823, *Ochlodes* sp. Scudder, 1872, *Emesis* sp. Fabricius, 1807, and *Mesene* sp. E. Doubleday, 1847 because no other species of the same genus were in the list.

We reported the species lists by dataset (Table 2) and explored how many and which species belong to each dataset, or are shared among datasets, with Venn diagrams (using online tool Venny, https://bioinfogp.cnb.csic.es/tools/venny/). We also looked at the species assembly (presence/absence) by sampling method and by region. We then collapsed datasets in a single list to analyze richness (whole data, as well as PEI traps: PEI trap, PEI fish, and PEI 1y datasets) and abundance (only PEI traps) per month and per season. For PEI traps, we separated the lists by datasets and divided the diversity metrics by the number of traps and trap days.

Lastly, focusing on baited traps (PEI traps and AG traps), to understand how the richness distribution match what has been found in other trap studies in the Atlantic Forest, we used a database of Nymphalidae species lists in Brazil called DnB (Shirai *et al*. 2019, downloaded from https://doi.org/10.5281/zenodo.2561408), filtering studies that exclusively used baited traps, exclusively at the Atlantic Forest. That is, we did not include studies of the DnB database that used *e.g.* active searches or active search plus traps; or done in other biomes or the Atlantic Forest plus other biomes, because the lists would not be strictly comparable. The DnB study used the same source for phylogenetic arrangement as ours (Wahlberg *et al.* 2009). In relation to species identities, we filtered species considered valid by Lamas (2004) which, despite being outdated, standardizes the taxonomy to a single source. To counterbalance this compromise, we also consulted the largest database of fruit-feeding butterflies in the Atlantic Forest (Santos *et al.* 2018), that has an updated taxonomy. Using Santos *et al.* (2018) classification of tribes, we compared our totals per tribe with their data (Figure 3 of Santos *et al.* 2018).

Results

In total, we found 312 species for PEI and surroundings (Table 2) that sum to 2,139 records (1,605 in active search, 509 in traps, 25 other). The museum contributed with 432 records for 229 species. We sampled 1,682 specimens belonging to 142 species, in a total effort of 36,679 sampling hours (36,432 trap and 247 net and observation hours, Table 1).

The richest families were Nymphalidae (148 species), the single family caught by all sampling methods, and Hesperiidae (100), followed by Riodinidae (23 species), Lycaenidae (19 species), Pieridae (14 species), and Papilionidae (8 species). Within nymphalids, the richest subfamily was Satyrinae (58 species), followed by Danainae (23), Heliconiinae (18), Biblidinae (16), Nymphalinae (13), Charaxinae (12), Limenitidinae (6), and Apaturinae (2). Within skippers, the richest subfamily was Hesperiinae (54 species), followed by Pyrginae (24), Eudaminae (16), Pyrrhopyginae (4), and Tagiadinae (2). None of the species were among the 63 threatened (that is, critically endangered, endangered, or vulnerable) butterfly species (ICMBio 2018, updated by Augusto H. B. Rosa).

The great majority of species was sampled exclusively by active methods (79.8%), with 18.3% having been collected by both active and passive methods, and only 1.9% sampled in traps only (Figure 2a). Additionally, the citizen science, iNaturalist and Santos *et al*. (2015)

Table 2. Butterfly species of PEI and surroundings per dataset. Museum data: PEI ZUEC (*Parque Estadual Intervales* in the ZUEC collection) and PENaP (*Parque Estadual Nascentes do Paranapanema*). Sampled in this study: PEI, PEI fish (carrion bait test in the Atlantic Forest), PEI 1y (one-year monitoring for capacity development and science outreach), and AG (Apiaí and Guapiara). "Other" refers to citizen science, iNaturalist and Santos *et al*. (2015) sources. Numbers are either the number of records or the abundance (only for standardized method of baited traps), while "x" denotes presence. In the "Month caught" column, PENaP did not have specify collection dates, only III–IV. This table is available at 10.5281/zenodo.7429126.

Family	Subfamily	Tribe	Taxon	Species description	PEI ZUEC	PENaP	PEI	AG	PEI	PEI fish	PEI 1y	AG	other	Month caught
					entomological net				baited traps					
Hesperiidae	Eudaminae		*Autochton* sp.	NA	x									NA
Hesperiidae	Eudaminae		*Cecropterus albimargo*	(Mabille, 1876)	1									I
Hesperiidae	Eudaminae		*Cecropterus zarex*	(Hübner, 1818)	2		1							I, III
Hesperiidae	Eudaminae		*Oechydrus chersis*	(Herrich-Schäffer, 1869)			1							IV
Hesperiidae	Eudaminae		*Perichares seneca*	(Latreille, [1824])			1							VII
Hesperiidae	Eudaminae		*Phanus australis*	L. Miller, 1965	2									I
Hesperiidae	Eudaminae		*Phocides pialia*	(Hewitson, 1857)	x									NA
Hesperiidae	Eudaminae		*Polygonus savigny*	(Latreille, [1824])	1									I
Hesperiidae	Eudaminae		*Spicauda procne*	(Plötz, 1881)			1							III
Hesperiidae	Eudaminae		*Spicauda simplicius*	(Stoll, 1790)			2							III, IV
Hesperiidae	Eudaminae		*Spicauda teleus*	(Hübner, 1821)		4								III–IV, IV
Hesperiidae	Eudaminae		*Telegonus alardus*	(Stoll, 1790)	1									I
Hesperiidae	Eudaminae		*Typhedanus aziris*	(Hewitson, 1867)	1									XII
Hesperiidae	Eudaminae		*Typhedanus stylites*	(Herrich-Schäffer, 1869)			1			1				XII
Hesperiidae	Eudaminae		*Urbanus esmeraldus*	(A. Butler, 1877)	2									I
Hesperiidae	Eudaminae		*Urbanus pronta*	Evans, 1952			3							III
Hesperiidae	Hesperiinae		*Anthoptus epictetus*	(Fabricius, 1793)	3									XII
Hesperiidae	Hesperiinae		*Callimormus rivera*	(Plötz, 1882)	2									XII
Hesperiidae	Hesperiinae		*Carystoides sicania*	(Hewitson, 1876)	x									NA
Hesperiidae	Hesperiinae		*Cobalopsis brema*	E. Bell, 1959	1									I
Hesperiidae	Hesperiinae		*Cobalopsis vorgia*	(Schaus, 1902)	1									I
Hesperiidae	Hesperiinae		*Conga immaculata*	(E. Bell, 1930)			1							XII
Hesperiidae	Hesperiinae		*Corticea lysias*	Evans, 1955	x									NA
Hesperiidae	Hesperiinae		*Corticea oblinita*	(Mabille, 1891)			1							III
Hesperiidae	Hesperiinae		*Decinea dama*	(Herrich-Schäffer, 1869)						1				III
Hesperiidae	Hesperiinae		*Decinea decinea*	(Hewitson, 1876)	1									I
Hesperiidae	Hesperiinae		*Enosis schausi*	O. Mielke & Casagrande, 2002	1									I
Hesperiidae	Hesperiinae		*Eutocus matildae*	(Hayward, 1941)	x									NA
Hesperiidae	Hesperiinae		*Eutychide physcella*	(Hewitson, 1866)	x									NA

Hesperiidae	Hesperiinae	*Lamponia lamponia*	(Hewitson, 1876)	x				NA
Hesperiidae	Hesperiinae	*Lerema duroca*	Evans, 1955	x				NA
Hesperiidae	Hesperiinae	*Levina levina*	(Plötz, 1884)	1				XII
Hesperiidae	Hesperiinae	*Libra aligula*	(Schaus, 1902)	2				II
Hesperiidae	Hesperiinae	*Lucida lucia*	(Capronnier, 1874)	x				NA
Hesperiidae	Hesperiinae	*Lucida ranesus*	(Schaus, 1902)	1				II
Hesperiidae	Hesperiinae	*Lucida schmithi*	(E. Bell, 1930)	1				XII
Hesperiidae	Hesperiinae	*Lychnuchoides ozias*	(Hewitson, 1878)	x				NA
Hesperiidae	Hesperiinae	*Lychnuchus celsus*	(Fabricius, 1793)	1	3			I, IV
Hesperiidae	Hesperiinae	*Metron oropa*	(Hewitson, 1877)	3				II, XII
Hesperiidae	Hesperiinae	*Miltomiges cinnamomea*	(Herrich-Schäffer, 1869)	1	3	4		I, III, IV, VII
Hesperiidae	Hesperiinae	*Mnasitheus ritans*	(Schaus, 1902)			3		IV, VII, XII
Hesperiidae	Hesperiinae	*Moeris remus*	(Fabricius, 1798)		1			IV
Hesperiidae	Hesperiinae	*Naevolus orius*	(Mabille, 1883)	x				NA
Hesperiidae	Hesperiinae	*Nastra lurida*	(Herrich-Schäffer, 1869)	1				XII
Hesperiidae	Hesperiinae	*Niconiades cydia*	(Hewitson, 1876)	4				I
Hesperiidae	Hesperiinae	*Niconiades merenda*	(Mabille, 1878)	1				I
Hesperiidae	Hesperiinae	*Ochlodes* sp.	NA	x				NA
Hesperiidae	Hesperiinae	*Papias phainis*	Godman, 1900				x	XII
Hesperiidae	Hesperiinae	*Perichares philetes*	(Gmelin, [1790])	x				NA
Hesperiidae	Hesperiinae	*Pheraeus fastus*	(Hayward, 1939)				x	NA
Hesperiidae	Hesperiinae	*Pheraeus odilia*	(Plötz, 1884)	1				XII
Hesperiidae	Hesperiinae	*Polites vibex*	(Geyer, 1832)				x	XII
Hesperiidae	Hesperiinae	*Pompeius pompeius*	(Latreille, [1824])	x				NA
Hesperiidae	Hesperiinae	*Psoralis coyana*	(Schaus, 1902)			1		VII
Hesperiidae	Hesperiinae	*Psoralis stacara*	(Schaus, 1902)		3	1		IV, VII
Hesperiidae	Hesperiinae	*Quasimellana nicomedes*	(Mabille, 1883)	2				I
Hesperiidae	Hesperiinae	*Saliana esperi*	Evans, 1955	x				NA
Hesperiidae	Hesperiinae	*Saliana saladin*	Evans, 1955	x				NA
Hesperiidae	Hesperiinae	*Sodalia dimassa*	(Hewitson, 1876)	1				XII
Hesperiidae	Hesperiinae	*Talides sergestus*	(Cramer, 1775)	x				NA
Hesperiidae	Hesperiinae	*Tirynthia conflua*	(Herrich-Schäffer, 1869)	2				I
Hesperiidae	Hesperiinae	*Vehilius clavicula*	(Plötz, 1884)	1	2	2		III, III–IV, XII
Hesperiidae	Hesperiinae	*Vehilius stictomenes*	(A. Butler, 1877)	1				I
Hesperiidae	Hesperiinae	*Vettius diana*	(Plötz, 1886)	x				NA
Hesperiidae	Hesperiinae	*Vettius lucretius*	(Latreille, [1824])	x				NA

Continue...

...Continuation

Family	Subfamily	Tribe	Taxon	Species description	PEI ZUEC	PENaP	PEI	AG	PEI	PEI fish	PEI 1y	AG	other	Month caught
					entomological net				baited traps					
Hesperiidae	Hesperiinae		*Vettius phyllus*	Evans, 1955	x									NA
Hesperiidae	Hesperiinae		*Vettius umbrata*	(Erschoff, 1876)	2		3							II, VII
Hesperiidae	Hesperiinae		*Vinius letis*	(Plötz, 1883)	1									XII
Hesperiidae	Hesperiinae		*Zariaspes mys*	(Hübner, [1808])		5								III–IV, IV
Hesperiidae	Hesperiinae		*Zenis jebus*	(Plötz, 1882)	x									NA
Hesperiidae	Pyrginae		*Achlyodes mithridates*	(Fabricius, 1793)	1									I
Hesperiidae	Pyrginae		*Anastrus obscurus*	Hübner, [1824]	x									NA
Hesperiidae	Pyrginae		*Anastrus ulpianus*	Poey, 1832	x									NA
Hesperiidae	Pyrginae		*Bolla catharina*	(E. Bell, 1937)	1									I
Hesperiidae	Pyrginae		*Burnsius orcus*	(Stoll, 1780)		1		x						IV, XII
Hesperiidae	Pyrginae		*Diaeus lacaena*	(Hewitson, 1869)			1							XII
Hesperiidae	Pyrginae		*Diaeus* sp.	NA		1								III–IV
Hesperiidae	Pyrginae		*Ebrietas infanda*	(A. Butler, 1877)			1							III
Hesperiidae	Pyrginae		*Gindanes brebisson*	(Latreille, [1824])	x									NA
Hesperiidae	Pyrginae		*Heliopetes (Heliopetes) arsalte*	(Linnaeus, 1758)		1								IV
Hesperiidae	Pyrginae		*Heliopetes (Heliopetes) ochroleuca*	J. Zikán, 1938	1		1							VII, XII
Hesperiidae	Pyrginae		*Heliopetes (Heliopyrgus) americanus*	(Blanchard, 1852)			1							III
Hesperiidae	Pyrginae		*Heliopetes (Leucoscirtes) omrina*	(A. Butler, 1870)		1								IV
Hesperiidae	Pyrginae		*Heliopetes alana*	(Reakirt, 1868)	x									NA
Hesperiidae	Pyrginae		*Mylon maimon*	(Fabricius, 1775)	1									I
Hesperiidae	Pyrginae		*Nisoniades castolus*	(Hewitson, 1878)			1							III
Hesperiidae	Pyrginae		*Noctuana diurna*	(A. Butler, 1870)	1									I
Hesperiidae	Pyrginae		*Pythonides lancea*	(Hewitson, 1868)	1								x	I
Hesperiidae	Pyrginae		*Quadrus cerialis*	(Stoll, 1782)	1									XII
Hesperiidae	Pyrginae		*Theagenes dichrous*	(Mabille, 1878)			1							VII
Hesperiidae	Pyrginae		*Trina geometrina*	(C. Felder & R. Felder, 1867)	x									NA
Hesperiidae	Pyrginae		*Viola violella*	(Mabille, 1898)									x	XII
Hesperiidae	Pyrginae		*Xenophanes tryxus*	(Stoll, 1780)		5	3							III, III–IV, VII
Hesperiidae	Pyrginae		*Zera hyacinthinus*	(Mabille, 1877)	x									NA

Hesperiidae	Pyrginae	*Zera zera*	(A. Butler, 1870)	x									NA
Hesperiidae	Pyrrhopyginae	*Elbella lamprus*	(Hopffer, 1874)	x									NA
Hesperiidae	Pyrrhopyginae	*Mimoniades (Mahotis) versicolor*	(Latreille, [1824])	2									I
Hesperiidae	Pyrrhopyginae	*Myscelus amystis*	(Hewitson, 1867)	1									XII
Hesperiidae	Pyrrhopyginae	*Pyrrhopyge charybdis*	Westwood, 1852	x									NA
Hesperiidae	Tagiadinae	*Celaenorrhinus eligius*	(Stoll, 1781)	1									I
Hesperiidae	Tagiadinae	*Celaenorrhinus similis*	Hayward, 1933	x									NA
Lycaenidae	Polyommatinae	*Elkalyce cogina*	(Schaus, 1902)	1									I
Lycaenidae	Polyommatinae	*Hemiargus hanno*	(Stoll, 1790)		1								IV
Lycaenidae	Polyommatinae	*Leptotes cassius*	(Cramer, 1775)			1							III
Lycaenidae	Polyommatinae	*Zizula cyna*	(W. H. Edwards, 1881)		3								III–IV, IV
Lycaenidae	Theclinae	*Arawacus meliboeus*	(Fabricius, 1793)		1								III–IV
Lycaenidae	Theclinae	*Arzecla arza*	(Hewitson, 1874)	x									NA
Lycaenidae	Theclinae	*Aubergina vanessoides*	(Prittwitz, 1865)	x									NA
Lycaenidae	Theclinae	*Brangas silumena*	(Hewitson, 1867)	x									NA
Lycaenidae	Theclinae	*Calycopis janeirica*	(C. Felder, 1862)	x									NA
Lycaenidae	Theclinae	*Chalybs janias or C. chloris*	NA	x									NA
Lycaenidae	Theclinae	*Evenus satyroides*	(Hewitson, 1865)	x									NA
Lycaenidae	Theclinae	*Janthecla flosculus*	(H. Druce, 1907)			1							III
Lycaenidae	Theclinae	*Laothus phydela*	(Hewitson, 1867)		1								IV
Lycaenidae	Theclinae	*Ocaria thales*	(Fabricius, 1793)	x									NA
Lycaenidae	Theclinae	*Ostrinotes empusa*	(Hewitson, 1867)			1							XII
Lycaenidae	Theclinae	*Rekoa palegon*	(Cramer, 1780)	x									NA
Lycaenidae	Theclinae	*Strephonota elika*	(Hewitson, 1867)				x						XII
Lycaenidae	Theclinae	*Theritas hemon*	(Cramer, 1775)		1								III–IV
Lycaenidae	Theclinae	*Theritas lisus*	(Stoll, 1790)	x									NA
Nymphalidae	Apaturinae	*Doxocopa laurentia*	(Godart, [1824])			1							IV
Nymphalidae	Apaturinae	*Doxocopa zunilda*	(Godart, [1824])									x	NA
Nymphalidae	Biblidinae	*Catonephele acontius*	(Linnaeus, 1771)				1	1					II, III
Nymphalidae	Biblidinae	*Catonephele numilia*	(Cramer, 1775)				1	2	1		1		III, IV
Nymphalidae	Biblidinae	*Diaethria clymena*	(Cramer, 1775)				7	1	3		4		II, III, V, XII
Nymphalidae	Biblidinae	*Diaethria eluina*	(Hewitson, [1855])					1					III
Nymphalidae	Biblidinae	*Dynamine athemon*	(Linnaeus, 1758)	x									NA
Nymphalidae	Biblidinae	*Dynamine tithia*	(Hübner, 1823)	x									NA

Continue...

...Continuation

Family	Subfamily	Tribe	Taxon	Species description	PEI ZUEC	PENaP	PEI	AG	PEI	PEI fish	PEI 1y	AG	other	Month caught
					entomological net				baited traps					
Nymphalidae	Biblidinae		*Ectima thecla*	(Fabricius, 1796)				2				3		II, IX
Nymphalidae	Biblidinae		*Epiphile orea*	(Hübner, [1823])			1	4	2	2	1	1		II to V, IX, XI, XII
Nymphalidae	Biblidinae		*Haematera pyrame*	(Hübner, [1819])				2				1		II
Nymphalidae	Biblidinae		*Hamadryas amphinome*	(Linnaeus, 1767)				2		1		1		II, XII
Nymphalidae	Biblidinae		*Hamadryas epinome*	(C. Felder & R. Felder, 1867)				41				17		II, V, IX, XI
Nymphalidae	Biblidinae		*Hamadryas februa*	(Hübner, [1823])	1			2						II
Nymphalidae	Biblidinae		*Hamadryas feronia*	(Linnaeus, 1758)	x									NA
Nymphalidae	Biblidinae		*Hamadryas fornax*	(Hübner, [1823])			1	3				1		II, III, XI
Nymphalidae	Biblidinae		*Myscelia orsis*	(Drury, 1782)		2	1	33	1			18		II to V, VIII, IX, XI
Nymphalidae	Biblidinae		*Temenis laothoe*	(Cramer, 1777)				2		2		1		V, XII
Nymphalidae	Charaxinae		*Archaeoprepona amphimachus*	(Fabricius, 1775)	1			2	1			1		I to III, V
Nymphalidae	Charaxinae		*Archaeoprepona chalciope*	(Hübner, [1823])				5		2				II, III, XII
Nymphalidae	Charaxinae		*Archaeoprepona demophon*	(Linnaeus, 1758)	2			4				1		I, II, XI
Nymphalidae	Charaxinae		*Consul fabius*	(Cramer, 1776)	1			1						I, II
Nymphalidae	Charaxinae		*Fountainea ryphea*	(Cramer, 1775)			1	2	10	6	2	2		II to VI, XII
Nymphalidae	Charaxinae		*Hypna clytemnestra*	(Cramer, 1777)	1		1				1			I, III, V
Nymphalidae	Charaxinae		*Memphis acidalia*	(Hübner, [1819])		1			1		1			III–IV, IV, V
Nymphalidae	Charaxinae		*Memphis appias*	(Hübner, [1825])			2	5	51	8	1			II to IV, XII
Nymphalidae	Charaxinae		*Memphis moruus*	(Fabricius, 1775)					4		1			II, III
Nymphalidae	Charaxinae		*Memphis otrere*	(Hübner, [1825])				4	2	2		2		II to IV, XI
Nymphalidae	Charaxinae		*Siderone galanthis*	(Cramer, 1775)							1			IV
Nymphalidae	Charaxinae		*Zaretis strigosus*	(Gmelin, [1790])				2	3	1	3			II to V, XI
Nymphalidae	Danainae		*Aeria olena*	Weymer, 1875	x									NA
Nymphalidae	Danainae		*Callithomia lenea*	(Cramer, 1779)	1		3							I, III
Nymphalidae	Danainae		*Danaus erippus*	(Cramer, 1775)			8							III, IV, VII, XII
Nymphalidae	Danainae		*Danaus gilippus*	(Cramer, 1775)		1							x	IV
Nymphalidae	Danainae		*Dircenna dero*	(Hübner, 1823)	2									I
Nymphalidae	Danainae		*Episcada hymenaea*	(Prittwitz, 1865)			1							III

Nymphalidae	Danainae	*Episcada philoclea*	(Hewitson, [1855])	x								NA
Nymphalidae	Danainae	*Episcada striposis*	Haensch, 1909	x								NA
Nymphalidae	Danainae	*Episcada sylvo*	(Geyer, 1832)	2								I
Nymphalidae	Danainae	*Epityches eupompe*	(Geyer, 1832)	5	2	52					x	I, III, IV, VI, VII, XII
Nymphalidae	Danainae	*Hypoleria adasa*	(Hewitson, [1855])	x								NA
Nymphalidae	Danainae	*Hypothyris ninonia*	(Hübner, [1806])			4			1	1		III to VI
Nymphalidae	Danainae	*Ithomia agnosia*	Hewitson, [1855]			1						IV
Nymphalidae	Danainae	*Ithomia drymo*	Hübner, 1816	4	4	6	x	2			x	I, III, IV, VI, VII, XII
Nymphalidae	Danainae	*Ithomia lichyi*	R.F. d'Almeida, 1939			3						III
Nymphalidae	Danainae	*Lycorea halia*	(Hübner, 1816)								x	NA
Nymphalidae	Danainae	*Mechanitis lysimnia*	(Fabricius, 1793)	2	1	4	x					I, III, III–IV, VI, XII
Nymphalidae	Danainae	*Melinaea ethra*	(Godart, 1819)	x								NA
Nymphalidae	Danainae	*Melinaea ludovica*	(Cramer, 1780)	2								I
Nymphalidae	Danainae	*Oleria aquata*	(Weymer, 1875)	1		1						I, III
Nymphalidae	Danainae	*Placidina euryanassa*	(C. Felder & R. Felder, 1860)			1						III
Nymphalidae	Danainae	*Pseudoscada erruca*	(Hewitson, 1855)	6		2	x					I, III, XII
Nymphalidae	Danainae	*Pteronymia carlia*	Schaus, 1902	4	3	5						I, III, IV, XII
Nymphalidae	Heliconiinae	*Actinote carycina*	Jordan, 1913			1						IV
Nymphalidae	Heliconiinae	*Actinote dalmeidai*	Francini, 1996	1								XII
Nymphalidae	Heliconiinae	*Actinote genitrix*	R.F. d'Almeida, 1922	2								XII
Nymphalidae	Heliconiinae	*Actinote melanisans*	Oberthür, 1917			1						XII
Nymphalidae	Heliconiinae	*Actinote parapheles*	Jordan, 1913	1								XII
Nymphalidae	Heliconiinae	*Actinote pyrrha*	(Fabricius, 1775)		1	5	x					IV, XII
Nymphalidae	Heliconiinae	*Actinote* sp.	NA	4	10						x	III–IV, IV, XI, XII
Nymphalidae	Heliconiinae	*Agraulis vanillae*	(Linnaeus, 1758)	x								NA
Nymphalidae	Heliconiinae	*Dione juno*	(Cramer, 1779)	x								NA
Nymphalidae	Heliconiinae	*Dryadula phaetusa*	(Linnaeus, 1758)								x	NA
Nymphalidae	Heliconiinae	*Dryas iulia*	(Fabricius, 1775)	1		5					x	I, III, IV, VI
Nymphalidae	Heliconiinae	*Eueides aliphera*	(Godart, 1819)		2							III–IV
Nymphalidae	Heliconiinae	*Eueides isabella*	(Stoll, 1781)			1	x					IV, XII
Nymphalidae	Heliconiinae	*Eueides pavana*	Ménétriés, 1857		1							IV
Nymphalidae	Heliconiinae	*Heliconius besckei*	(Ménétriés, 1857)	3	1	8	x					I, III, IV, VII, XII

Continue...

...Continuation

Family	Subfamily	Tribe	Taxon	Species description	PEI ZUEC	PENaP	PEI	AG	PEI	PEI fish	PEI 1y	AG	other	Month caught
					entomological net				baited traps					
Nymphalidae	Heliconiinae		*Heliconius erato*	(Linnaeus, 1758)	3	1	10							I, III, IV, XII
Nymphalidae	Heliconiinae		*Heliconius ethilla*	(Godart, 1819)	1		4							III, VII, XII
Nymphalidae	Heliconiinae		*Heliconius sara*	(Fabricius, 1793)	1	2	1							III, III–IV, XII
Nymphalidae	Heliconiinae		*Philaethria wernickei*	(Röber, 1906)		1	3						x	III, IV
Nymphalidae	Limenitidinae		*Adelpha cocala*	(Cramer, 1779)	1									I
Nymphalidae	Limenitidinae		*Adelpha gavina*	Fruhstorfer, 1915	x									NA
Nymphalidae	Limenitidinae		*Adelpha lycorias*	(Godart, [1824])	x									NA
Nymphalidae	Limenitidinae		*Adelpha mythra*	(Godart, [1824])	1									I
Nymphalidae	Limenitidinae		*Adelpha serpa*	(Boisduval, 1836)	1			x						I, XII
Nymphalidae	Limenitidinae		*Adelpha* sp.	NA	1	3							x	III–IV, IV, XI
Nymphalidae	Limenitidinae		*Adelpha syma*	(Godart, [1824])			1							IV
Nymphalidae	Nymphalinae		*Anartia amathea*	(Linnaeus, 1758)	1	1	10	x					x	I, III, IV, XII
Nymphalidae	Nymphalinae		*Eresia lansdorfi*	(Godart, 1819)		1	1	x						III, III–IV, XII
Nymphalidae	Nymphalinae		*Eresia perna*	Hewitson, 1852	x									NA
Nymphalidae	Nymphalinae		*Historis odius*	(Fabricius, 1775)	1									I
Nymphalidae	Nymphalinae		*Hypanartia bella*	(Fabricius, 1793)		2	3							III–IV, XII
Nymphalidae	Nymphalinae		*Hypanartia lethe*	(Fabricius, 1793)	x									NA
Nymphalidae	Nymphalinae		*Junonia evarete*	(Cramer, 1779)			1	x						IV, XII
Nymphalidae	Nymphalinae		*Ortilia ithra*	(W. F. Kirby, 1900)		1								III–IV
Nymphalidae	Nymphalinae		*Siproeta epaphus*	(Latreille, [1813])	1			x						I, XII
Nymphalidae	Nymphalinae		*Smyrna blomfildia*	(Fabricius, 1781)	2				2					I, III
Nymphalidae	Nymphalinae		*Tegosa claudina*	(Eschscholtz, 1821)	1	1	3							III, IV, XII
Nymphalidae	Nymphalinae		*Tegosa* sp.	NA			2							III
Nymphalidae	Nymphalinae		*Telenassa teletusa*	(Godart, [1824])	3	4		x						I, III–IV, XII
Nymphalidae	Nymphalinae		*Vanessa braziliensis*	(Moore, 1883)	1		1							VII, XI
Nymphalidae	Satyrinae	Brassolini	*Blepolenis bassus*	(C. Felder & R. Felder, 1867)	5						1			I, II
Nymphalidae	Satyrinae	Brassolini	*Blepolenis batea*	(Hübner, [1821])	1	2	3		2		3	3		I to III, III–IV
Nymphalidae	Satyrinae	Brassolini	*Blepolenis catharinae*	(Stichel, 1902)				1				1		II
Nymphalidae	Satyrinae	Brassolini	*Caligo arisbe*	Hübner, [1822]								1		II
Nymphalidae	Satyrinae	Brassolini	*Caligo beltrao*	(Illiger, 1801)	1								x	XI
Nymphalidae	Satyrinae	Brassolini	*Caligo brasiliensis*	(C. Felder, 1862)	x									NA
Nymphalidae	Satyrinae	Brassolini	*Catoblepia amphirhoe*	(Hübner, [1825])				1						II

Nymphalidae	Satyrinae	Brassolini	*Dasyophthalma creusa*	(Hübner, [1821])			1	8				4		II, III
Nymphalidae	Satyrinae	Brassolini	*Dasyophthalma rusina*	(Godart, [1824])		1		1			1			II, IV
Nymphalidae	Satyrinae	Brassolini	*Eryphanis reevesii*	(E. Doubleday, [1849])	1			16	2		1	2		II, III, V, X to XII
Nymphalidae	Satyrinae	Brassolini	*Narope cyllene*	C. Felder & R. Felder, 1859				2	2	1		1		II to IV, XII
Nymphalidae	Satyrinae	Brassolini	*Opoptera aorsa*	(Godart, [1824])	1		3							III, XII
Nymphalidae	Satyrinae	Brassolini	*Opoptera sulcius*	(Staudinger, 1887)		1	2	12	1			2		II to IV
Nymphalidae	Satyrinae	Brassolini	*Opsiphanes cassiae*	(Linnaeus, 1758)	1									I
Nymphalidae	Satyrinae	Brassolini	*Opsiphanes invirae*	(Hübner, [1808])	1			4						I
Nymphalidae	Satyrinae	Haeterini	*Pierella lamia*	(Sulzer, 1776)	1									I
Nymphalidae	Satyrinae	Haeterini	*Pierella nereis*	(Drury, 1782)	1									XII
Nymphalidae	Satyrinae	Morphini	*Antirrhea archaea*	Hübner, [1822]	x									NA
Nymphalidae	Satyrinae	Morphini	*Morpho aega*	(Hübner, [1822])	3	1	2	80	1			10		all but VI, VII, IX, X
Nymphalidae	Satyrinae	Morphini	*Morpho anaxibia*	(Esper, [1801])		2	1							III–IV, IV
Nymphalidae	Satyrinae	Morphini	*Morpho epistrophus*	(Fabricius, 1796)			5	125	37	24	4	9	x	II to IV
Nymphalidae	Satyrinae	Morphini	*Morpho helenor*	(Cramer, 1776)		1	3	46				3		II, III, III–IV, XI
Nymphalidae	Satyrinae	Morphini	*Morpho hercules*	(Dalman, 1823)		1								IV
Nymphalidae	Satyrinae	Satyrini	*Archeuptychia cluena*	(Drury, 1782)	1									VIII
Nymphalidae	Satyrinae	Satyrini	*Capronnieria galesus*	(Godart, [1824])	2	2					2			I, III, IV
Nymphalidae	Satyrinae	Satyrini	*Carminda griseldis*	(Weymer, 1911)	2							1		I, II, XII
Nymphalidae	Satyrinae	Satyrini	*Carminda paeon*	(Godart, [1824])	2	5	5	7	2	1		8		all but VI, VII, X
Nymphalidae	Satyrinae	Satyrini	*Cissia eous*	(A. Butler, 1867)		3								III–IV
Nymphalidae	Satyrinae	Satyrini	*Cissia phronius*	(Godart, [1824])	4	3	6	10	1		7	4		I, III to V, IX, XII
Nymphalidae	Satyrinae	Satyrini	*Eteona tisiphone*	(Boisduval, 1836)				12	6		1	7		II to V, IX, XI
Nymphalidae	Satyrinae	Satyrini	*Euptychoides castrensis*	(Schaus, 1902)	3	2		16				8		I, II, III–IV, XI, XII
Nymphalidae	Satyrinae	Satyrini	*Forsterinaria necys*	(Godart, [1824])	1		4	115	10	2		12		all but VI, X
Nymphalidae	Satyrinae	Satyrini	*Forsterinaria pronophila*	(A. Butler, 1867)	1		1	44		3		11		I to III, V, VIII, IX, XI
Nymphalidae	Satyrinae	Satyrini	*Forsterinaria quantius*	(Godart, [1824])	4	2		52				2		all but III, VI, VII, X
Nymphalidae	Satyrinae	Satyrini	*Godartiana muscosa*	(A. Butler, 1870)	2		4	133	3			41		all but VII, X
Nymphalidae	Satyrinae	Satyrini	*Hermeuptychia aff. hermes*	NA			10		1					III, IV, VI, VII, XII

Continue...

...Continuation

Family	Subfamily	Tribe	Taxon	Species description	PEI ZUEC	PENaP	PEI	AG	PEI	PEI fish	PEI 1y	AG	other	Month caught
					entomological net				baited traps					
Nymphalidae	Satyrinae	Satyrini	*Hermeuptychia hermes*	(Fabricius, 1775)	2			33				14		I, II, XII
Nymphalidae	Satyrinae	Satyrini	*Hermeuptychia* sp.	NA			11				3			III to V
Nymphalidae	Satyrinae	Satyrini	*Moneuptychia soter*	(A. Butler, 1877)	1			3	1		4	2		I, III to VI, XI
Nymphalidae	Satyrinae	Satyrini	*Pareuptychia ocirrhoe*	(Fabricius, 1776)	1									IV
Nymphalidae	Satyrinae	Satyrini	*Paryphthimoides grimon*	(Godart, [1824])	1									IV
Nymphalidae	Satyrinae	Satyrini	*Paryphthimoides poltys*	(Prittwitz, 1865)	x									NA
Nymphalidae	Satyrinae	Satyrini	*Praepedaliodes phanias*	(Hewitson, 1862)			1					5		II, III, V
Nymphalidae	Satyrinae	Satyrini	*Praepedaliodes* sp.	NA	1									II
Nymphalidae	Satyrinae	Satyrini	*Pseudodebis celia*	(Cramer, 1779)				2	1	1				III, XI
Nymphalidae	Satyrinae	Satyrini	*Pseudodebis euptychidia*	(A. Butler, 1868)	5		3		8		10			all but II, VII, VIII
Nymphalidae	Satyrinae	Satyrini	*Splendeuptychia aff. boliviensis*	NA				6				18		V, VIII, IX, XI
Nymphalidae	Satyrinae	Satyrini	*Splendeuptychia ambra*	(Weymer, [1911])				11				2		XI
Nymphalidae	Satyrinae	Satyrini	*Splendeuptychia* sp.	NA	1									XII
Nymphalidae	Satyrinae	Satyrini	*Taygetina kerea*	(A. Butler, 1869)	x									NA
Nymphalidae	Satyrinae	Satyrini	*Taygetis acuta*	Weymer, 1910				1			1			II, V
Nymphalidae	Satyrinae	Satyrini	*Taygetis mermeria*	(Cramer, 1776)			2		3					III, VI
Nymphalidae	Satyrinae	Satyrini	*Taygetis rectifascia*	(Weymer, 1907)	14							1		I, II, XII
Nymphalidae	Satyrinae	Satyrini	*Taygetis rufomarginata*	Staudinger, 1888	x									NA
Nymphalidae	Satyrinae	Satyrini	*Taygetis sosis*	Hopffer, 1874	x									NA
Nymphalidae	Satyrinae	Satyrini	*Taygetis* sp.	NA	2									I
Nymphalidae	Satyrinae	Satyrini	*Taygetis tripunctata*	Weymer, 1907	x									NA
Nymphalidae	Satyrinae	Satyrini	*Taygetis ypthima*	(Hübner, [1821])	12					1	3			I to III, VI, XII
Nymphalidae	Satyrinae	Satyrini	*Yphthimoides affinis*	(A. Butler, 1867)	1									I
Nymphalidae	Satyrinae	Satyrini	*Yphthimoides ochracea*	(A. Butler, 1867)	1									IV
Nymphalidae	Satyrinae	Satyrini	*Yphthimoides ordinaria*	A.V.L. Freitas, L. Kaminski & O.H.H. Mielke, 2012	1									II
Nymphalidae	Satyrinae	Satyrini	*Zischkaia pacarus*	(Godart, [1824])								1		NA
Papilionidae	Papilioninae		*Eurytides bellerophon*	(Dalman, 1823)	1									I
Papilionidae	Papilioninae		*Heraclides thoas*	(Rothschild & Jordan, 1906)	x									NA
Papilionidae	Papilioninae		*Mimoides lysithous*	(Hübner, [1821])	1								x	I
Papilionidae	Papilioninae		*Mimoides protodamas*	(Godart, 1819)	1									I
Papilionidae	Papilioninae		*Parides agavus*	(Drury, 1782)			2							III
Papilionidae	Papilioninae		*Parides anchises*	(Linnaeus, 1758)	2									I
Papilionidae	Papilioninae		*Parides proneus*	(Hübner, [1831])	2									I
Papilionidae	Papilioninae		*Pterourus scamander*	(Boisduval, 1836)		1								IV

Pieridae	Coliadinae	*Eurema albula*	(Cramer, 1775)	1	5	5	x						III, IV, VI, XII
Pieridae	Coliadinae	*Phoebis argante*	(Fabricius, 1775)		3		x						III–IV, IV, XII
Pieridae	Coliadinae	*Phoebis philea*	(Linnaeus, 1763)	2	1	2	x					x	I, III, IV, XII
Pieridae	Coliadinae	*Phoebis sennae*	(Linnaeus, 1758)		1								IV
Pieridae	Coliadinae	*Pyrisitia nise*	(Cramer, 1775)		1								III–IV
Pieridae	Coliadinae	*Rhabdodryas trite*	(Linnaeus, 1758)	1	1	1	x					x	III, IV, XII
Pieridae	Dismorphiinae	*Dismorphia amphione*	(Cramer, 1779)			1							III
Pieridae	Dismorphiinae	*Dismorphia crisia*	(Drury, 1782)			2							III
Pieridae	Dismorphiinae	*Dismorphia thermesia*	(Godart, 1819)	1	1								I, III–IV
Pieridae	Dismorphiinae	*Enantia clarissa*	(Weymer, 1895)				x						XII
Pieridae	Dismorphiinae	*Pseudopieris nehemia*	(Boisduval, 1836)	1									XII
Pieridae	Pierinae	*Archonias brassolis*	(Fabricius, 1776)	1									I
Pieridae	Pierinae	*Melete lycimnia*	(Cramer, 1777)		2								IV
Pieridae	Pierinae	*Pereute swainsoni*	(G. Gray, 1832)			4						x	III
Riodinidae	Riodininae	*Adelotypa bolena*	(A. Butler, 1867)	x								x	NA
Riodinidae	Riodininae	*Ancyluris aulestes*	(Saunders, 1850)	x								x	NA
Riodinidae	Riodininae	*Calospila apotheta*	(H. Bates, 1868)	x									NA
Riodinidae	Riodininae	*Emesis* sp.	NA			1							III
Riodinidae	Riodininae	*Eurybia carolina*	Godart, [1824]			1							IV
Riodinidae	Riodininae	*Eurybia molochina*	Stichel, 1910	x									NA
Riodinidae	Riodininae	*Eurybia pergaea*	(Geyer, 1832)	1	2								IV, XII
Riodinidae	Riodininae	*Euselasia hygenius*	(Stoll, 1787)	x									NA
Riodinidae	Riodininae	*Ionotus alector*	(Geyer, 1837)	x									NA
Riodinidae	Riodininae	*Leucochimona icare*	(Hübner, [1819])			1							III
Riodinidae	Riodininae	*Mesene* sp.	NA	1									I
Riodinidae	Riodininae	*Mesosemia mayi*	Lathy, 1958	x									NA
Riodinidae	Riodininae	*Mesosemia odice*	(Godart, [1824])	4	1	1							I, IV, VI, XII
Riodinidae	Riodininae	*Mesosemia rhodia*	(Godart, [1824])	4									XII
Riodinidae	Riodininae	*Metacharis ptolomaeus*	(Fabricius, 1793)			1							III
Riodinidae	Riodininae	*Napaea elisae*	(J. Zikán, 1952)	x									XII
Riodinidae	Riodininae	*Napaea nepos*	(Fabricius, 1793)	1									XII
Riodinidae	Riodininae	*Panara soana*	Hewitson, 1875	x									NA
Riodinidae	Riodininae	*Rhetus periander*	(Cramer, 1777)			1							III
Riodinidae	Riodininae	*Rhetus arcius*	(Linnaeus, 1763)									x	NA
Riodinidae	Riodininae	*Stichelia bocchoris*	(Hewitson, 1876)	x									NA
Riodinidae	Riodininae	*Symmachia aconia*	Hewitson, 1876	x									NA
Riodinidae	Riodininae	*Voltinia cebrenia*	(Hewitson, [1873])	x									NA
			Total n	**230 + 74**	**128**	**288**	**866 + 19**	**165**	**64**	**53**	**227**	**25**	
			Total richness	**195**	**60**	**92**	**64**	**31**	**20**	**21**	**40**	**23**	

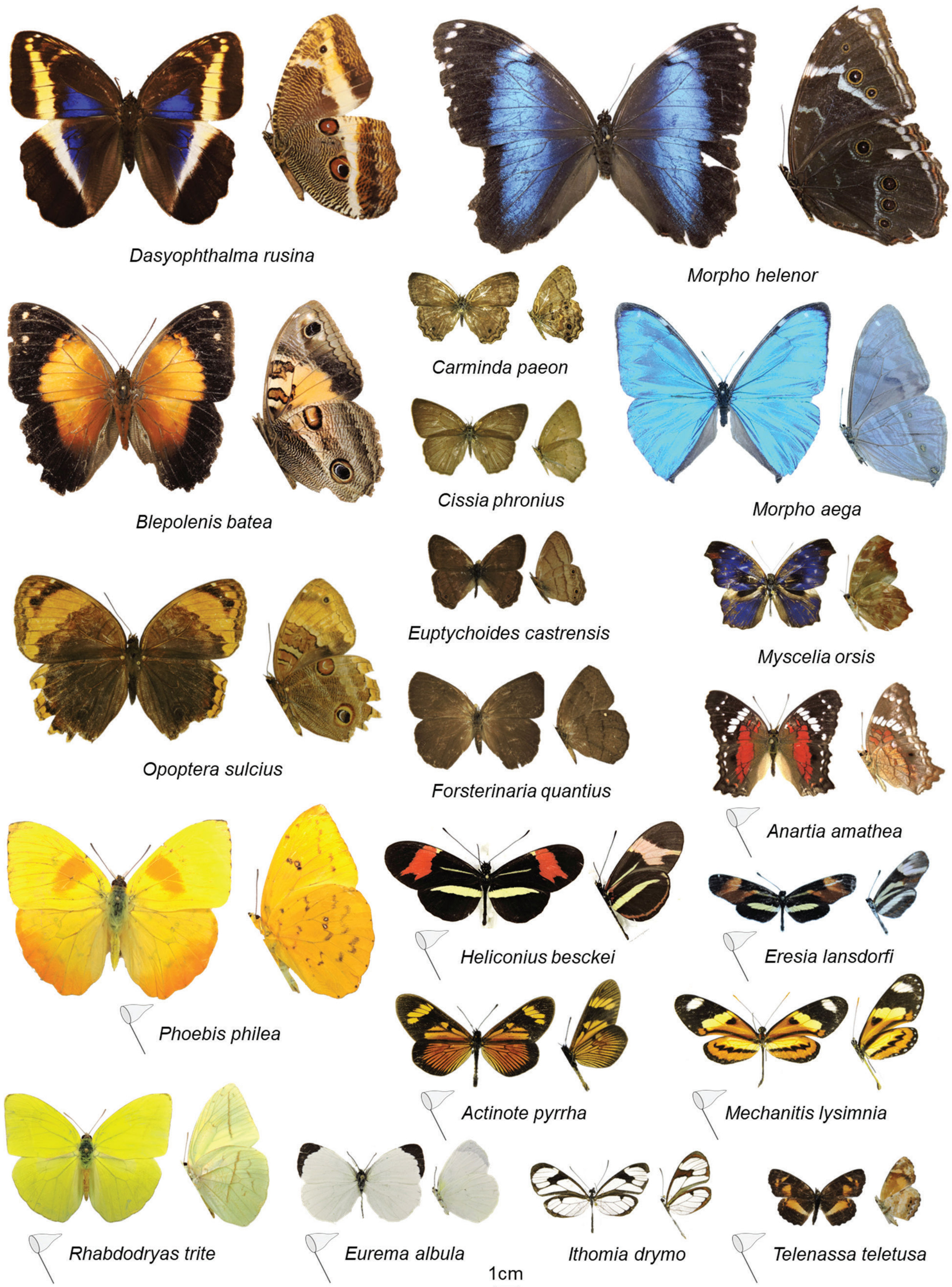

Figure 3. Butterfly species common to all sampling regions (intersection of PEI, PENaP, AG datasets in Figure 2). These are likely the species that any person will see at the PEI and surroundings. Sizes are proportional to their real size, with dorsal wing surfaces shown spread, and ventral surfaces tilted similar to the butterfly's resting position. The net symbol indicates species caught exclusively with the entomological net, while all others were caught with the net and traps. A more complete illustrated guide can be found in the companion paper (Shirai *et al*. 2022).

sources (“other” dataset, not included in Figure 2a due to the uncertain methodology) contributed with eight species not found by any of the active or passive methods: the skippers *Papias phainis* Godman, 1900, *Pheraeus fastus* (Hayward, 1939), *Polites vibex* (Geyer, 1832), and *Viola violella* (Mabille, 1898); the nymphalids *Doxocopa zunilda* (Godart, [1824]), *Lycorea halia* (Hübner, 1816), *Dryadula phaetusa* (Linnaeus, 1758); and the metalmark *Rhetus arcius* (Linnaeus, 1763).

The comparison by region (Figure 2b) showed, expectedly, that the majority was collected at the focal region (PEI, with 64.5% being exclusive). The 20 species common to all regions (Figure 3) included both forest dwellers and open area species.

We found the highest diversity in warmer months (Figure 4), as can be seen in the total richness (Figure 4a, using all data but not standardized by sampling effort). The information of which month each species was sampled (last column of Table 2) should, however, be used with caution because it does not necessarily reflect the true phenology of species due to sampling bias, such as the overrepresentation of summer months. Also, the number of individuals is subject to methodological bias, so looking at the PEI trap data, where we can compare abundance, March, April, and May outstood in both richness and abundance (Figure 4b). We would not biologically interpret the results in September since it seems an effect of statistic inflation: only 5 individuals of 4 species were sampled but, as they were caught only the PEI 1y dataset (3 days in 11 traps), the metrics end up higher than other datasets that sampled for more days in a higher number of traps.

As we sampled in every month of the year, we can demonstrate that winter months have lower richness compared to any other season in the whole dataset. We found in June: 13 species, July: 16 species, August: nine species, September: 13 species (Figure 4a); which is much lower compared to remaining seasons (average ± standard deviation 142.3 ± 47.2). This result can also be observed in the comparable data of baited traps (Figure 4b-c). Lastly, 15 out of the 19 Brassolini and Morphini species were reported exclusively from December to April.

Despite our sampling bias, we found that nine species should, with some certainty, be observed most of the year (species caught in seven-10 months in the year, Table 2): biblidines *Epiphile orea* (Hübner, [1823]) and *Myscelia orsis* (Drury, 1782), and satyrines *Carminda paeon* (Godart, [1824]), *Forsterinaria necys* (Godart, [1824]), *F. pronophila* (A. Butler, 1867), *F. quantius* (Godart, [1824]), *Godartiana muscosa* (A. Butler, 1870), *Morpho aega* (Hübner, [1822]), and *Pseudodebis euptychidia* (A. Butler, 1868). Except from *E. orea* and *F. quantius*, the remaining seven species also ranked among the 15 most abundant species the trap sampling.

Among trap datasets, we caught 505 individuals of 63 species. The richest and most abundant subfamily was Satyrinae, followed by Charaxinae, Biblidinae, and Nymphalinae (Table 3). We found that 51.3% of the individuals belong to seven species: *Morpho epistrophus* (Fabricius, 1796) (74), *Memphis appias* (Hübner, [1825]) (60), *G. muscosa* (44), *F. necys* (24), *Fountainea ryphea* (Cramer, 1775) (20), *M. orsis* (19), and *P. euptychidia* (18). On average, we caught 0.19 individuals/trap/day in the PEI (0.34), PEI-fish (0.22), PEI 1y (0.13) and AG (0.09) datasets. The comparison between PEI habitats and vertical stratification using the PEI and PEI fish datasets can be found in the Supplementary Material.

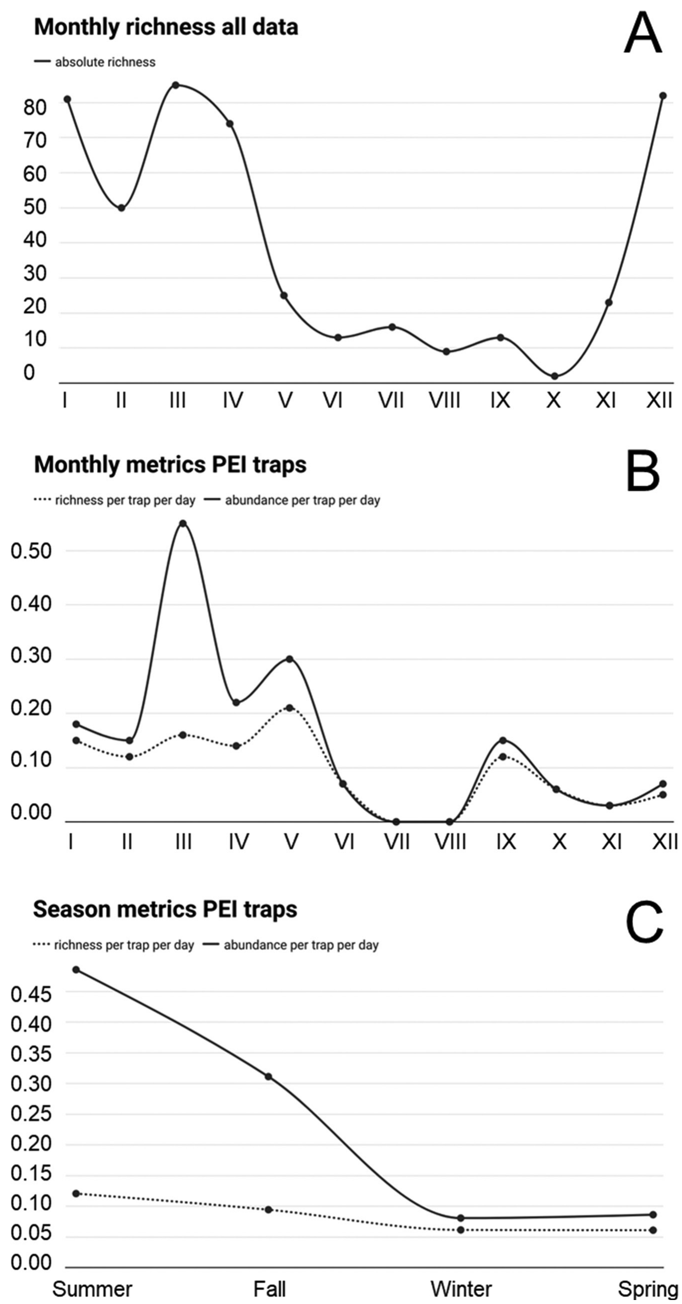

Figure 4. Diversity metrics for (a) all data, under the three sampling methods (absolute number of species) by month; and for PEI traps (richness, in dashed line, and abundance, in solid line, per trap and per trap day) reporting them by (b) month, and (c) by season.

Discussion

Butterflies are excellent model organisms for scientific research (reviewed in *e.g.* Brown & Freitas 1999, Santos *et al*. 2008, Sourakov & Shirai 2020) and for conservation purposes (see Introduction). However, even considering they are among the best-studied insects, important gaps in basic information still exist, such as for species description and mapping species distributions (Linnean and Wallacean shortfalls *c.f.* Hortal *et al*. 2015). That results in the exclusion of butterflies, as well as other invertebrates, from studies like those that established hotspots

Table 3. Richness and abundance of trap studies: "Our study" sums the trap individuals from the PEI, PEI fish, PEI 1y and AG datasets; "DnB" refers to "Database of nymphalids in Brazil" (Shirai *et al*. 2019), with species lists exclusively caught with traps at the Atlantic Forest (numbers are sums of 40 studies, see Material and Methods); and "Atl butterflies" refers to the "Atlantic Butterflies" database (Santos *et al*. 2018), the largest database for fruit-feeding butterflies. The number of individuals refers to abundance (our study) or to the number of records (= presence) in the literature (for DnB and Atl butterflies). Nymphalidae classification follows Wahlberg *et al*. 2009 (subfamilies) and Santos *et al*. 2018 (tribes).

Taxa	Richness			Abundance (our study)/records		
	Our study	DnB	Atl butterflies	Our study	DnB	Atl butterflies
Biblidinae	**12**	**45**	**63**	**66**	**513**	**2336**
Ageroniini	4	11	11	23	208	639
Biblidini	0	1	2	0	29	136
Callicorini	3	11	11	10	81	448
Epicaliini (Catonephilini)	3	5	6	24	79	244
Epiphilini	2	6	6	9	61	272
Eubagini	0	3	12	0	5	362
Eunicini	0	8	15	0	50	235
Charaxinae	**11**	**30**	**34**	**107**	**331**	**1010**
Anaeini	8	19	20	102	190	656
Preponini	3	11	14	5	141	354
Nymphalinae	**1**	**5**	**5**	**2**	**100**	**268**
Coeini	1	5	5	2	100	268
Satyrinae	**37**	**126**	**176**	**328**	**897**	**3434**
Brassolini	9	28	36	28	244	881
Haeterini	0	4	5	0	17	79
Morphini	3	12	9	88	73	354
Satyrini	25	82	126	212	563	2120
Total	**61**	**206**	**278**	**503**	**1841**	**7048**

(Myers *et al*. 2000) or other global conservation efforts (Bossart & Carlton 2002, Barua *et al.* 2012), even considering that butterflies are a bioindicator, flagship, and umbrella group. In this study, we aimed at filling this gap by inventorying butterfly diversity in a region within the Paranapiacaba Continuum, a key network of protected areas for the preservation of the Atlantic Forest hotspot. We also provided several resources to aid conservation efforts (see Introduction), and a discussion for both academics and non-academics.

Most of butterfly richness is in the tropics. While Nymphalidae diversified in the Asian, African and American tropics, several lineages of Hesperiidae and Riodinidae adaptive radiations happened exclusively in the Neotropics (Toussaint *et al.* 2018, Seraphim *et al*. 2018), with metalmarks being mainly Amazonian. Thus, it is not surprising that the richest families we found were Nymphalidae and Hesperiidae, frequently reported as the most diverse in the Atlantic Forest (Brown & Freitas 2000, Francini *et al*. 2011). Hesperiidae is probably the richest butterfly family in Brazil and the fact that we caught them less than Nymphalidae probably reflects the suggestion of Francini *et al*. (2011): in relatively complete inventories, the small and inconspicuous Hesperiidae are better sampled, but in short term studies, Nymphalidae appears with higher richness because they are easily captured, with both net and traps. Lycaenidae and Riodinidae, despite having less species in the Atlantic Forest than the two families above, equally suffer in shorter inventories by being harder to catch. Moreover, most Pieridae species we caught are found in open areas across the country but important species are expected to be caught only at the higher altitudes in the park, above 800 m a.s.l. (Francini *et al*. 2011).

Focusing on nymphalids, a review of species lists in Brazil (Shirai *et al.* 2019) listed the presence of 162-315 butterfly species in the top-10 richest places in the country. The corresponding biomes (Amazon, Atlantic Forest and one in the Cerrado) rank among the richest because 1) they are biologically rich but also, and importantly, 2) they were well-sampled (Shirai *et al.* 2019). The most complete dataset of a subset of Nymphalidae, fruit-feeding butterflies, listed 279 species for the whole Atlantic Forest (Santos *et al*. 2018), which is a substantial increment over previously available data, such as the 88–127 fruit-feeding species found in the same biome (Brown 2005). We were able to record 148 nymphalids (61 fruit-feeding), but these numbers as well as our total of 312 butterfly species are certainly not the true richness of the PEI and surroundings. The richness in the region could easily surpass 500 species or more, as compared to similar areas in the same region (Francini *et al*. 2011). More sampling, and sampling by different strategies (like comparing to an estimate of the "true richness" by maximized sampling, Uehara-Prado *et al*. 2007), are necessary to reflect the diversity of this region. Despite the attempt to combine different datasets to enhance this inventory, some obvious gaps remained, such as 1) a thorough expedition to the other PEI station at the East lowlands (Saibadela), that has a different climate and altitude (Morellato *et al.* 2000); 2) the most inaccessible and pristine region of the PEI, at the South, as well as the few but important high altitude sites in the park; 3) the contiguous protected areas PECB, PETAR and Xitué (see Figure 1); and 4) more sampling with different baits.

The use of different sampling methods certainly adds to more comprehensive inventories (*e.g*. Clench 1949, 1979, Brown 1972, Brown & Freitas 2000, Iserhard *et al.* 2013, Freitas *et al*. 2014, Checa *et al.* 2018). For example, most of the species we found were sampled by active methods (Figure 2a), collected by highly experienced people, during different years. However, despite having caught only about a fifth (63/312 species) of the total richness with traps, *Siderone galanthis* (Cramer 1775) was among the six trap-exclusive species (Figure 2a), caught by a recently trained PEI guide ("PEI 1y" dataset). *Decinea dama* (Herrich-Schäffer 1869) was also among the trap-exclusive species, only caught with fish carrion ("PEI fish" dataset). It is also worth mentioning that the citizen science approaches and a field course experiment ("other" dataset) contributed with eight species not found with neither active nor passive methods.

Including efforts of other places than the PEI also enhanced the inventory for the region adding, in our case, 33 species (20 exclusive of PENaP, 11 exclusive of AG, plus two in common, Figure 2b) not found at the park, that had much more sampling effort (Table 1). Several of the 20 species common to all regions (Figure 2b) are associated with open or fragmented areas; we illustrated them (Figure 3) because they are, aside from beautiful, colorful and diverse, likely the species that any person will observe at the region.

An interesting statistic, both biologically and in terms of planning, is the number of individuals caught per trap per day, as the 0.5 individuals/trap/day reported in an Amazonian *terra firme* site (Ribeiro & Freitas 2012). Traps at open and/or fragmented habitats tend to catch more individuals, but not species (*e.g*. Uehara-Prado *et al*. 2007). We caught 0.19 individuals/trap/day, but the highest values were not necessarily at the open, disturbed or fragmented sites, such as PEI 1y (0.13) and AG (0.09) datasets, but rather at the PEI (0.34) and PEI-fish (0.22). It would be interesting to gather more data like this to uncover differences, for instances, between environments and biomes.

The best months to collect in South Eastern Brazil are March to May (end of the rainy season, Ebert 1970, Brown 1972, 1992, Freitas *et al.* 2014) but other seasonal intervals are also relevant. Here, we chose when to collect aiming to span different seasons and months, complementing the effort from museum data at the same time as revisiting good months (like December and March) and we indeed found the highest diversity metrics in warmer months, particularly from March to May (Figure 4).

We thus reinforce a previous suggestion (Brown 1972, Uehara-Prado *et al*. 2007) that the best sampling months in the Atlantic Forest should be extended to the period between December to May (summer plus end of rainy season). The summer months of December to February are mandatory for tribes like Morphini and Brassolini that only fly in this period (Freitas *et al.* 2014). Here, we found 79% of brassolines and morphines within the months of December and April. However, winter months should not be completely disregarded since some species were exclusively caught in this season and, in the case of the PEI, it is the only season when an *Epityches eupompe* (Geyer, 1832) aggregation can be observed (Shirai *et al*. 2017, https://youtu.be/bUO4kpYS2uo).

For Brazilian rainforests standards, the PEI is chilly, partly because it has a lot of forest and in high altitudes, but also because it has a cold winter: the annual average temperature ranged from 15.1 to 19.2°C (Table S5), similar to what is reported in other sources (17.5°C from WorldClim and 17–18°C from Leonel 2010). More importantly, the temperature is seasonal, with a warm summer and a cold winter (Figure S1, Table S5): maximum temperatures of the warmest month (26–27°C) and minimum temperature of the coldest month (7.5–11°C), also like the WorldClim data.

The dominant species in our trap datasets was *Morpho epistrophus* (15%), the only *Morpho* species in the Atlantic Forest that is iridescent white (Pablos *et al.* 2021, Figure 5) – caught on traps with black mesh (see Freitas *et al*. 2014). Although we caught them in traps only from February to April (many of which with worn wings, LTS pers. obs.), this large butterfly is visible anywhere in the park since November. Butterflies are good flagship invertebrates because of their aesthetic appeal which, in

Figure 5. The emblematic white morpho, *Morpho epistrophus*, adult and caterpillar, that we suggest becoming the PEI invertebrate mascot. Images used with permission of the author Almir Cândido Almeida.

turn, relates to species attributes of size and brightness/color (Barua *et al.* 2012). The iridescent white color of *M. epistrophus* might seem dull to some visitors, but the fact that tourists in Neotropical forests are somewhat used to seeing, and being amused by, the blue morphos, might raise interest to their phylogenetic relationship, enhancing the color feature. Another important aspect of butterflies as invertebrate flagships is the fact that they are harmless. The elegant flight of *M. epistrophus* does not incite danger or disgust like wasps or mosquitoes might do. Although caterpillars are not always understood as an early stage of butterflies (Barua *et al.* 2012), they can trigger fear or dislike because of damage to crops. *M. epistrophus* harmless caterpillars are large, gregarious, bright red, and visible in many *Inga* Mill. tree trunks near the PEI reception (Figure 5). The contrasting effect of beauty and fear/disgust of these caterpillars could be used as an opportunity: the particularly skilled, enthusiastic and charismatic PEI staff could educate visitors about holometabolous life cycle, what makes a caterpillar harmful or harmless, what are aposematic colors, and warn visitors about moth caterpillars found at PEI (like *Megalopyge* Hübner 1820) that actually sting. Thus, the easy identification, large size, omnipresence in the park, relationship with famous blue morphos, elegant flight, and inciting caterpillars make *M. epistrophus* eligible to become the emblematic invertebrate mascot of the PEI.

Overexploitation of resources (like the juçara palm), illegal hunting and mining are permanent issues that endanger threatened species (such as the jaguar, Beisiegel & Nakano-Oliveira 2020) and a threatened biome (Tabarelli *et al*. 2005, Maxwell *et al.* 2016, ICMBio 2018). Even in the largest and strongest continuous of Atlantic Forest, we face the danger of (irreversibly) losing species and natural resources. More involvement and investment in the area would result in much more for the people and the planet than *e.g.* the end goals of several "perverse subsidies" (Myers 1998, Tabarelli *et al*. 2005). For example, investing in a geographically wider and temporally longer sampling of butterflies, together with more science outreach seminars and material, capacity development and teaching in local schools are ideas that need dedicated, almost exclusive, time of trained people but, surprisingly, they do not require a substantial financial investment (see Shirai *et al*. 2022). Another better idea, though, would be to invest in bioliteracy (*c.f.* Janzen 2010), which is a strong chance to save tropical diversity.

Supplementary Material

The following online material is available for this article:

A – Full description of our data.
B – Climatic data from the weather station at the PEI, in English and in Portuguese.
C – PEI trap datasets: habitats and vertical stratification in the PEI.

Acknowledgments

This work is dedicated to Benedito Amaral, a highly skilled citizen scientist and fine observer of the natural world. We would like to thank: Benedito Amaral, Thiago B. Conforti, Eliseu C. de Paula, Faustino A. Ribeiro, José Floido, Mara C. F. Paiva, Irene A. Ribeiro, Zarife O. Mora, and the remaining PEI staff for their outstanding contribution; Benedito Amaral, Massuo J. Kato, Lydia F. Yamaguchi, Mariana A. Stanton, Dimitre Ivanov, Sidneia C. C. do Nascimento, Adilson R. Moreira, Tamara M. C. Aguiar, André R. Nascimento, and Joel Lastra-Valdés for their help during field sampling; Patrício A. Salazar for designing and performing the preliminary analysis of the carrion test with LTS; Jessie P. dos Santos for teaching LTS how to use the slingshot; Wesley R. Silva for kindly lending his car in the first field trip; Thamara Zacca for identifying many Satyrinae, Ronaldo B. Francini for identifying the *Actinote*; Augusto H. B. Rosa and Olaf H. H. Mielke for museum information and access; Marcelo Duarte for allowing LTS to access the MZUSP collection; Tamara M. C. Aguiar, Artur N. Furegatti, and Michela Borges for their assistance at the ZUEC-LEP collection; Almir C. Almeida for allowing with such enthusiasm to use his photographs; and Renato R. Ramos, Eduardo P. Barbosa and Junia Y. O. Carreira for remote assistance during the COVID-19 lockdown. LTS acknowledges FAPESP (14/23504-7) for a fellowship, and ALCR thanks CNPq (166036/2020-0). AVLF thanks (FAPESP) (2021/03868-8) and the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) (421248/2017-3, 304291/2020-0). This study was done under environmental licenses ICMBio 8585-1 (sol. 10438), IBAMA 02001.000480/2014-38 (820/2017), and COTEC 260108-004.611/2016. The SisGen registry is AE4A636. All authors declare no conflict of interest.

Associate Editor

Gustavo Graciolli

Author Contributions

Leila T. Shirai: contribution to data analysis and interpretation; contribution to critical revision, adding intellectual content; read and approved the manuscript; substantial contribution to the conception and design of the study; contribution to data collection; contribution to manuscript preparation.

Renato O. Silva: contribution to data analysis and interpretation; contribution to critical revision, adding intellectual content; read and approved the manuscript; substantial contribution to the conception and design of the study; contribution to data collection.

Fernando M. S. Dias: contribution to data analysis and interpretation; contribution to critical revision, adding intellectual content; read and approved the manuscript.

André L. C. Rochelle: contribution to data analysis and interpretation; contribution to critical revision, adding intellectual content; read and approved the manuscript.

André V. L. Freitas: contribution to data analysis and interpretation; contribution to critical revision, adding intellectual content; read and approved the manuscript; substantial contribution to the conception and design of the study.

Conflicts of Interest

The authors declare that they have no conflict of interest related to the publication of this manuscript

Ethics

This study did not involve human beings and/or clinical trials that should be approved by one Institutional Committee.

Data Availability

The primary data is reported in the main text as Table 2 and is also available at the public repository https://doi.org/10.5281/zenodo.7429126, with the metadata in the readme file available at https://doi.org/10.5281/zenodo.7439430.

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Received: 16/12/2022
Accepted: 01/05/2023
Published online: 10/07/2023