ECOSYSTEMS

Species composition and abundance of free-living turbellarians in three different wetlands in Southern Brazil

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Abstract: Free-living turbellarians mostly live in marine and freshwater environments, but they have been little considered in ecological studies. The coastal plain in southern Brazil harbors a diverse fauna and flora, but only a few studies have related environmental factors to the abundance, richness, and distribution of turbellarians. Hence, we analyzed the structure of turbellarian communities in floating vegetation in three differently sized limnic environments. We aimed to determine differences in abundance, density, and richness of turbellarians among these habitats in relation to environmental factors. We found 1,225 turbellarians (34 species) distributed among 6 taxa. The most abundant was Tricladida with 49.4% relative abundance; the remaining (Catenulida, Dalytyphloplanida, Macrostomorpha, Kalyptorhynchia, and Prorhynchida) were less abundant. We found no significant differences among population parameters and environments. Small shallow lakes are a potentially rich environment, while water channels are the richest environment. Larger shallow lakes are not very diverse for turbellarians. Similarities regarding turbellarian communities among close limnic systems could be explained by the connectivity of bodies of water that presents similar aquatic macrophytes and hydrological subsystems, while larger shallow lakes have a different community because they have their own contributing basin and could be influenced by other factors.

Key words: Aquatic invertebrate, diversity, Neotropical region, Ramsar site, turbellaria.

INTRODUCTION

Humid areas such as wetlands cover an area of ~30,300 km^2 in southern Brazil (Maltchik et al. 2003). In this sense, the coastal plain of southern Brazil has several limnic ecosystems (shallow lakes, ponds, and wetlands) (Marques et al. 2013) that determine diversity. Aquatic macrophytes are an important part of communities, contributing to high biomass and high productivity (Wetzel 1993, Esteves 2011). Aquatic macrophytes influence the physical and chemical characteristics of water, are a source of organic matter (living or dead) for various organisms (bacteria, invertebrates, and vertebrates), and change the spatial structure of aquatic ecosystems due to the increase of spatial complexity of habitats (Thomaz & Esteves 2011). In addition, shallow lakes are characterized by a coastal zone that strongly interacts with the pelagic and pleuston zones, thus favoring high species richness (Meerhoff & Jeppesen 2009).

Habitat structures are important in determining the diversity of species, in which complex habitats usually have greater diversity (Bell et al. 1991, Jeffries 1993, Taniguchi et al. 2003, Thomaz et al. 2008, Mykrä et al. 2011). The presence of vegetation in an aquatic ecosystem result in a considerable increase in the area available for invertebrate colonization (Pinder

1995). Thus, shallow lakes and wetlands in southern Brazil are likely to present a great biodiversity of aquatic invertebrates (Braccini & Leal-Zanchet 2013). Aquatic invertebrates are important components of the food web and perform other functional food groups (predators, collectors, shredder, filter feeders, etc.) in limnic aquatic communities. However, most environmental research involving aquatic invertebrates is conducted using insects (larvae and adults) owing to the relative ease of sampling, fixing and identifying species. Nonetheless, many other groups, including the free-living turbellarians, are usually left out of environmental research (Braccini et al. 2016).

Free-living turbellarians are hermaphroditic, and some have the capacity for asexual reproduction (Tyler & Hooge 2004, Noreña et al. 2016). According to the body size, and for practical reasons, turbellarians are grouped into microturbellarians (~1 mm) and macroturbellarians (0.2–10 cm). Microturbellarians are represented by Catenulida, Macrostomorpha, Rhabdocoela, Proseriata, Prorhynchida, Prolecithophora and Bothrioplanida, while larger animals belonging to Tricladida and Polycladida are considered macroturbellarians (Schockaert et al. 2008, Noreña et al. 2015). A conservative approach reveals that approximately 1,500 species of turbellarians inhabit freshwater environments (Schockaert et al. 2008, Balsamo et al. 2020). Turbellarians are inhabitants of several inland ecosystems, such as wetlands, lakes, humid forest, soils/mosses and rivers (Noreña-Janssen 1995, Young 2001, Gamo & Leal-Zanchet 2004, Noreña et al. 2005, Braccini & Leal-Zanchet 2013, Reyes & Brusa 2017, Balsamo et al. 2020); they are also found in wet agricultural environments (Vara & Leal-Zanchet 2013). Moreover, turbellarians are associated with pleuston communities living

among floating aquatic plants (Damborenea et al. 2005, Noreña et al. 2006).

Turbellarians are abundant and diverse in aquatic ecosystems; however, most investigations on these organisms have been based on taxonomic issues and have disregarded ecological aspects (Kolasa 2000, Schockaert et al. 2008). The main reasons comprise the need to use living turbellarians (especially microturbellarians) for the species identification, the paucity of research in the area, the lack of taxonomist specialized in the group, lack of appropriate equipment for sample processing, and knowledge of a scattered literature in different languages and hardly any existing identification keys (Eitam et al. 2004, Schockaert et al. 2008, Balsamo et al. 2020). Yet, some efforts have been made to understand turbellarian communities in differently sized pools. In Israel, Eitam et al. (2004) determined that species richness and community dissimilarity were positively related to surface area, while community dissimilarity was also positively related to permanence. Young (2001) indicated that abiotic variables such as temperature, food availability, and the water regime in the ecosystem influence the seasonal occurrence of microturbellarians.

With respect to the Neotropical region, only a few studies have been performed to assess the turbellarian fauna in lentic waters (Heckman 1998). Noreña-Janssen (1995) indicated that the abundance dynamics of the microturbellarian species mainly depend on the type of life cycle adopted by the species, the water flow regime, and the coverage of aquatic plants. In addition, the highest population densities were found within macrophytes (Noreña-Janssen 1995). Similarly, Braccini & Leal-Zanchet (2013) studied differently sized permanent coastal wetlands in southern Brazil and found that the highest estimated species richness was present in

smaller rather than in more intermediate and larger wetlands. Besides, summer influenced the variation in abundance among wetlands.

The fact that freshwater turbellarians are abundant and diverse makes them an important group in structuring the community of other freshwater invertebrates (e.g., mosquito larvae, chironomid larvae, and crustaceans), causing oscillations in the abundance dynamics (Maly et al. 1980, Schwartz & Hebert 1986, Blaustein & Dumont 1990, Dumont et al. 2014). Triclads can also be important predators in small streams when fish are not present (Noreña et al. 2015).

Southern Brazil is a biodiversity hotspot (Guadagnin 1999), presenting an extent mosaic of flooded areas (about 37,300 km^2) (Schwarzbolt & Schäfer 1984). This coastal ecosystem harbors high abundance and diversity of fauna (Artioli et al. 2009, Garcias & Bager 2009, Stenert et al. 2012, Moreira et al. 2014, Kirst et al. 2017) and aquatic vegetation (Maltchik et al. 2004, Rolon et al. 2010) as a function of the heterogenic geography (Vieira & Rangel 1983) and seasonal hydrographic modifications (Vieira & Rangel 1988). Hence, the complexity of environmental factors (especially aquatic rooted and floating vegetation) in these temporally or permanently flooded areas of southern Brazil potentially represents an adequate habitat for turbellarians. However, there are only a few studies on the ecology of turbellarians (Braccini & Leal-Zanchet 2013). In this context, we indagated the species richness and abundance of turbellarians in different shallow limnic systems of the coastal plain of southern Brazil. In addition, we tested whether abiotic factors such as temperature and pH could explain turbellarian abundance and richness in distinct environments. As a model, we used the nature reserve of Taim (ESEC Taim), which is one of the most consistently flooded areas in southern Brazil. This area is composed of a mosaic of temporal and permanent ponds,

including part of a large lake within its area (Charão Kurtz et al. 2003). The ESEC Taim shelters a great diversity of vertebrates (including endangered species) (Gomes & Krause 1982, Pires Gayer et al. 1988, Garcia et al. 2006a, Azambuja 2010, Correa et al. 2011, Sponchiado et al. 2012, MMA 2018) and aquatic plants (Marques & Villanueva 2001). Due to its importance as a humid ecosystem, the ESEC Taim was recently designated as a Ramsar site (Ramsar 2017).

MATERIALS AND METHODS

Research area

The study area is situated at the ESEC Taim, located in the coastal plain of Rio Grande do Sul, southern Brazil. The ESEC Taim is a strictly protected area located between the Lake Mirim and the Atlantic Ocean. The ESEC Taim has a plain relief and is composed of several shallow freshwater ecosystems (channels, lakes, and wetlands). This area also has sand dunes and grass fields (Waechter & Jarenkow 1998). For this study, we collected samples in three different environments: in a shallow lake (site 1 [S1]), in a water channel (site 2 [S2]), and in a large shallow lake (site 3 [S3]) (Fig. 1). Water temperature and pH were recorded for each sampling site using a multiparameter (Hanna HI 9126). Details on sites are given as follows.

Lake Nicola (S1; 32°33′18.07″ S; 52°31′18.50″ W) is a 2.58 km^2 shallow lake, located in the northernmost area of the ESEC Taim. This lake has a remarkable seasonal water volume variation. Seasonally, the lake Nicola is covered by different kinds of marsh vegetation that is generally arranged in banks. Diffusely, the lake Nicola receives water from external agricultural channels and from the lake Mangueira (Würdig et al. 2007).

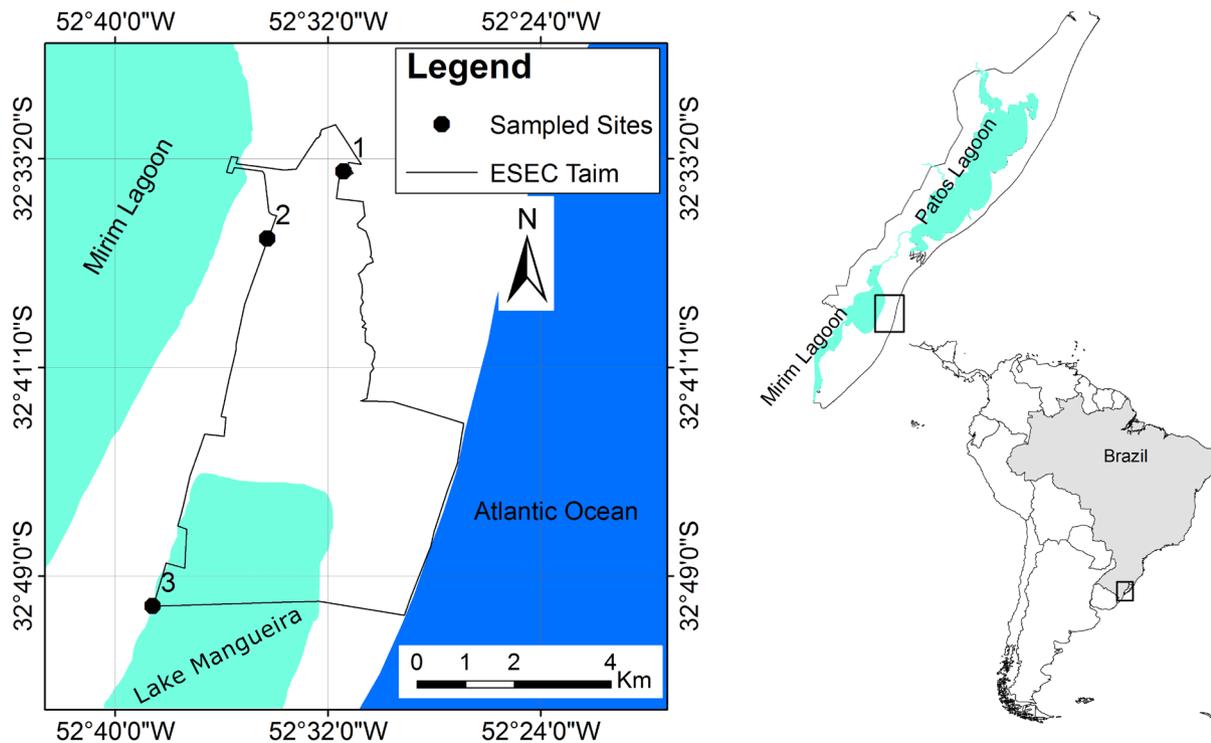A water channel (S2; 32°35′50.52″ S; 52°34′5.40″ W) is located at the western limit of

Figure 1. Sampling sites at the Taim nature reserve (ESEC Taim) in the Coastal Plain of southern Brazil.

the protected area. This channel was formed as a result of the construction of the main road (BR 471) which runs parallelly along almost the entire western limit of the ESEC Taim. Depending on the rainfall regime and water availability, this channel diffusely connects lake Nicola to lake Mangueira. This channel is long and presents floating and rooted plants according to seasonal rainfall and eutrophication processes.

Lake Mangueira (S3; 32°50'11.42" S; 52°38'37.45" W) is a vast body of freshwater (820 km^2), which diffusely supplies water to the lake Nicola. The north area of the lake is within the boundaries of the ESEC Taim. Littoral aquatic plants are composed by rooted and floating vegetation (Würdig et al. 2007).

Sample collection and preparation

Sampling campaigns were carried out between September 2018 and July 2019. We performed five samplings in S1 (September and November 2018; February, May, and July 2019) and S2 (October

and November 2018; March, April, and May 2019). We sampled S3 four times (October 2018; February, April, and June 2019). All samples were collected approximately every two months to remove potential bias, namely that the frequency of sampling campaigns could influence the determination of the turbellarian community at the sampled sites. We used a 0.25 m^2 square to set a known area to collect turbellarians, from which free-living turbellarians on floating vegetation microhabitats (0.5 m maximum depth) were randomly collected.

Floating vegetation was gathered using a plankton sieve (335 μm mesh size), then washed with water from the environment and transferred to 1.25 L plastic jars. The sampling procedure was randomly repeated three times in each site. Live specimens were transported to the laboratory and stored in a temperature-controlled room (~20°C) with air pumps.

Subsequently, samples underwent an oxygen depletion method (Schockaert 1996). In

brief, samples were left without any source of oxygen, causing turbellarians to swim toward the surface of the jar to reach dissolved oxygen at the upper layers of water, where turbellarians can be picked up easily. In addition, we looked for turbellarians among leaves, roots, and detritus, as had been suggested by Brusa et al. (2003).

Live animals collected from jars were observed *in vivo* using an optical microscopy to identify turbellarians (Young 2001), whenever possible, to the species level. When needed, after live observations, some specimens were whole mounted using Hoyer's medium (Humason 1979) for a detailed study. We compared all species found at the ESEC Taim against specialized literature concerning turbellarian fauna mainly from the Neotropics, but also from other biogeographic regions. All material studied here was described and registered in Reyes et al. (2021). All specimens were counted and recorded according to the site from where they were collected. Sampling permits were granted by the Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio), permits number 60291-2/2017 and 64551-1/2018.

Data analyses

We estimated species richness, total abundance, mean abundance, relative abundance, mean density, and density of individuals (per square meter) for each sampling site. We defined species abundance according to Chodorowski (1959), with modifications: (a) rare (only 1 individual), (b) less abundant (2–19 individuals), and (c) abundant (≥ 20 individuals). The composition of the turbellarian assemblages was compared among the sites by cluster analysis, using data of relative species abundance to explore differences in turbellarian assemblage composition among the sampling sites. We used a similarity percentage analysis (SIMPER)

test to identify the most important turbellarian species to generate the patterns of dissimilarity observed among the sites. Both analyses were based on the Bray–Curtis similarity coefficient (Gotelli & Ellison 2004).

The simplest method to assess diversity in a locality is by counting the number of species. The use of non-parametric richness estimators such as Chao, Jacknife, and Bootstrap have been suggested as a solution to determine richness in many cases involving freshwater organisms (Melo & Froehlich 2001, Foggo et al. 2003). Rarefaction curves are also used if samplings were performed properly to determine richness (Gotelli & Colwell 2001). Both richness estimators and rarefaction curves have been used in southern coastal wetlands in Brazil to estimate freshwater turbellarian diversity in these environments, suggesting that they are efficient tools to represent turbellarian richness (Braccini & Leal-Zanchet 2013).

Species rarefaction curves for each site were built based on the accumulation of individuals (abundance) (Colwell & Elsensohn 2014), so that biases in comparisons due to differences in the abundance of some turbellarians species were corrected (Gotelli & Colwell 2001). Because the ESEC Taim has few previously published records of turbellarian species (Reyes et al. 2019, Reyes et al. 2021), we tested the performance of five non-parametric richness estimators, based on abundance (Chao 1) and incidence (Chao 2, Jacknife 1, Jacknife 2, and Bootstrap), to determine the completeness of the species inventory. Hence, richness estimators were used to understand the alpha diversity in each sampling site and considering the ESEC Taim as a whole (14 samples). Species accumulation curves and richness estimators were computed using Past 3.x (Hammer et al. 2001), performing 999 randomizations in each analysis. In addition, analysis of variance (ANOVA) was performed

to test whether richness or mean density of turbellarian differed among the sampling sites. When necessary, the data were log-transformed to meet ANOVA assumptions. In addition, a multiple regression analysis (generalized linear models [GLMs]) (McCullagh & Nelder 1989) was performed to investigate the effects of temperature and pH (independent variables) on mean density and richness (dependent variables) of turbellarians.

RESULTS

A total of 1,225 turbellarians were found in the floating vegetation microhabitat. They were identified as follows: 34 species belonging to 12 genera, distributed among 6 taxa. Tricladida had the highest relative abundance (49.4%), while Catenulida, Dalytyphloplanida, Macrostomorpha, Kalyptorhynchia, and Prorhynchida were less abundant (Table I). Triclads were represented by the most abundant species, *Girardia* sp. 1; this species was abundant at all sampling sites (Table I).

Catenulida was the second most abundant taxon with 12 species and 27.7% relative abundance. Within Catenulida, the most abundant species was *Stenostomum* sp. 1, with 14.4% relative abundance and present in all sampling sites. The remaining species within Catenulida appeared with less relative abundance (~ 0.1–7%), with *Stenostomum cryptops* and *Stenostomum* cf. *pegephilum* considered rare species (Table I).

Dalytyphloplanida contributed with 13 species and 16.2% relative abundance, being the third most abundant taxon. Within Dalytyphloplanida, the three most abundant species were *Gieysztoria* sp. 2, *Gieysztoria falx*, and *Gieysztoria duopunctata*, with 6.2%, 4.4%, and 1.6% relative abundance, respectively. The rarest species were *Gieysztoria evelinae*,

Gieysztoria sp. 1, and *Bothromesostoma evelinae* (~ 0.1% relative abundance each) (Table I).

The taxa with the lowest relative abundances were Macrostomorpha (3.8%), Kalyptorhynchia (2.7%), and Prorhynchida (0.2%). Within Macrostomorpha, *Macrostomum tuba* and *Microstomum* sp. 1, were rare species. Within Kalyptorhynchia, *Gyratrix hermaphroditus* was abundant. Regarding Prorhynchida, only two individuals were found (Table I).

The turbellarian mean density at each sampling site was 34 ± 18.4 (S1), 156.6 ± 165.9 (S2) and 160.3 ± 74.7 (S3) individuals/m^2. There were no significant differences between the three sampling sites (F = 1.48, df = 2, p > 0.05).

The turbellarian species richness in each sampling site varied from 13 to 25 (Table I) without significant differences between the three sites (F = 0.61, df = 2, p > 0.05). Rarefaction curves based on individual abundance showed that S2 and S3 are nearing their asymptote of alpha diversity, while S1 did not. S3 presented less richness than S1 and S2 (Fig. 2). These results are similar to those for the observed richness. The microhabitat at S1 showed 18 observed species against 22 predicted species by the Bootstrap estimator, representing 83% completeness; Chao 1 and Chao 2 each predicted 31 species, and thus the number of observed species represented 59% completeness. On average, 66% of turbellarian fauna were observed at S1 (Fig. 3a). The microhabitat at S2 presented 25 observed species, while Bootstrap estimated 30 species, indicating 83% completeness with respect to the observed species. Chao 2 and Jacknife 2 calculated 41 and 42 species, respectively, representing 60% completeness with respect to the observed species. On average, there was 71% completeness at S2 (Fig. 3b). In the microhabitat at S3, 13 species were found; the same richness was predicted by Chao 1, indicating 100% completeness. Jacknife 2 calculated 18 species,

Table I. Total and relative (%) abundances, and density of Turbellaria in three floating vegetation microhabitats (S1, S2 and S3) of the ESEC Taim.

| Species	Abundance				Density (indiv./m2)		
	Site 1	Site 2	Site 3	% Lumped individuals	Site 1	Site 2	Site 3
Catenulida				27.7			
Catenula lemnae	29	0	8		7.7 ± 14.4	0	2.7 ± 3.3
Stenostomum grande	11	80	3		2.9 ± 3.3	21.3 ± 36.4	1 ± 1.3
Stenostomum cryptops	0	1	0		0	0.3 ± 0.6	0
Stenostomum cf. pegephilum	0	1	0		0	0.3 ± 0.6	0
Stenostomum saliens	0	5	0		0	1.3 ± 3	0
Stenostomum paraguayense	0	3	0		0	0.8 ± 1.2	0
Stenostomum tuberculosum	0	2	0		0	0.5 ± 0.7	0
Stenostomum glandulosum	0	6	0		0	1.6 ± 3.6	0
Stenostomum sp. 1	17	136	30		4.5 ± 5.6	36.3 ± 39.5	10 ± 13
Stenostomum sp. 2	0	2	0		0	0.5 ± 0.7	0
Stenostomum sp. 3	0	2	0		0	0.5 ± 1.2	0
Myostenostomum vanderlandi	2	1	0		0.5 ± 0.7	0.3 ± 0.6	0
Macrostomorpha				3.8			
Macrostomum quiritium	9	0	8		2.4 ± 5.37	0	2.7 ± 2.9
Macrostomum itai	1	1	0		0.3 ± 0.6	0.3 ± 0.6	0
Macrostomum sp. 1	9	2	15		2.4 ± 5.4	0.5 ± 0.7	5 ± 6.9
Macrostomum tuba	1	0	0		0.3 ± 0.6	0	0
Microstomum sp. 1	1	0	0		0.3 ± 0.6	0	0
Tricladida				49.4			
Girardia sp. 1	17	196	392		4.5 ± 7.3	52.3 ± 69	130.7 ± 90.9
Dalytyphloplanida				16.2			
Gieysztoria falx	18	36	0		4.8 ± 6.6	9.6 ± 15.2	0
Gieysztoria evelinae	0	0	1		0	0	0.3 ± 0.7
Gieysztoria hymanae	0	0	7		0	0	2.3 ± 4.7
Gieysztoria hermes	3	0	0		0.8 ± 1.8	0	0
Gieysztoria duopunctata	0	15	5		0	4 ± 8.9	1.7 ± 2
Gieysztoria sp. 1	1	0	0		0.3 ± 0.6	0	0
Gieysztoria sp. 2	5	60	11		1.3 ± 2.3	16 ± 20.2	3.7 ± 7.3
Mesostoma ehrenbergii	2	2	0		0.5 ± 1.2	0.5 ± 1.2	0
Mesostoma sp. 1	0	7	0		0	1.9 ± 4.2	0
Mesostoma sp. 2	0	2	0		0	0.5 ± 1.2	0
Strongylostoma elongatum	5	0	1		1.3 ± 3	0	0.3 ± 0.7
Strongylostoma sp. 1	1	15	1		0.3 ± 0.6	4 ± 4.6	0.3 ± 0.7
Bothromesostoma evelinae	0	1	0		0	0.3 ± 0.6	0
Kalyptorhynchia				2.7			
Gyratrix hermaphroditus	3	22	7		0.8 ± 1.2	5.9 ± 11	2.3 ± 3.2
Gyratrix sp. 1	0	1	0		0	0.3 ± 0.6	0
Prorhynchida				0.2			
Prorhynchus stagnalis	0	2	0		0	0.5 ± 1.2	0
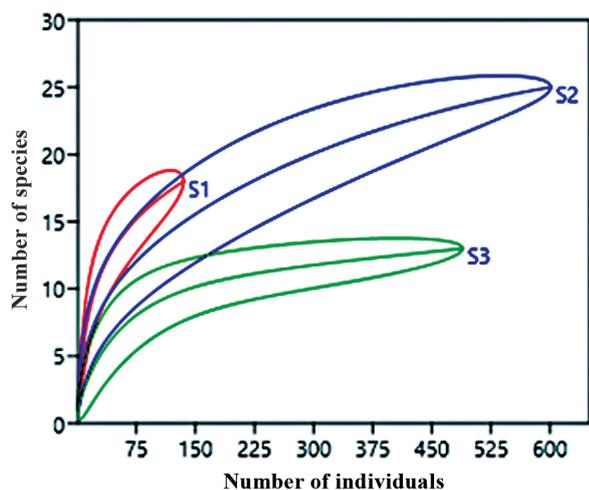
Figure 2. Species accumulation curves with their respective intervals of confidence for lake Nicola (S1), the water channel (S2), and lake Mangueira (S3).

estimating 74% completeness. On average, 85% completeness at S3 was calculated (Fig. 3c, Table II). When the analyses were performed with all samples, Chao 1 and Chao 2 estimated 76% and 61% completeness, respectively. Jacknife 1 and Jacknife 2 calculated 49 and 58 species, respectively, representing 70% and 59% completeness. Bootstrap calculated 40 species, representing 84% of completeness; this was the highest percentage of species estimated using the Bootstrap method at the ESEC Taim. Considering the five estimators, the species richness found in the present study represented an average 70% completeness of the inventory of species for this protected area (Fig. 4 and Table II).

During the study, water temperature varied from 10.6 to 26.1°C (mean ± standard deviation = 20.09 ± 5.12°C), and pH ranged from 6.5 to 8.2 (mean ± standard deviation = 7.37 ± 0.69). These abiotic factors did not affect the turbellarian richness and mean density at the ESEC Taim (Table III).

The cluster analysis grouped seven (A–G) categories, indicating that there was not a chief factor in faunal similarity, especially in S1 and S2. On the contrary, samples from S3 fell into the same category in the analysis, suggesting similar turbellarian composition (Fig. 5). Groups A, B, and C were different from the remaining groups; each was composed of one sample and had distinct assemblages with very few individuals from a few species. At a 39.7% level of similarity, group D was composed of three samples dominated by *Stenostomum* sp. 1 (42.3%). Group E comprised samples at S1, at a 45% level of similarity; the dominant species was *G. falx* (41.6 %). Similarly, group F (61.9% similarity) comprised samples of S2, in which *Girardia* sp. 1 contributed with 32.6%. At a 52.7% level of similarity, group G clustered all samples from S3; *Girardia* sp. 1 was dominant in all samples, contributing to 70% to the formation of this group (Fig. 5, Table IV). The species that contributed the most to the dissimilarities (on average 67.9%) among the groups were *Girardia* sp. 1, *G. falx*, and *Stenostomum* sp. 1 (Table V).

DISCUSSION

There have been only few studies in the southern part of the Neotropical region dealing with ecological aspects of turbellarian assemblages because individuals must be observed alive to identify diagnostic characteristics and classify them to the species level (Braccini & Leal-Zanchet 2013, Noreña et al. 2015). In our dataset, the most abundant taxon was Tricladida (*Girardia* sp. 1); within Tricladida, *Girardia* sp. 1 occurred in all the three sampling sites, more abundant in Lake Mangueira (S3). By contrast, Braccini & Leal-Zanchet (2013) found a few individuals of the genus *Girardia* in both benthic substrate and vegetation in large coastal wetlands (2–6 km) in southern Brazil. Lake Mangueira (S3) is a large coastal lake (820 km²) where the littoral zone is covered by aquatic vegetation (Meerhoff & Jeppesen 2009) and, because aquatic vegetation influences the macroinvertebrate colonization
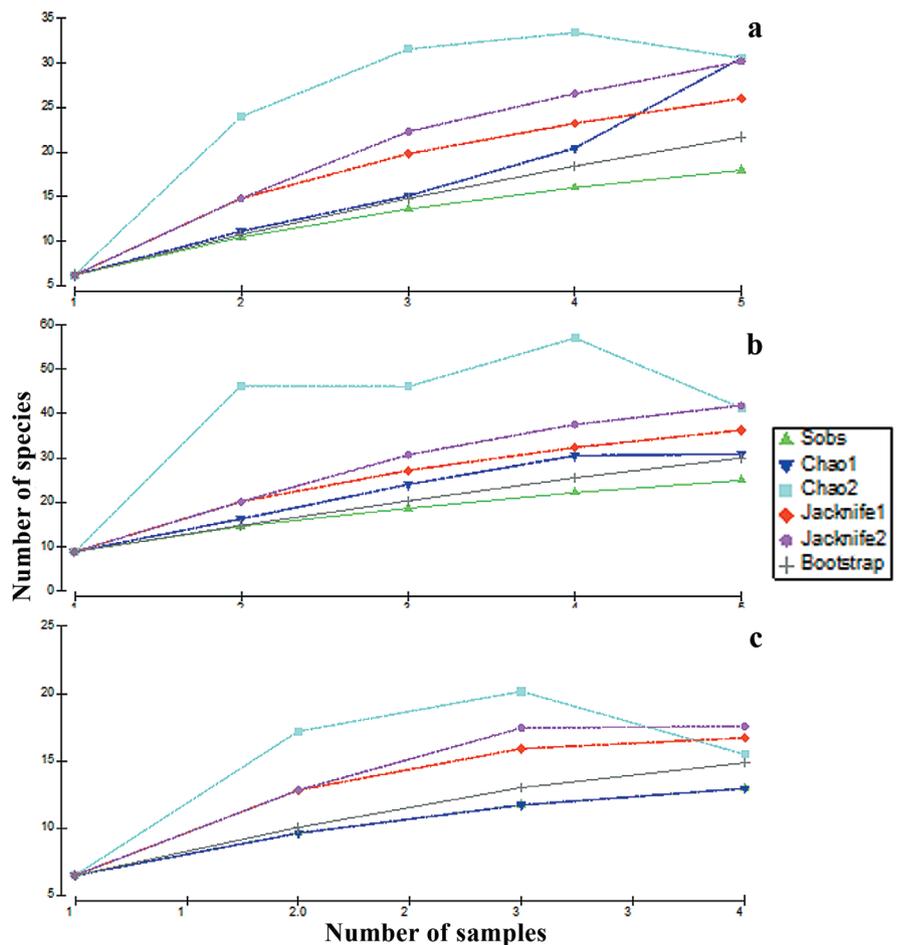
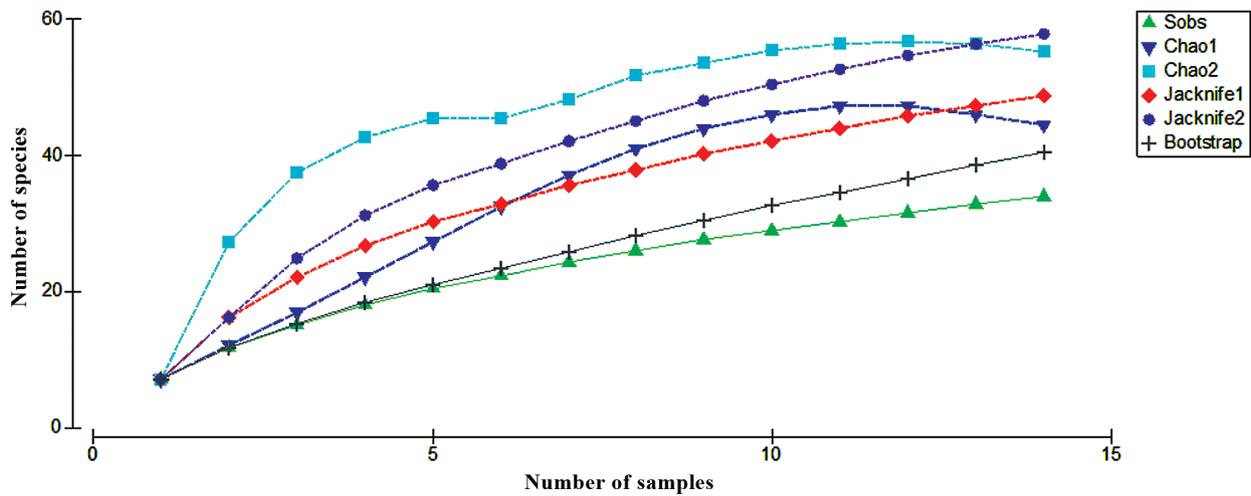**Figure 3.** Completeness of the inventory of species determined by different richness estimators at the Taim nature reserve (ESEC Taim). a, Estimations for lake Nicola (S1); b, Estimations for the water channel (S2); c, Estimations for lake Mangueira (S3) (Sobs: observed richness).

(Pinder 1995, De Szalay & Resh 2000), this could be responsible for the abundance of *Girardia* sp. 1 in S3. It is noteworthy that in the northern hemisphere the water temperature may limit the abundance of triclad species (Reynoldson 1977, Reynoldson & Young 2000, Harrath et al. 2004), while in the southern hemisphere triclads can tolerate greater thermal windows (9–31°C), thus maintaining their abundance (Lenguas-Francavilla et al. 2018). In this sense, a combination of other factors is likely to influence the abundance of triclads in southern coastal wetlands. Regarding less dominant species, such as microturbellarians, most of them are widely distributed and well-known. We found a few species that are representatives of the Neotropical region (e.g., *Gieysztoria falx*, *G. duopunctata*, *G. evelinae*, *G. hermes*, *G. hymanae*, *Stenostomum paraguayense*, and *Bothromesostoma evelinae*) as other authors reported earlier (Martín 1908, Marcus 1946, Brusa et al. 2003, Reyes et al. 2019, Reyes et al. 2021). The abundance of microturbellarians in the neotropics depends mainly on the life cycle and flooding (Noreña-Janssen 1995). Moreover, these animals take advantage of the growing expansion of aquatic vegetation on littoral areas of bodies of water, where they find protection and a suitable substratum for community support (Noreña-Janssen 1995). In addition, turbellarians are much more diverse in littoral zones that are covered with vegetation than in deeper zones (Young 2001).

Table II. Number of species expected and percentages of inventory completeness according to different richness estimators at the three sampling sites (S1, S2 and S3) and lumped samples (ESEC Taim).

	S1	S1 (%)	S2	S2 (%)	S3	S3 (%)	ESEC Taim	ESEC Taim (%)
Cumulative alfa diversity	18		25		13		34	
Chao 1	31	59	31	81	13	100	45	76
Chao 2	31	59	41	60	16	84	55	61
Jacknife 1	26	69	36	69	17	78	49	70
Jacknife 2	30	60	42	60	18	74	58	59
Bootstrap	22	83	30	83	15	87	40	84
Average of inventory completeness		66		71		85		70

Figure 4. Completeness of the inventory of the species determined by different richness estimators (using lumped samples) at the Taim nature reserve (ESEC Taim) (Sobs: observed richness).

Aquatic environments like the ones at the ESEC Taim are highly variable systems due to the presence of macrophytes, the abundance of detritus, and water hydrodynamics (Würdig et al. 2007, Marques et al. 2013). In the present study, there were no significant differences in richness, abundance, and mean density between sampling sites. The rarefaction curves showed that Lake Nicola (S1) did not reach its asymptote and could have an incomplete inventory of species. On the other hand, the richness estimators (66% completeness) predicted 27 species in S1, which would indicate that this site has the second highest diversity at the ESEC Taim. In fact, the S1

environment is highly diverse in microhabitats and presents notable water fluctuations (Würdig et al. 2007). Braccini & Leal-Zanchet (2013) also reported that large (2–24 km perimeter) wetlands have lower estimated richness of turbellarian species. If we follow the considerations of Braccini & Leal-Zanchet (2013), Lake Nicola (S1) could be considered a large body of water (2.58 km^2). Thus, a low diversity of turbellarian species would be expected in Lake Nicola, contrary to the estimated results of the rarefaction curves. In this sense, our results could represent the real alpha diversity at S1 without a bias in the sampling effort.

Table III. Summary results of a GLM - multiple regression analysis, testing the relationship between richness and mean density of freshwater turbellarians as a function of temperature and pH (physical variables).

Physical variables	richness					Density				
	r2	Df	MS	F	p	r2	Df	MS	F	p
pH	5.057	1	5.057	0.241	0.634	140.900	1	140.900	0.010	0.922
Temperature	16.805	1	16.805	0.801	0.392	29489.639	1	29489.639	2.116	0.176
Error	209.846	10	20.985			139376.223	10	13937.622		

Both S2 and S3 reached their asymptotes in the rarefaction curves. For the water channel (S2), estimators calculated an average of 71% completeness, showing that this site is expected to have 35 species. This would indicate that this site has the highest diversity at the ESEC Taim. In addition, the water channel (S2) could be seasonally interrupted along its littoral surface by marsh or rooted vegetation, with these plants delimiting small 'ponds'. This feature is considered an important factor for diversification and generation of diversity and density (Pinder 1995, Panatta et al. 2006, Thomaz et al. 2008), because small ponds may harbor higher species diversity than larger and less isolated waterbodies (Scheffer et al. 2006, Braccini & Leal-Zanchet 2013).

As mentioned above, the rarefaction curve of Lake Mangueira (S3) reached its asymptote with fewer species and individuals; this indicates that diversity at S3 is low. Similarly, on average, estimators computed 85% completeness over the four sampling campaigns. Of note, Chao 1, an estimator that considers abundance, computed 100% completeness for S3. Thus, it would be expected that no more species would be recorded in new surveys. These results are also in agreement with those of Braccini & Leal-Zanchet (2013), who indicated that larger wetlands present lower estimated richness of turbellarian species. So, this principle could be observed in larger coastal lakes such as S3. When all sites were gathered for analysis, the estimators predicted 70% (on average)

completeness of the species inventory. This result indicates that the collected samples represent most of the turbellarian fauna at the ESEC Taim if we consider that S1, S2, and S3 are different habitats with different factors affecting the development of the communities of the species (Würdig et al. 2007).

The water temperature varied from 10.6 to 24.1°C and the pH varied from 6.5 to 8.2 between the three sampling sites. These factors did not affect the richness and mean density of turbellarians. The main factors that influence turbellarian distribution are oxygen content and availability of food (Noreña et al. 2015); other factors such as temperature and water regime could influence reproduction of turbellarians (laying eggs) (Young 2001, Noreña et al. 2015). Moreover, several microturbellarians can tolerate ecological changes and temperature variation (Young 1976, Gamo García 1987, Noreña et al. 2006). Turbellarians can tolerate relatively wide pH ranges (Young 2001, Noreña et al. 2004). The richness, abundance and species composition of turbellarians in South America is likely to be more closely related to geographic location, types of microhabitat and seasonal variations. These characteristics are associated with the availability of food and other abiotic and biotic factors that would shape the communities of wetlands (Balsamo et al. 2020). Thus, new samples measuring oxygen, type of microhabitat, seasonality and/or food items would be necessary to understand whether
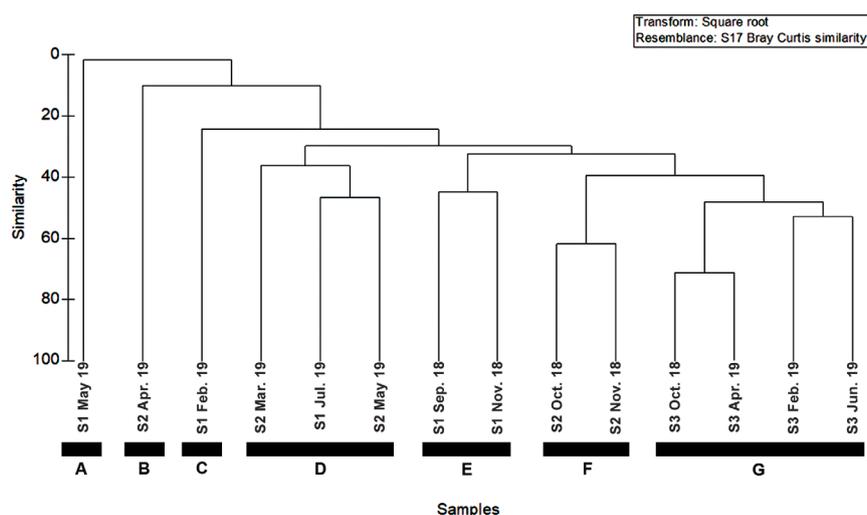
Figure 5. Cluster diagram by group-average clustering based on the Bray-Curtis similarity index using the relative abundance of species.

turbellarian communities could be influenced by these factors at the ESEC Taim.

The cluster analysis revealed that the turbellarian community at Lake Nicola (S1) and the water channel (S2) was relatively similar, while the community at Lake Mangueira (S3) was constant and notably different. This could be explained by the fact that S1 and S2 are relatively close to each other and share similar detritus load and aquatic macrophytes (Würdig et al. 2007). Besides, aquatic macrophytes generate habitat complexity that affects species richness and abundance (Bell et al. 1991, Jeffries 1993, Thomaz et al. 2008). Thus, S1 and S2 would be expected to have a similar turbellarian community. Moreover, water diffusion between these two bodies of water could contribute to the sharing of species and connectivity (Würdig et al. 2007).

Interestingly, group G clustered all samples from Lake Mangueira (S3); this finding suggests that the turbellarian community at this lake was mostly the same along the sampling campaigns. This result can be explained because Lake Mangueira has its own contributing basin (hydrological South subsystem) (Würdig et al. 2007), so this lake could have its own community composition. Moreover, turbellarian

assemblages in larger bodies of water are known to have low species diversity (Braccini & Leal-Zanchet 2013). Because S3 presents a well-established vertebrate fauna such as fish communities (Garcia et al. 2006b, Correa et

Table IV. Percentage contribution of typifying taxa to within-group similarity for the identified turbellarian assemblages by cluster analysis (cut off for low contributions: 90%). The first two species with the greatest contribution are shown.

Group D Average similarity: 39.74	Average Abundance	Contribution (%)
Species		
Stenostomum sp. 1	3.35	42.29
Girardia sp. 1	1.76	24.12
Group E Average similarity: 45.02		
Species		
Gieysztoria falx	3.46	41.56
Catenula lemnae	1.57	14.1
Group F Average similarity: 61.86		
Species		
Girardia sp. 1	11.27	32.6
Stenostomum sp. 1	8.7	23.18
Group G Average similarity: 52.70		
Species		
Girardia sp. 1	10.85	70.32
Stenostomum sp. 1	2.72	13.43

Table V. Dissimilarity values among clusters of sampling campaigns (Bray-Curtis coefficient was used as a measure of similarity). The first three species with the greatest contribution are shown.

SIMPER			
Pairwise comparison	**Average dissimilarity (%)**	**Species**	**Contribution (%)**
D-F	72.3	*Girardia* sp. 1	18.81
		Stenostomum sp. 1	10.69
		Gieysztoria falx	9.26
D-G	68.1	*Girardia* sp. 1	35.28
		Gieysztoria sp. 2	8.55
		Macrostomum sp. 1	6.45
E-F	65.5	*Girardia* sp. 1	19.85
		Stenostomum sp. 1	13.8
		Stenostomum grande	12.42
E-G	68.6	*Girardia* sp. 1	32.82
		Gieysztoria falx	12.86
		Gieysztoria sp. 2	6.63
E-D	72.1	*Gieysztoria falx*	14.16
		Gieysztoria sp. 2	10.9
		Girardia sp. 1	8.56
F-G	60.6	*Stenostomum* sp. 1	13.46
		Stenostomum grande	12.77
		Gieysztoria falx	10.29

al. 2011), these vertebrates are likely to shape the invertebrate communities, influencing abundance and species richness, as it was stated for other shallow lakes (Scheffer et al. 2006, Meerhoff & Jeppesen 2009).

Girardia sp. 1, *G. falx*, and *Stenostomum* sp. 1 contributed the most to the dissimilarity between S1, S2, and S3. Differences based on these three species should be interpreted with caution because two species were recognized according to appreciable morphological structures. The genus *Stenostomum* is very diverse and difficult to classify taxonomically (Larsson & Willems 2010), while *Girardia* is less diverse (Carbayo & Froehlich 2008), but there can be sibling species. This uncertainty could generate misinterpretations in determining the abundance and diversity of turbellarians at different sites. Thus, analyses using (meta) barcoding as an additional identification tool are highly recommended for ecological and biodiversity studies that would help us to detect sibling species that would otherwise be difficult to identify (Balsamo et al. 2020, Tessens et al. 2021). The present analysis of the turbellarian community on floating vegetation microhabitats provides an overview that is useful in understanding the dynamics of ecological systems in wetlands in southern Brazil, particularly inside the ESEC Taim nature reserve. Thus, this study represents the first contribution to the knowledge of the diversity and assemblage structure of this scarce-studied group of invertebrates in a protected area in southern Brazil. Therefore, a continuous monitoring of turbellarians in the following years will be valuable to gain a better understanding of how this ecosystem behaves and develops with respect to these free-living flatworms.

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