

Alpha and beta diversity of phytoplankton in two subtropical eutrophic streams in southern Brazil

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ABSTRACT

This paper reports the alpha and beta diversity of phytoplankton communities of two streams differing in land use. We analyzed which environmental conditions affect diversity of the phytoplankton communities and tested the hypothesis that stream stretches protected by forests will have lower species richness, whereas higher beta-diversity would occur among the non-protected stretches. Samples were taken quarterly, from February to November 2012, in nine stretches, including four non-protected stretches, two partially protected stretches and three totally forest-protected stretches. Eleven abiotic variables and their coefficients of variation were analyzed. Phytoplankton was analyzed for species richness (alpha diversity), frequency of occurrence and beta-diversity. Species richness was calculated by first and second order jackknife indexes. Biotic data were submitted to Sørensen similarity analysis. A total of 429 infrageneric taxa were reported, representing 88% of the estimated expected species richness. The phytoplankton composition was typical for lotic environments with high richness values of Bacillariophyceae (66.0%). High phytoplankton richness values occurred in all the sampled stretches, even though beta-diversity was moderate and indicated similarity between the sampled stretches, therefore only a part of the original hypothesis was corroborated.

Keywords: Diatomaceae, phytoplankton community, Preservation Unit, taxonomical composition, western region of the state of Santa Catarina

Introduction

Phytoplankton communities consist of photoautotrophic microorganisms (Reynolds 1984) suspended in aquatic environments that move passively due to the movements of winds and currents. Phytoplankton comprise a great diversity of species of a wide variety of forms and sizes, each with its own unique physiological requirements (Reynolds 2006). Information on phytoplankton communities (such as composition, structure, and spatial and temporal variability) in continental water ecosystems can elucidate the functioning of, and the energy flow within, these environments (Rodrigues 2004). Since phytoplankton communities respond rapidly to a wide variety of environmental disturbances (Cottingham & Carpenter 1998; Lepistö *et al.* 2004; Paerl & Huisman 2009) and present a high diversity of species in natural conditions (Hutchinson 1961; 1976; Reynolds 1984; 2006), they can serve as important indicators of environmental disarray.

River algae are controlled by different factors than phytoplankton in lentic habitats, such as physical (light,

temperature), chemical (gases, inorganic nutrients, ions) and biotic (herbivory) variables, and their responses, in contrast to species in lentic waters, are mainly affected by river flow. Anthropogenic activities (disposal of organic wastes and removal of riparian vegetation) may cause significant changes to phytoplankton communities (Leland & Porter 2000; Lewis *et al.* 2001; Munn *et al.* 2002). In fact, species richness may be the simplest method to assess and quantify the complexity of a given environment (Nabout *et al.* 2007). Since it is coupled to taxonomic composition, species richness provides a measure of the main components of biological diversity for characterizing an ecosystem and, thus, is important for defining preservation strategies (Nogueira *et al.* 2008). Consequently, species diversity is a quality of biological communities that is deeply related to stability, productivity, trophic structure and migratory processes (Stirling & Wilsey 2001).

Alpha-diversity and beta-diversity have been employed to determine patterns of species diversity (Whittaker 1972; Legendre *et al.* 2005), where α -diversity is defined as the local diversity (within the community) and thus a meas-

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ure of richness, whereas γ -diversity (regional diversity) represents the total number of sampled species (Whittaker 1972). Beta-diversity encompasses the differences in species composition of different sites. It is thus a simple method to characterize species heterogeneity of a given region (Whittaker 1972; Gering *et al.* 2003). In contrast to species richness (alpha-diversity), beta-diversity tests hypotheses about the patterns of distribution of species biodiversity among different places (Baselga 2010).

There have been very few studies on phytoplankton beta-diversity in Brazil. Of these, the studies of Huszar *et al.* (1990), Nabout *et al.* (2007), Nogueira *et al.* (2008), Nogueira *et al.* (2010) and Pires (2014) should be highlighted, although these studies only evaluated alpha- and beta-diversity in lentic environments.

The current paper assesses variation in alpha- and beta-diversity of species of phytoplankton in two streams with different land use and levels of occupation, and analyzes whether changes, such as in environmental conditions, affect phytoplankton diversity. Specifically, this paper tests the hypothesis that stream stretches protected by the forest will have lower species richness, whereas non-protected stretches will have higher beta-diversity. The influence of physical and chemical variables of the water on the components of alpha- and beta-diversity of algae will also be tested.

Materials and methods

The hydrographical region under study lies in the western region of the state of Santa Catarina, Brazil, on the border between the municipalities of Chapecó and Guatambu, and comprises an area of the most salient agricultural activities in the state. The area can be divided into two hydrographic microbasins, namely the Tigre River and the Retiro Stream. The cultivation of bean, soybean and corn, and the breeding of swine, broilers and livestock, on small farms are the main agricultural activities in these two micro basins.

The microbasin of the Tigre River has almost 20% of its area covered by the preservation unit 'Floresta Nacional de Chapecó' (FLONA), Chapecó, SC, Brazil. Six sampling sites (P1 to P6) were selected throughout the length of the Tigre River. The first two sites (P1 and P2) are not protected by forest and lie upstream in an area subjected to human impacts. P3 lies in an area of transition between forest and the agricultural area upstream, whereas sites P4, P5 and P6 lie within the forest and are protected by it. The Retiro Stream only borders the forest and has three sampling sites: P7 is upstream of the forest, and P8 and P9 have only one of their margins protected by the forest (Fig. 1). Table 1 provides the geographic sites on the stretches analyzed, and the characteristics of each site.

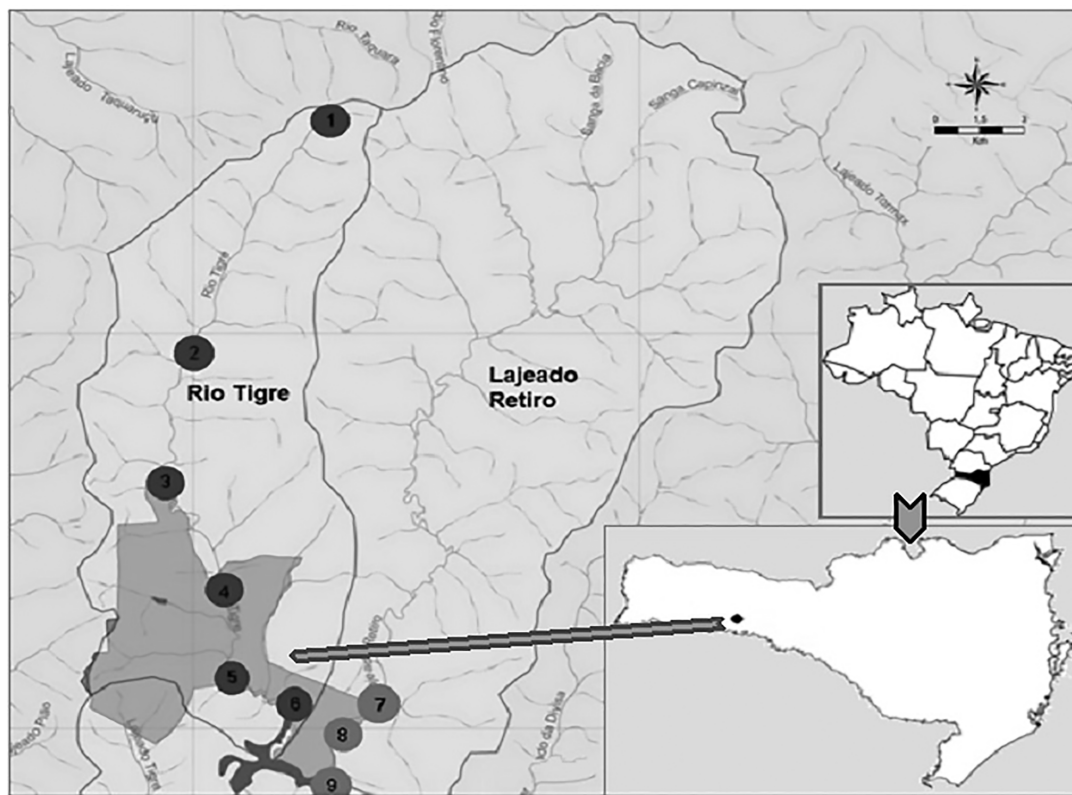


Figure 1. Sampling sites on the river Tigre and stream Retiro in the western region of the state of Santa Catarina, Brazil, between February and November 2012. Source: ICMBio, 2013.

Table 1. General characteristics of the sampled sites. Localization by geographic coordinates, altitude, mean depth, mean width of the streams and discharge (m³/s) in the stretches under analysis.

Stream	Identification of the sampled site	Geographic coordinates	Altitude (m)	Mean depth (m)	Mean width (m)	Discharge (m ³ .s ⁻¹)	Order stream	Additional observations
Tigre	P1	27°01'07"S/ 52°45'46"W	613	0.2	3.0	0.12	1 ^a	Small strip of riparian vegetation, adjacent areas with corn crops.
	P2	27°02'50"S/ 52°46'37"W	586	0.2	2.7	0.36	3 ^a	Strip of non-extant riparian vegetation; adjacent areas planted with eucalyptus and mate trees; marked erosion process.
	P3	27°04'11"S/ 52°46'50"W	583	0.3	4.8	0.37	3 ^a	Small strip of riparian vegetation; transition area between pastureland and preserved forest.
	P4	27°05'39"S/ 52°46'10"W	560	0.4	4.8	0.50	3 ^a	Preserved riparian vegetation, area protected by the forest
	P5	27°06'05"S/ 52°46'12"W	545	0.3	4.9	0.57	3 ^a	Preserved riparian vegetation, area protected by the forest
	P6	27°06'37"S/ 52°45'29"W	539	0.3	9.5	0.59	4 ^a	Preserved riparian vegetation, area protected by the forest, low water velocity; river's mouth.
	P7	27°05'05"S/ 52°43'55"W	560	1.2	8.5	0.29	4 ^a	Lack of riparian vegetation, modified river course with small barrages.
Retiro	P8	27°06'46"S/ 52°45'01"W	542	0.3	8.3	1.17	4 ^a	Left margin protected by the forest; right margin with a small strip of riparian vegetation; adjacent area with crops; stretch with small rapids
	P9	27°07'17"S/ 52°45'06"W	540	1.2	6.8	1.26	4 ^a	Left margin protected by the forest; adjacent areas with pastureland, erosion processes; river's mouth

Sampling

Sub-surface samplings were made at the nine sampling sites during February (summer), May (autumn), August (winter) and November (spring) of 2012. Data on latitude, longitude, elevation (GPS - Garmin Etrex H), river width and depth (measuring tape), and river discharge (floating method, EPA 1997) were recorded at each sampling site.

Samples were collected by successive horizontal hauls with 20 µm mesh nets, and fixed with 4% formaldehyde solution analysis of species richness. Species were identified using an optical microscope with magnifications of 400x and 1000x. Eight slides per sample were analyzed, for a total of 32 slides per sampling site. Classification followed: Round (1971) for classes of Chlorophyta; Komárek & Anagnostidis (1989; 1998; 2005) and Hoffmam *et al.* (2005) for Cyanobacteria; and Hoek *et al.* (1995) for the other classes. Identification to genus and species were performed using the specialized works of Komárek & Fott (1983), Sant'Anna (1984), Nogueira (1991), Comas (1996), Godinho (2009), Godinho *et al.* (2010), Rodrigues *et al.* (2010), Rosini *et al.* (2012; 2013a), and Ramos *et al.* (2012) for green algae; Hüber-Pestalozzi (1955), Tell & Conforti (1986), and Menezes (1994) for Euglenophyceae; Castro *et al.* (1991), and Menezes (1994) for Cryptophyceae; Komár-

ková-Legnerová & Cronberg (1994), Azevedo *et al.* (1996), Azevedo & Sant'Anna (1999; 2003), Komárek & Azevedo (2000), Sant'Anna *et al.* (2004), and Rosini *et al.* (2013b) for Cyanobacteria; Krammer & Lange-Bertalot (1986; 1988; 1991), Metzeltin & Lange-Bertalot (1998; 2007), and Metzeltin *et al.* (2005) for Bacillariophyceae; Sant'Anna *et al.* (1989), Ferragut *et al.* (2005), Tucci *et al.* (2006), and Sant'Anna *et al.* (2012) for the overall community. Updating of the taxonomy followed An *et al.* (1999), Hegewald (1997; 2000), Hegewald & Hanagata (2000), Hegewald & Wolf (2003), Krienitz *et al.* (2003), Buchheim *et al.* (2005), and Krienitz & Bock (2012). Samples were deposited in the herbarium of the Universidade Comunitária Regional de Chapecó.

Abiotic variables, such as water temperature and dissolved oxygen (oximeter HANNA HI 9146), were also measured. An aliquot of water was retrieved for measuring pH (digital pHmeter Thermo Scientific Orion 4 Start), electrical conductivity and total dissolved solids (multi-parameter water quality monitoring system, Orion), turbidity (turbidity meter Hach 2100N), total alkalinity (titration meter method), total nitrogen (APHA 2012), total phosphorus (Goltermam *et al.* 1978), biochemical oxygen demand (BOD respiration meter method) and chlorophyll-a (APHA 2012).

Data analysis

Means, standard deviations and amplitudes were calculated for the physical and chemical variables of the water from the seasonal samplings at each collection site. The trophic level of the site was analyzed for phosphorus and chlorophyll-a concentrations, following Lampareli (2004). One-way analysis of variance (ANOVA) was used to evaluate differences among sampling sites with each sampling site being treated as an independent variable. The physical and chemical variables were treated as the response variables with each seasonal sampling as a replication for the sampling sites. Physical and chemical variables with significant differences ($p < 0.05$) were correlated with alpha- and beta-diversity.

The species richness of each sampling site (alpha-diversity) was determined from the number of species collected throughout the entire study period, taking into consideration data from qualitative analysis, and was estimated by first and second order jackknife indexes (Nabout *et al.* 2007) with StimatS (Colwell 2006). Occurrence frequencies were categorized as: constant for species found in more than 50% of the collections; common when found between 25% and 50% of the collections; and accidental or rare species when found in less than 25% of the collections (Lobo & Leighton 1986).

The β -1-diversity index (Harrison *et al.* 1992) measures the amount that regional diversity exceeds mean alpha-diversity, and is calculated by the formula $\beta-1 = [(S/\alpha_{\text{mean}}) - 1] / [N - 1] \times 100$, where S is the regional diversity or total richness (the number of species per each sampling site); α_{mean} is the mean alpha diversity (mean number of species) for each site in each period; N is the number of sites of the period.

The β -1 index varies between 0 and 100, with 100 indicating a set of dissimilar sampling sites and 0 indicating a set of totally similar sampling sites. Beta-diversity over 50% indicates high heterogeneity in phytoplankton composition among systems; between 20 and 50% indicates intermediate heterogeneity; and below 20% indicates low heterogeneity (Harrison *et al.* 1992; Nabout *et al.* 2007).

Floral similarity between sites was measured by Sørensen's similarity index (Magurran 1988). Similarity in phytoplankton composition among sites was determined using cluster analyses with Sørensen's distance, employing the UPGMA-type method (mean of groups). Analysis was performed using PC-ORD (McCune & Mefford 1999) and the reliability of the dendrogram was assessed by the coefficient of the co-phenetic co-relationship with PAST (Hammer *et al.* 2001). This analysis was employed to increase the reliability of the conclusions drawn from the interpretation of the dendrogram (Kopp *et al.* 2007).

Results

Phosphorus concentrations for the Tigre River (mean $0.2 \text{ mg.L}^{-1} (\pm 0.1)$) and for the Retiro Stream ($0.3 \pm 0.2 \text{ mg.L}^{-1}$) classified the sampling stretches of P1, P2, P3, P4, P5, P7

and P8 as eutrophic and P6 and P9 as super-eutrophic, with no significant difference between the sites ($p > 0.05$). However, analysis of the concentration of chlorophyll-a in the stretches showed that P2 ($7.4 \text{ } \mu\text{g.L}^{-1}$), P6 ($7.7 \text{ } \mu\text{g.L}^{-1}$), P8 ($6.7 \text{ } \mu\text{g.L}^{-1}$) and P9 ($21.1 \text{ } \mu\text{g.L}^{-1}$) were super-eutrophic (Tab. 2). All the sampling sites had a pH close to neutral and were well oxygenated, (between 5.5 and 9.8 mg.L^{-1}). Turbidity for the stretches of the Retiro Stream were higher than those for the Tigre River ($F_{(2,33)} = 9.6964$; $p = 0.0005$). Rates for Electrical conductivity, total dissolved solids and total alkalinity in the upstream sections of the rivers were higher than those in sections further downstream (river mouth) (Tab. 2), and the differences among sites in total alkalinity were significant ($F_{(2,33)} = 4.5393$; $p = 0.0181$). The total number of infrageneric taxa of the phytoplankton communities of both rivers was 429. This amount corresponded to 88% of the estimated expected richness (Jackknife 1=491 sp; Jackknife 2=501 sp) (Fig. 2). The phytoplankton community comprised nine classes among which the most represented were Bacillariophyceae (283 taxa) Chlorophyceae (47 taxa), Zygnemaphyceae (36 taxa), Cyanophyceae (35 taxa), followed by Euglenophyceae (17 taxa), Xanthophyceae (five taxa), Chrysophyceae (three taxa), Dinophyceae (two taxa) and Chryptophyceae (one taxon) (Fig. 3). Bacillariophyceae had the greatest richness at all sampling sites.

Fifty-nine out of the 429 taxa had constant occurrence (relative occurrence frequency - FR% above 51%); 91 taxa were common (between 25 and 50%) and 279 taxa were rare (lower than 24%). Further, 96% of the 59 taxa with constant occurrence were specimens of the class Bacillariophyceae, whereas the others (4%) comprised the species *Pediastrum duplex* var. *duplex* and *Desmodesmus communis*, both of the class Chlorophyceae. Of Cyanophyceae, the greatest occurrence was *Microcystis aeruginosa* (42%), a potentially toxin-producing species. The Bacillariophyceae comprised *Amphipleura chiapasensis*, *Cymbella aspera*, *Melosira varians*, *Navicula cryptocephala*, *N. rostellata*, *N. simulata*, *N. germainii*, *Nitzschia palea*, *N. recta*, *Ulnaria ulna*, *Achnantheidium minutissimum*, *Aulacoseira pusilla*, *A. calypsi*, *Gyrosigma scalpoides*, *G. acuminatum*, *G. nodiferum*, *Luticola acidoclinata* and *Gomphonema parvulum*, which occurred in at least 80% of the collected samples.

Species richness (alpha-diversity) ranged between 248 taxa at P1 and 147 taxa at P3 (Fig. 4), with a decreasing gradient in the number of taxa from the river source to its mouth in both rivers. The beta-diversity index was applied to quantify renewal or substitution of the species among sites. In fact, the β -1 diversity index for the period under analysis ranged between 23.8 (P1 and P7) and 38.9 (P9) (Fig. 4) and indicated an intermediate level of similarity among the sampled environments.

Sørensen's similarity analysis revealed two groups of taxa (Fig. 5), with a similarity of 60%: sampling sites P1, P2 and P7 were the most similar in phytoplankton composition, followed by P3, P4 and P5. Sites P8, P6 and P9 were dis-

Table 2. Means, standard deviation (in brackets) and amplitude of physical and chemical characteristics of stream water measured at the subsurface of each sampled site throughout the four sampled periods. Water T = Water temperature; pH = hydrogenionic potential; DO = dissolved oxygen; BOD = Biochemical Oxygen Demand; TN = total nitrogen; TP = total phosphorus; EC = Electrical conductivity; Talk = total alkalinity; TDS = total dissolved solids; Turb = turbidity.

Physical and chemical characteristics	P1	P2	P3	P4	P5	P6	P7	P8	P9
Water T (°C)	21.1 (±3.2)	18.5(±3.8)	19.8(±3.9)	18.2 (±3.1)	18.3 (±3.7)	21.0 (±4.3)	20.7 (±4.7)	19.6 (±3.1)	20.3 (±4.7)
	17.6-25.0	15.0-22.6	14.5-23.7	14.4-22.0	13.9-23.0	17.7-27.0	14.8-26.0	15.5-23.0	15.0-26.0
pH	7.3(±0.1)	7.2 (±0.1)	7.2 (±0.1)	7.0 (±0.06)	7.1 (±0.1)	7.3 (±0.5)	6.9(±0.03)	7.2 (±0.1)	7.7 (±0.9)
	7.2-7.5	7.1-7.3	7.1-7.4	6.9-7.1	6.9-7.1	7.0-8.0	6.8-6.9	7.1-7.4	7.1-9.2
DO (mg.L ⁻¹)	7.6 (±1.5)	6.9 (±0.7)	7.4 (±0.6)	6.93 (±0.4)	7.0 (±0.8)	7.7 (±0.6)	6.5 (±0.8)	6.9 (±0.4)	8.03 (±0.4)
	6.4-9.8	5.5-7.4	6.6-7.9	6.4-7.4	6.2-8.1	6.7-8.2	5.7-7.7	6.6-7.6	7.5-8.4
BOD (mg.L ⁻¹)	1.9 (±1.1)	1.3 (±1.0)	1.6 (±1.2)	2.0 (±1.4)	2.8 (±0.7)	1.7 (±1.6)	1.2 (±1.2)	3.1 (±1.7)	2.0 (±1.7)
	0.7-2.9	0.6-2.4	0.3-2.5	0.6-3.4	2.0-3.3	0.0-3.3	0.0-2.5	0.9-5.1	0.3-3.5
TN (mg.L ⁻¹)	0.6 (±0.8)	0.3 (±0.1)	1.1 (±1.7)	0.7 (±0.8)	0.5 (±0.6)	0.7 (±0.8)	2.4 (±3.7)	1.2 (±0.7)	1.0 (±0.6)
	0.0-1.7	0.2-0.4	0.0-3.7	0.0-1.5	0.0-1.4	0.1-1.9	0.3-8.0	0.5-2.1	0.4-1.5
TP (mg.L ⁻¹)	0.2 (±0.1)	0.2 (±0.1)	0.2 (±0.1)	0.2 (±0.1)	0.1 (±0.1)	0.3 (±0.5)	0.3 (±0.4)	0.2 (±0.2)	0.3 (±0.4)
	0.06-0.43	0.06-0.25	0.08-0.33	0.08-0.43	0.00-0.25	0.00-1.02	0.06-0.83	0.08-0.54	0.06-0.97
EC (µS.cm ⁻¹)	83.6 (±18.2)	66.5 (±17.1)	64.0 (±18.6)	61.2 (±16.7)	58.3 (±15.4)	43.4 (±4.3)	65.2 (±26.6)	64.0 (±14.5)	54.8 (±17.1)
	68.0-106.7	48.6-86.7	46.2-86.7	46.9-83.0	45.8-78.2	38.5-48.5	50.1-105.0	49.1-80.5	39.8-79.5
TDS (mg.L ⁻¹)	39.7 (±9.0)	31.5 (±8.0)	30.5 (±8.6)	28.7 (±7.9)	27.7 (±7.3)	21.0 (±2.1)	30.7 (±12.8)	31.0 (±8.1)	25.7 (±7.8)
	32.0-51.0	23.0-41.0	22.0-41.0	22.0-39.0	22.0-37.0	18.0-23.0	23.0-50.0	23.0-41.0	19.0-37.0
Turb (NTU)	7.8 (±3.2)	9.6 (±3.9)	7.6 (±0.6)	7.56 (±1.3)	10.6 (±1.4)	14.5 (±5.1)	22.6 (±4.4)	18.2 (±10.7)	14.1 (±4.6)
	3.2-10.6	6.4-15.3	6.8-8.2	6.1-8.9	8.6-11.8	8.3-20.1	18.6-27.3	10.8-34.2	10.7-20.8
Talk (mg.L ⁻¹ CaCO ₃)	27.3 (±4.4)	22.3 (±4.1)	19.5 (±2.5)	18.1 (±2.5)	17.0 (±2.6)	17.7 (±4.1)	17.8 (±2.7)	17.3 (±4.1)	15.5 (±2.6)
	22.0-32.8	18.7-28.3	17.3-22.8	15.5-20.4	14.5-19.4	15.0-23.7	15.5-21.6	14.0-22.7	13.0-19.0
Chlorophyll a (µg.L ⁻¹)	1.1 (±2.2)	7.4 (±8.3)	0.1 (±0.2)	0.2 (±0.3)	0.1 (±0.3)	7.7 (±12.0)	1.9 (±1.5)	6.7 (±5.0)	21.1 (±3.2)
	0.0-4.53	0.0-16.4	0.0-0.5	0.0-0.7	0.0-0.6	0.0-25.5	0.0-3.8	2.7-13.3	0.2-29.9

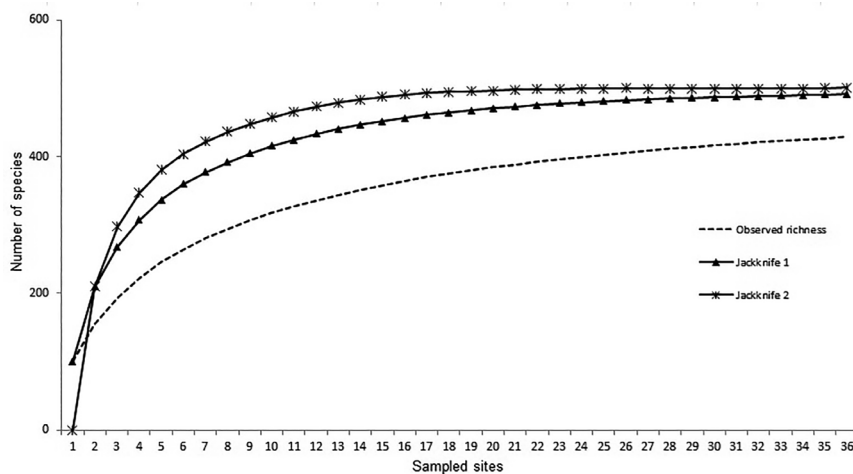


Figure 2. Species rarefaction curve for the 36 sampled sites between February and November 2012 on the streams Tigre and Retiro, western Santa Catarina, Brazil.

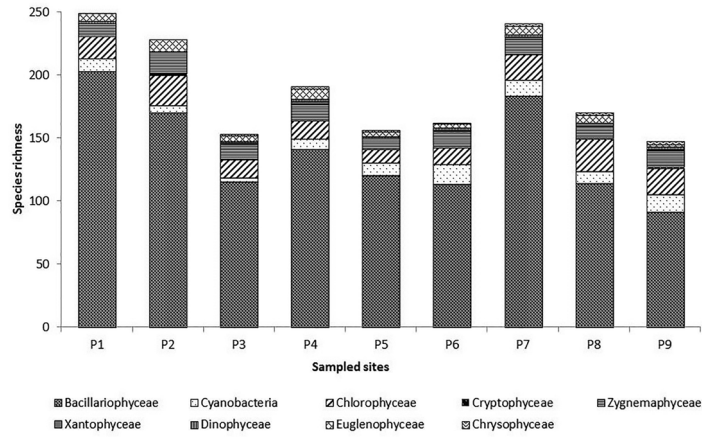


Figure 3. Number of species per sampling sites during the study period (gamma diversity), distributed in taxonomic classes in each sampled river stretch.

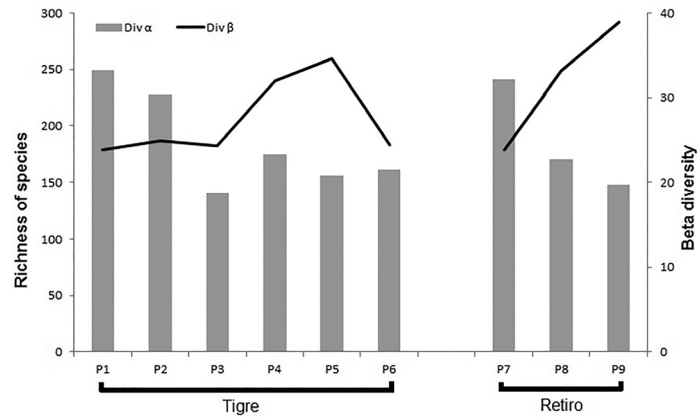


Figure 4. Species richness (alpha diversity) and beta diversity for each sampling site on the streams Tigre and Retiro, western Santa Catarina, Brazil, between February and November 2012.

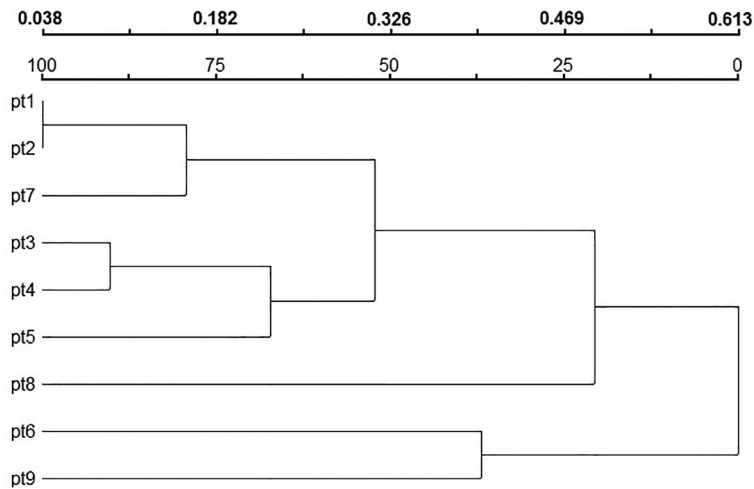


Figure 5. Dendrogram of Sørensen coefficient of similarity based on the floristic inventory of each sampling site. Co-phenetic Coefficient (r) = 0.86. pt 1 = site 1; pt2 = site 2; pt3 = site 3; pt4 = site 4; pt5 = site 5; pt6 = site 6; pt7 = site 7; pt8 = site 8; pt9 = site 9.

similar to all the other sites. However, similarity coefficients at the sites ranged between 0.038 and 0.613, which revealed high flora similarity. Since the phytoplankton composition among the sites was very similar, it reinforced the low heterogeneity found for beta-diversity among the stretches. The co-phenetic coefficient rate was $r = 0.86$.

Discussion

As a whole, the hydrographical region exhibited high phytoplankton richness with 429 registered taxa or rather, 88% of the expected richness. These results show that the sampling effort was successful at representing the richness present and in indicating consistent patterns of diversity. Although rarely employed in studies on phytoplankton, the jackknife indexes of the first and second orders (Nabout *et al.* 2007; Nogueira *et al.* 2008) were an important tool for assessing the efficacy of the sampling effort. It should be emphasized that closeness between the observed and the estimated expected species richness indicates that the sampling effort was adequate to reveal the diversity of phytoplankton in the two study rivers (Nogueira *et al.* 2010).

According to Vannote *et al.* (1980), a hypothetical longitudinal profile can be identified in rivers in which it is expected that there would be a lower amount of suspended substances and a lower nutrient load in the stretches closer to a river's source. The conditions of the two study-rivers were far from this prediction. The highest rates for electrical conductivity, total alkalinity and total dissolved solids were reported in the stretches closest to the source, whereas phosphorus load did not vary among all of the sampling sites. This fact indicates that, regardless of a stretch being protected or not by the forest, exposure to human interference occurs at some level. The environment's trophic level may have contributed to the high richness of algae along the rivers since phosphorus is usually a limiting resource for primary productivity (Ternus *et al.* 2011). The de-characterization of the longitudinal profile may be attributed to land use and occupation in the areas close to the sources of the two rivers, where crop cultivation and intensive animal breeding predominate and the streams are not protected by riparian vegetation. The current investigation shows the importance of surface drainage in contributing electrolytes, which were much higher in the stretches close to the sources of the rivers. Research by Ternus *et al.* (2011) showed that nitrogen and phosphorus concentrations in lotic environments can be greatly affected by the use and occupation of the soil by agricultural activities.

Bacillariophyceae and Chlorophyceae were the two taxonomic classes with the greatest number of species recorded out of the nine classes encountered during the study. These groups are usually found in limnic environments and their occurrence demonstrates their ability to adapt to water fluxes. Similar results were reported in other Brazilian rivers by Monteiro *et al.* (2009) and Rodrigues *et*

al. (2009), who reported that Diatomaceae, Chloroficeae and groups of cyanobacteria were the most abundant taxa. Although, according to Reviers (2006), Diatomaceae are widely distributed and may colonize all types of aquatic environments, Reynolds *et al.* (1994) states that the richness of Diatomaceae may be affected by water flow, intensity of light on the river, high nutrient availability, water quality and herbivory. This is due to the fact that species of Diatomaceae are fast growing organisms, especially in conditions of high nitrogen concentration, and to their status as opportunistic or colonizing algae (r- and C-strategists, respectively) (Reynolds 1984; Sommer 1988). The above facts corroborate the results of the present study since the greatest richness of Diatomaceae occurred at P1, a site close to the source of the Tigre River and a site with low water flux, many stones, and scant shade, but a high degree of electrical conductivity.

Among the Diatomaceae with the highest occurrence frequencies, *Melosira varians* and *Cymbella* are associated with slow water flow due to their weak fixing structure and their preference for habitats with high luminosity (Hermany 2005). Maier & Rott (1988) and Salomoni *et al.* (2006) underscored that *Nitzschia palea*, *Achnantheidium minutissimum*, *Navicula cryptocephala* and *Ulnaria ulna* are widely distributed species and are generally dominant in eutrophic environments. Moreover, *Gomphonema parvum* has been classified by Van Dam *et al.* (1994) and Lobo *et al.* (2002) as a moderately pollution-tolerant species. On the other hand, the ubiquitous genus *Aulacoseira* is the most successful of all the centric Diatomaceae, being greatly abundant in the plankton of lakes, reservoirs and large rivers (Wetzel 2011).

Green algae (Chlorophyceae) form the second most diverse group of the floristic composition of the studied rivers. According to Silva *et al.* (2009), Chlorophyceae encompasses some of the most widely distributed phytoplankton groups, occurring in all types of continental waters, whereas Luzia (2009) reports that they typically occur in rivers, lakes and shallow reservoirs with turbulence. These observations corroborate the results of current study since the greatest richness of Chlorophyceae occurred at the sampling sites P8 and P2, which are shallow and with few rapids. According to Peres & Senna (2000), Chlorophyceae survive in very different environments, ranging from slightly polluted waters to highly eutrophized environments, and exhibit several survival strategies due to their great diversity.

In the present study, β -diversity proved to be moderate (β -1 ranged between 24 and 38%) and revealed a relatively low level of heterogeneity in the species of the sampled stretches. Dissimilarities may be found among stretches of the same river and stretches of the two rivers, since other environmental characteristics may affect phytoplankton composition. These environmental characteristics may include either different limnological features of lakes, as registered by Nogueira *et al.* (2008) when they estimated the three diversity components (alpha, beta, gamma) of the four urban lakes in the municipality of Goiânia GO Brazil,

or interference by/with flood cycles (Nabout *et al.* 2007) or even the environment's trophic degree (Pires 2014). Consequently, the hypothesis regarding beta-diversity tested in by the present research is rejected since no significant co-relationship between the results has been observed.

The similarity analysis of species composition among sample sites found a co-phenetic coefficient of 0.86, revealing a good representation of the actual distances between genotypes (Sokal & Rohlf 1962). The dendrogram indicated that stretches without forest protection (P1, P2 and P7) were dissimilar from sites with extensive or total forest protection (P3, P4, P5), and sites P6, P8 and P9 had a composition that differed widely from the other sampled stretches. The classification shows that community composition can be affected by local environmental variables. Group 1, with approximately 78% similarity, was composed of only stream stretches with no forest protection. The above indicates that the removal of riparian vegetation increased the erosion of river-banks, thereby substituting the biota and changing the functional dynamics. The ecosystem did not remain predominantly heterotrophic, in which allochthonous energy is derived from leaves from riverside vegetation, but became a predominantly autotrophic ecosystem. In this case, the removal of riparian vegetation and, consequently, a greater penetration of light, benefitted autochthonous energy related to the algae of the streams (Novaes 2010).

Group 2, with approximately 65% similarity, was composed of forest-protected stretches of the Tigre River (P3 is partially protected; P4 and P5 are totally protected). Consequently, there was low alpha-diversity due to the low concentration of chlorophyll-a, which is associated with canopy density and shading of the river margins of the stretches. Luminosity is one of the factors that can limit the rate of development of a phytoplankton community (Silveira 2004; Tundisi & Matsumura-Tundisi 2008). Algae inserted in ecosystems with scant light must adapt to such conditions by improving their photosynthetic capacity with cells featuring great pigment amounts or by speeding their life cycle (Reynolds *et al.* 1994; Soares *et al.* 2007).

The stretches P6, P8 and P9 differed from all the other stretches (Groups 1 and 2), and with no similar composition among themselves. Since P6 and P9 lie at the mouths of the rivers where they empty into reservoirs, they are lotic to lentic transition environments. The two sites had low similarity, although they had the highest cyanobacteria concentrations. Cyanobacteria are organisms that exhibit ecological plasticity since they grow in almost all aquatic and land media, and are distributed throughout the planet and may be the most widespread photosynthetic organisms with regard to habitat (Badger *et al.* 2006; Melcher 2007). These organisms are sensitive to nutrient availability, and often bloom in eutrophized environments, thereby causing serious environmental changes and putting public health at risk due to the production of toxins (Yunes *et al.* 1996; Ferrão-Filho *et al.* 2002).

A high concentration of chlorophyll-a, an indirect indication of algal biomass, could be perceived at site P9. Increase in algal biomass at this specific site may be associated with several factors, but mainly greater biological activity that usually occurs during the months with higher temperatures, hydraulic characteristics (less water movement due to the lotic-lentic transition environment), and the features of the hydrographic basin in which the river lies (Cunha & Calijuri 2008).

The current investigation found high values of species richness for all of the sampled stretches, where beta-diversity, however, was moderate. A high degree of similarity between sampled stretches with only slight differences in species composition was observed among the sites along the gradient of the two rivers. Bacillariophyceae and Chlorophyceae were the most represented classes. Consequently, the hypothesis that river stretches protected by the forest will have lower species richness and greater beta-diversity than non-protected stretches was only partially supported. Protected sites did have lower species richness in comparison to non-protected stretches, however, beta-diversity did not differ significantly among these sites.

All of the 429 taxa registered in the present study are first records for the two study-rivers. This clearly illustrates the need for further floristic studies of larger streams and rivers in remote areas of the state of Santa Catarina, Brazil. Because of their rarity, some taxa could not be identified to the species level and, consequently, their distinguishing features could not be analyzed.

Since human activities are more and more intense in this specific region due to the development of towns and cities or due to the broadening of agricultural frontiers, the need for further study on the biodiversity of hundreds of other lotic environments of the region should be emphasized.

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