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Phytoplankton relationship with bacterioplankton, dissolved carbohydrates and water characteristics in a subtropical coastal lagoon

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ABSTRACT

Release of carbohydrates by phytoplankton enhances microbial diversity, promoting associations between algae and heterotrophic organisms. Thus, this work aimed to characterise the dissolved carbohydrates at a Brazilian subtropical coastal lagoon (Merin lagoon), in addition to determining their relationships with environmental parameters and phyto/bacterioplankton communities over one year. We analysed the relationships among physical, chemical and biological parameters by a principal component analysis (PCA) after normalisation of data as z *scores. Chlorophyceae showed the highest richness, although Bacillariophyceae and Cyanophyceae showed the highest densities. These classes are essentially represented by centric diatoms (*Aulacoseira *cf.* muzzanensis*) and filamentous cyanobacteria (*Planktolyngbya limnetica *and* Planktolyngbya *cf.* contorta*). Merin lagoon showed a strong seasonal behaviour for most of parameters and phytoplanktonic density was mainly correlated with temperature, specific conductance, phosphate and total bright sunshine duration. Only combined dissolved carbohydrates (CDCHOs) were found and their main components were glucose (31.6%), mannose/xylose (20.6%), ribose (13.9%), arabinose (8.9%) and galacturonic acid (8.1%). The CDCHO amounts were higher in November, March-April and September and the December/January and July/August periods showed lower ones. Ribose was first detected only in the warm months and it gradually decreased with bacterial density. The carbohydrate concentration was coupled to phytoplanktonic density, except in December and January, when the bacterial density was increased. These results supported the significance of dissolved carbohydrates in associations with algae and bacteria in the freshwater planktonic environment. Our data reinforced the influence of phytoplankton community on the natural dissolved carbohydrate pool, besides the significance of such carbon source on the bacterial community dynamic.*

Key words: algae, coastal lagoon, plankton, polysaccharides.

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INTRODUCTION

Phytoplankton biomass is composed of significant amounts of carbohydrates (Urbani *et al.,* 2005) both in particulate and dissolved fractions (Biersmith and Benner 1998). Release of carbohydrates increased the microbial diversity in aquatic systems, mainly through the enhancement of algae/heterotrophic organism associations, in which extracellular polysaccharides (EPS) frequently act as key compounds, supplying both mechanical support and carbon source (Freire-Nordi and Vieira, 1998; Giroldo and Vieira, 2005; Giroldo *et al.,* 2005a, 2007). Additionally, several ecological functions have been assigned to EPS, such as floating regulation, protection against predation, nutrient and metal tracking, among others (Reynolds, 2007).

Extracellular polysaccharides production by phytoplankton depends on the species, their physiological state and environmental conditions (Urbani *et al.,* 2005; Hanamachi *et al.,* 2008). As a consequence, this process promotes qualitative and quantitative variations in the dissolved organic carbon (DOC) pool in the planktonic environment depending on the abundant species of primary producers and heterotrophic organisms (Dellamano-Oliveira *et al.,* 2007). Chemical and physical water characteristics also influence such fluctuations, as they act directly in both photosynthesis and heterotrophic processes (Myklestad, 1995; Myklestad and Børsheim, 2007).

Although several reports have been published on seasonal fluctuations in phytoplankton (Pérez *et al.,* 1999; Pérez and Odebrecht, 2005) and on carbohydrate production by isolated phytoplanktonic strains (Vieira and Myklestad, 1986; Giroldo and Vieira, 2002, 2005), integrated studies on phytoplankton, bacteria, carbohydrates and

water characteristic dynamics are scarce. However, this approach contributes to understanding the significance of the phytoplankton/bacteria community in the carbohydrate pool and energy fluxes in the pelagic environment (Dellamano-Oliveira *et al.,* 2007).

This work aims to characterise dissolved carbohydrates from the pelagic zone of a high phytoplankton diversity site in Merin lagoon (Rio Grande do Sul, Brazil) and to analyse their relationships with phyto/bacterioplankton communities as well as with water physical and chemical characteristics. The present study also aims to contribute to understanding carbohydrate release by phytoplankton as well as the roles of these compounds in carbon fluxes among the ecological compartments.

METHODS

Study area

Merin lagoon (Fig. 1), which has a total area of 3748 km^2 and a maximum depth of 7 m, is partially located within Taim ecological station, along with several other wetlands and coastal lagoons (Vieira and Rangel, 1988). The major part of Merin lagoon is located in the Brazilian territory, although almost a quarter of its area belongs to Uruguay. The climate is subtropical humid (Fragoso *et al.,* 2008), and the water level is quite variable depending on the intensity and direction of winds, which can result in flooding of adjacent floodplains.

The margin of Merin lagoon is covered by two types of vegetation. Its eastern margin is covered by floodplains and wetlands, which are mainly flooded during winter. The western shore is covered with native woods, marsh in some areas, *Utricularia* insectivores, grasses and sedges (Vieira and Rangel, 1988). Merin lagoon is connected through Sao Gonçalo channel to the estuary of Patos lagoon, which is considered more eutrophic than Merin lagoon (Yunes *et al.,* 1996; Perez and Odebrecht, 2005). Despite being linked to a salt coastal lagoon, salinisation has not been observed in Merin lagoon since 1977, when a dam was built in São Gonçalo channel (Vieira and Rangel, 1988).

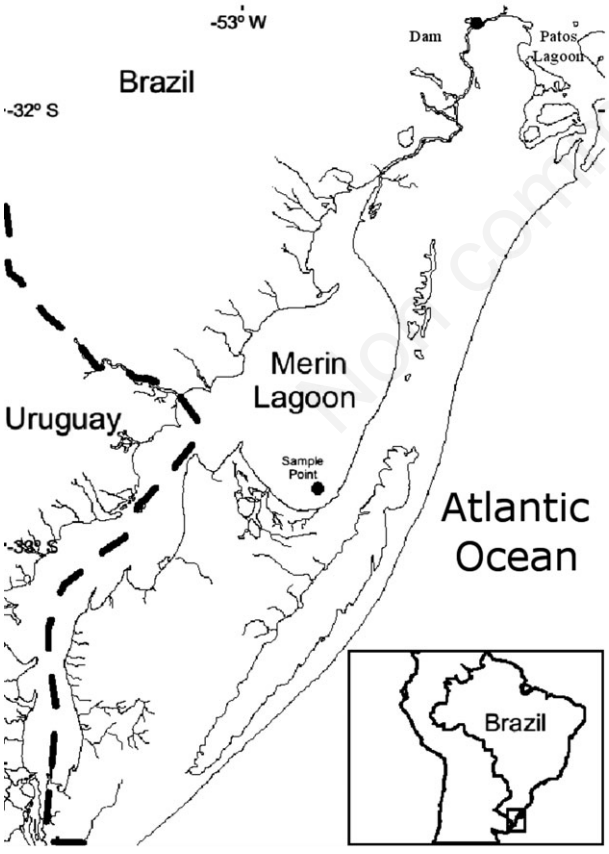

Fig. 1. Location of Merin lagoon in the extreme southern territory of Brazil (Rio Grande do Sul state), showing the Vila Anselmi sampling site (32°52'44"S and 52°46'04"W).

Sampling and field analysis

Samples were collected monthly from September 2009 to August 2010 at Vila Anselmi sampling site located 1000 m from the eastern margin of Merin lagoon (32°52'44"S and 52°46'04"W). This site was chosen because previous phytoplanktonic analysis had shown the presence of a high phytoplanktonic diversity, which was suitable for the process studied in this work. We analysed the physical and chemical parameters of water at the site and collected four water samples, as described below.

We performed two horizontal hauls with a plankton net (mesh 20 μm). In the first haul (sample 1), a 100 mL sample was stored fresh in a sterile amber vial for observation of living material. In the second (sample 2), a 100 mL sample was immediately fixed with 4% formalin for qualitative analysis of phytoplankton. In addition to the hauls, we collected two 500 mL sub-surface (30 cm) samples using PVC flasks. The first (sample 3) was immediately fixed with 0.5% Lugol for quantitative analysis of phytoplankton and bacterioplankton. The second (sample 4) was kept fresh for laboratory analysis of dissolved nutrients and carbohydrates. At the time of sampling, the following parameters were also determined: temperature (accuracy±0.01°C), hydrogenionic potential (pH accuracy of±0.01) and specific conductance (accuracy±0.000001 μMHOS) with a portable Hanna Combo-HI98130 probe (Hanna Instruments, Woonsocket, RI, USA); and depth and transparency using a Secchi disc. Additionally, we obtained information from the National Institute of Meteorology (INMET), including the monthly average wind speed (m s^{-1}), total precipitation (mm), total bright sunshine duration (h) and cloudiness expressed in tenths of

sky covered for the city of Santa Vitoria do Palmar, near the sampling site.

Phytoplankton

Qualitative analysis consisted of observing sample 2 using an Olympus CX21 microscope (Olympus, Tokyo, Japan) at 100×1000 magnification. Identification was made to the genus level when possible and, for abundant and dominant species, at the species level. We used several keys to this identification of the genus (Barber and Haworth, 1981; Round *et al.,* 1990; Wehr and Sheath, 2002; Bicudo and Menezes, 2006) and specific level (Prescott *et al.,* 1975; Croasdale *et al.,* 1983; Komárek, 1983; Komárek and Fott, 1983; Parra *et al.,* 1983; Komárek and Anagnostidis, 1998; Flôres *et al.,* 1999a, 1999b; Ludwig *et al.,* 2004; Komárek and Anagnostidis, 2005).

Quantitative analyses were performed on sample 3 by the method of Whipple (Whipple *et al.,* 1927) using a Sedgwick-Rafter counting chamber with a 1 mL capacity. Individual phytoplankton was counted along the entire chamber (APHA, 1995) on an Olympus CX21 microscope (Olympus) with up to 200 times magnification. Cells, colonies, filaments or cenobium were considered individuals. We considered dominant taxa those with densities that exceeded 50% of the average density of each sample and abundant taxa those with densities that exceeded the average density of each sample (Lobo and Leighton, 1986).

All taxa were organised according to two classifications. A phylogenetic approach (Van den Hoek *et al.,* 1995) was used in both qualitative and quantitative analysis. A second classification, consisting of a morpho-functional group (MFG) approach modified from Salmaso and Pádisak (2007), was used only for quantitative analysis. In the MFG classification, all flagellated organisms were divided into four groups based on their potential mixotrophy or autotrophy and structural features: i) colonial mixotrophic flagellates (CMF), which included organisms of the class Chrysophyceae; ii) unicellular mixotrophic flagellates (UMF), which included Euglenophyceae, Dinophyceae, Cryptophyceae, Chrysophyceae and Raphidophyceae; iii) unicellular autotrophic flagellates (UAF), including some Prasinophyceae and Chlorophyceae; and iv) colonial autotrophic flagellates (CAF), which included some Chlorophyceae (Salmaso and Pádisak, 2007). The Cyanophyceae and Bacillariophyceae were divided into two groups each based on structural characteristics. The first is divided into filamentous (FCY) and colonial Cyanobacteria (CCY), and diatoms are classified as centric (CDI) and pennate diatoms (PDI). The Zygnematophyceae, Chlorophyceae, Xanthophyceae and other classes are divided into three distinct groups: i) green coccoid and other unicellular species (GCU); ii) green colonial/cenobial and other colonials (GCC); and iii) green and other filamentous algae (GFI) (Salmaso and Pádisak, 2007).

Bacterioplankton

Quantitative bacterioplankton analyses were performed on sample 3 following sodium thiosulphate addition, filtering through 0.2 µm Nucleopore black membranes (Millipore, Billerica, MA, USA) and Acridine orange treatment (Hobbie *et al.,* 1977). Bacteria were counted in 30 random fields using an epifluorescent microscope (Zeiss Axioplan, Jena, Germany) with a blue filter (487709-BP 450-490; FT 510; LT 520).

Dissolved carbohydrates and anion analyses

Sample 4 was filtered through 0.45 µm pore size glass fibre filters GF/3 (Macherey-Nagels, Düren, Germany) and the filtrate was frozen and stored at -4°C for nutrient and carbohydrate analysis. Dissolved carbohydrates and anions were evaluated qualitatively and quantitatively in filtered samples by high performance ion chromatography (HPIC) with pulsed amperometric detection (PAD) for dissolved carbohydrates and specific conductance detection for anions. Carbohydrates were analysed in two fractions: free dissolved carbohydrates (FDCHOs), including free mono- and disaccharides, and total dissolved carbohydrates (TDCHOs), also including oligo- and polysaccharides. Combined dissolved carbohydrates (CDCHOs), including only oligo- and polysaccharides, were obtained by subtraction of FDCHOs from TDCHOs.

We used a Dionex (Dionex, Sunnyvale, CA, USA) ion chromatographic device, model ICS3000, equipped with a Single Gradient Degas pump and an ED-50 detector. For FDCHO analysis, a 0.10-mL aliquot was directly injected into the HPIC device, while for TDCHO analysis, a 1-mL aliquot was hydrolysed with 0.64 M HCl (final concentration) at 100°C for 12 h (Gremm and Kaplan, 1997) prior to injection. A CarboPac PA-1 column (2×250 mm) with the corresponding guard column (2×50 mm) was employed for carbohydrate separation using 18 mM NaOH for elution of neutral monosaccharides, 500 mM sodium acetate in a gradient for uronic acid elution, and 200 mM NaOH to recover the column at a flow rate of 0.25 mL min^{-1} (Wicks *et al.,* 1991). Fucose, rhamnose, N-acetyl-galactosamine, arabinose, N-acetyl-glucosamine, galactose, glucose, mannose, xylose, fructose, ribose, galacturonic acid and glucuronic acid standards (Sigma-Aldrich, St. Louis, MO, USA) were used to identify the monosaccharides.

Dissolved anions were analysed by injection of 0.10-mL aliquots to detect the following dissolved anions: bromide, chloride, nitrate, phosphate and sulphate. An IonPac AS22 column (2×250 mm) with the corresponding guard column (2×50 mm) was used with 4.5 mM sodium carbonate and 1.4 mM sodium bicarbonate for anion separation at a 0.25 mL min^{-1} flow rate (Groussac *et al.,* 2000; Huang *et al.,* 2000). All samples were analysed in triplicate and quantified using calibration curves including 8 concentrations close to those observed in the samples.

Statistical analysis

We analysed the relationships among physical, chemical and biological parameters by principal component analysis (PCA) using PAST software (Hammer *et al.,* 2001) after normalisation of data as *z* scores (Bini, 2007; Mann, 2003).

RESULTS

Tab. 1 shows all minimum, maximum and average values, with the corresponding standard deviation for all physical, chemical and meteorological parameters. Water transparency varied from 20 to 55 cm (30.69±10.73 cm on average), with higher values occurring from April to September. Specific conductance, temperature and pH showed higher values between October and March, varying from 90 to 130 µMHOS (111.1±14.46 µMHOS on average), 15.2 to 25.4°C (19.5±3.89°C on average) and 7.24 to 8.28 (7.8±0.32 on average), respectively. Depth varied from 1.5 to 4.5 m, and two different periods were characterised by low water levels: January to March and June to July. The total precipitation and the average cloudiness varied from 42.6 to 160.2 mm (108.7±43.31 mm on average) and 4 to 7 tenths (5±1 tenths), respectively. The total bright sunshine duration showed higher values between December and April and varied from 120 to 269 h (182.4±47.82 h). The wind speed varied from 2.8 to 5.1 m/s (4±0.68 m/s on average). The nitrate concentration varied from 1.47 to 0.025 µM (0.451±0.493 µM), while the phosphate concentration varied from 0.003 to 0.062 µM (0.087±0.061 µM on average). The total dissolved anion concentration varied from 0.38 to 1.99 µM (1.038±0.489 µM on average), with lower values being observed in December and July. The annual variation of these parameters is shown in Figs. 2 and 3.

The identified phytoplanktonic taxa were grouped in 8 different classes: Chlorophyceae (36%), Bacillariophyceae (30%), Cyanophyceae (16%), Zygnematophyceae (14%), Euglenophyceae (2%), Xanthophyceae (2%), Chrysophyceae (<1%) and Dinophyceae (<1%). *Oscillatoria* sp., *Pseudanabaena* cf. *limnetica, Cyclotella* sp., *Staurosira longirostris*, *Closterium aciculare* and *Closterium* cf. *kuetzingii* were abundant in at least one

Tab. 1. Minimum, maximum, average and standard deviation values of all physical, chemical and meteorological parameters in 2009-2010.

Parameters	Min	Max	Average±SD
Water transparency (cm)	20	55	30.7±10.73
Specific conductance (µMHOS)	90	30	111.1±14.46
Temperature (°C)	15.2	25.4	19.5±3.89
pH	7.24	8.28	7.8±0.32
Depth (m)	1.5	4.5	3.1±1.07
Total precipitation (mm)	42.6	160.2	108.7±43.31
Cloudiness (tenths)	4	7	5±1
Total bright sunshine (h)	120	269	182.4±47.82
Wind speed (m/s)	2.8	5.1	4±0.68
Nitrate (µM)	1.47	0.025	0,451±0.493
Phosphate (µM)	0.003	0.062	0.087±0.061
Dissolved anion concentration (µM)	0.38	1.99	1.038±0.489

Min, minimum; max, maximum; SD, standard deviation.

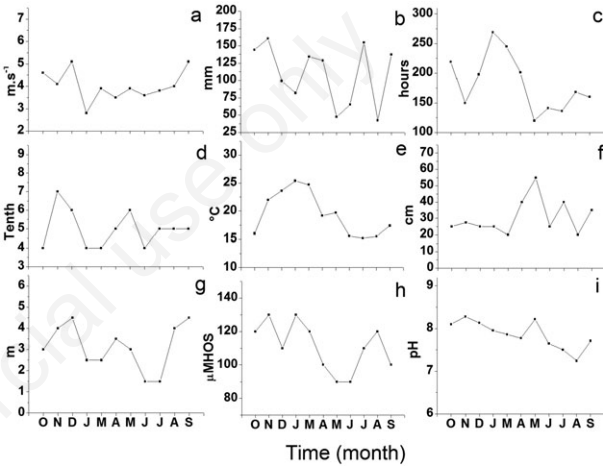

Fig. 2. Average wind speed (a), total precipitation (b), total bright sunshine duration (c), average cloudiness (d), temperature (e), water transparency (f), depth (g), specific conductance (h) and pH (i) during 2009-2010.

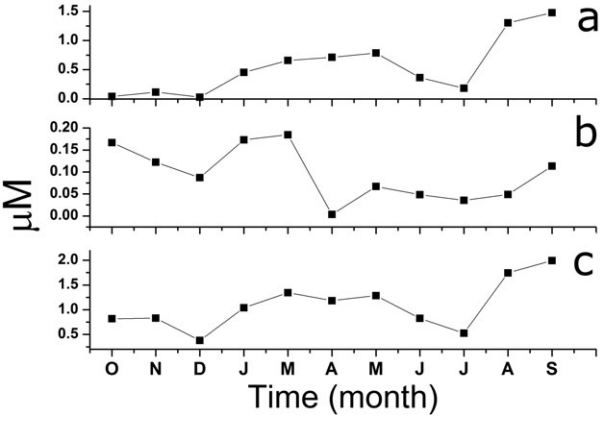

Fig. 3. Dissolved nitrate (a), phosphate (b) and total anions (c) during 2009-2010.

month, and *Planktolyngbya* cf. *contorta*, *Planktolyngbya limnetica* and *Aulacoseira* cf. *muzzanensis* were abundant all throughout the study.

January and August showed the highest richness values, with 75 and 70 taxa observed, respectively, while June, July and September presented the lowest values, with 46, 42 and 46 taxa found, respectively, as shown in Fig. 4a. The proportions of the classes showed only slight variations during the study, with Bacillariophyceae, Chlorophyceae, Cyanophyceae and Zygnematophyceae being the main classes found (Fig. 4b).

We observed the highest phytoplanktonic densities over December and March, when there was an average density of 1299 ind. mL^{-1}, while September showed the lowest value of 453 ind. mL^{-1} (Fig 5a). Bacillariophyceae and Cyanophyceae were the classes presenting the highest densities (Fig. 5b), although these values were mainly related to centric diatoms (CDI) (*Aulacoseira* cf. *muzzanensis*) and filamentous cyanobacteria (FCY) (*Planktolyngbya* cf. *contorta* and *P. limnetica*), as shown in Figs. 6 and 7.

Fig. 8 shows the annual variation of total dissolved carbohydrates in comparison with the total phytoplanktonic and bacterioplanktonic density. Except for December and January, the total dissolved carbohydrate variation was adjusted to the phytoplankton and bacterioplankton dynamics, although only phyto- and bacterioplankton density showed a significant correlation (Tab. 2). The period from December to January, when the total dissolved

Tab. 2. Pearson's correlation test among the total dissolved carbohydrate concentration and phytoplanktonic and bacterioplanktonic total densities at Vila Anselmi sampling site in Merin lagoon (Brazil) during 2009-2010.

	Total carbohydrates	Phytoplankton density	Bacterial density
Whole study			
Total carbohydrates	-		
Phytoplankton density	0.29	-	
Bacterial density	0.18	0.75*	-
January and December excluded			
Total carbohydrates	-		
Phytoplankton density	0.71*	-	
Bacterial density	0.77*	0.84*	-

**Significant correlation ($P \leq 0.05$).*

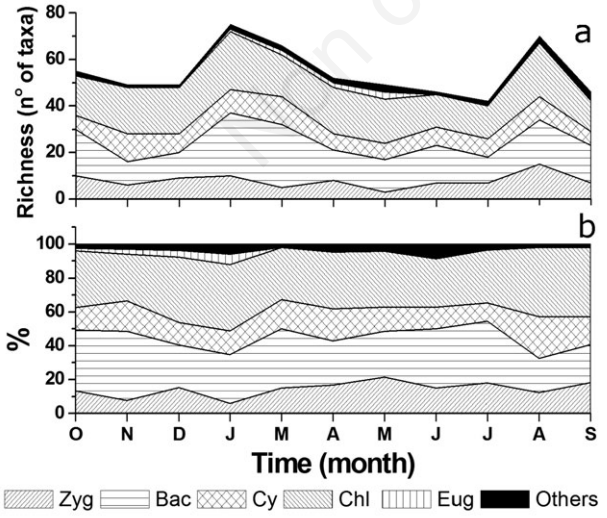

Fig. 4. Absolute (a) and relative (b) richness of taxa during 2009-2010. Zyg=Zygnematophyceae; Bac=Bacillariophyceae; Cy=Cyano-phyceae; Chl=Chlorophyceae; Eug=Euglenophyceae; others= Xanthophycea, Chrysophyceae and Dynophyceae.

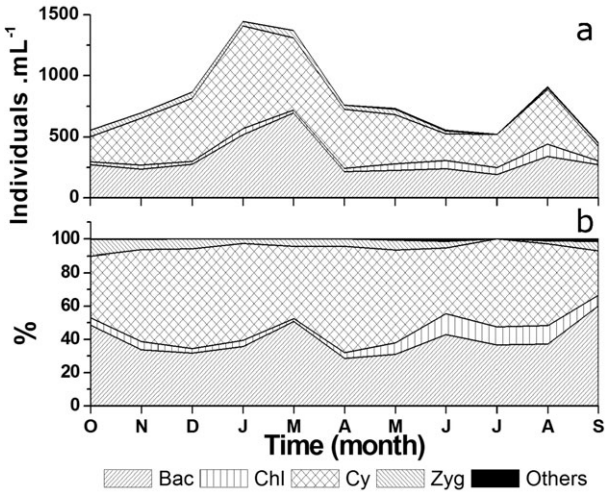

Fig. 5. Density of identified phytoplanktonic classes (a) and its respective relative abundance (b) during 2009-2010. Bac=Bacillariophyceae; Chl=Chlorophyceae; Cy=Cyanophyceae; Zyg=Zygnematophyceae; others=Dynophyceae, Euglenophyceae and Xanthophyceae.

carbohydrates showed a different trend than phyto- and bacterioplankton, coincided with the highest bacterioplankton density detected during the study. When January and December were removed from this analysis, the correlation among total dissolved carbohydrates, phytoplankton and bacterial density became significant (Tab. 2).

In TDCHOs, only CDCHOs were identified during the whole study, indicating that all dissolved carbohydrates at this sampling site in Merin lagoon were oligosaccharides or polysaccharides. Fig. 9 shows the annual absolute (Fig. 9a) and relative (Fig. 9b) CDCHO composition variation. We observed higher total values in November, March-April and September (0.8, 1.55, 0.85, and 0.71 mg L^{-1}, respectively). The period between December/January (0.32 and 0.25 mg L^{-1}, respectively) and July/August (0.34 and 0.32 mg L^{-1}, respectively) showed lower carbohydrate concentrations. The main monosaccharides constituting the CDCHO throughout the study were glucose (31.6%), mannose/xylose (20.6%), ribose (13.9%), arabinose (8.9%) and galacturonic acid (8.1%) (Fig. 9a). Fructose and glucuronic acid were only found in March, while ribose was only detected among March and June. Additionally, fucose, rhamnose, N-acetyl-glucosamine and galactose were found in minor concentrations (Fig. 9).

Fig. 10 shows the results of the PCA, in which it was observed that components 1 and 2 explained 32.3 and 17.3% of the data variation, respectively. We found positive correlations of temperature, phosphate, specific conductance, total bright sunshine duration and bacterial and phytoplankton densities with component 1, which was associated with October, November, December and, mainly, January and March. Nitrate and water transparency showed the strongest negative correlations with component 1. Component 2 was mainly explained on its positive side by cloudiness, wind speed, depth, pH and total precipitation. Total dissolved anions and carbohydrates showed weak positive correlations associated with both components 1 and 2, although their positions in the PCA diagram were close to pH and total precipitation.

DISCUSSION

Phytoplankton and bacteria were mainly correlated with temperature, specific conductance, phosphate and total bright sunshine duration. On the other hand, the parameters that showed the greatest influence on water level (depth) were cloudiness, total precipitation and wind speed. In previous studies carried out between 1998 and 1999, a similar pattern of higher phytoplanktonic densities has been observed and was associated with higher temperature and conductivity, mainly in summer time (Pérez and Odebrecht, 2005; Sophia and Pérez, 2010). Our data confirmed such a trend for Vila Anselmi sampling site in Merin lagoon.

Among the most abundant taxa, we found members

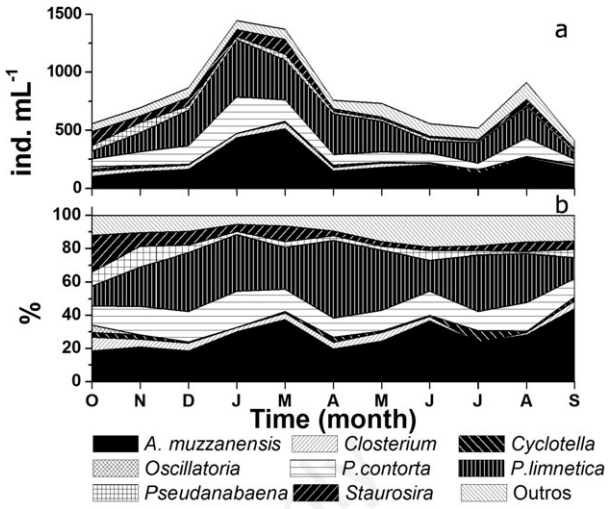

Fig. 6. Densities of abundant taxa (a) and their respective relative abundance (b) during 2009-2010. *A. muzzanensis=Aulacoseira* cf. *muzzanensis; P. contorta=Planktolyngbya* cf. *contorta; P. limnetica=Planktolyngbya limnetica; Closterium=Closterium kuetzingii* and *Closterium aciculare*.

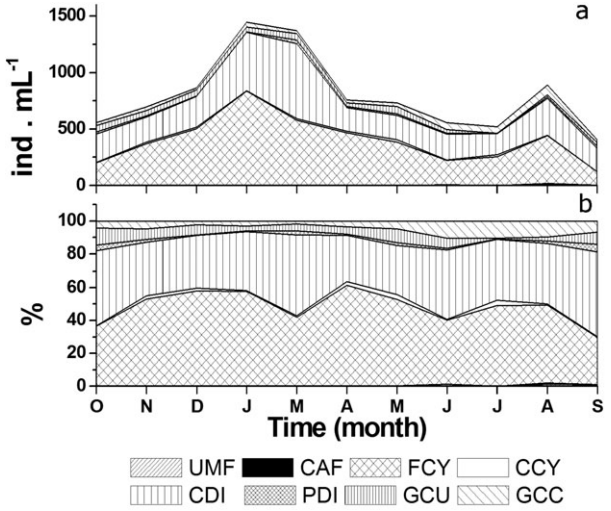

Fig. 7. Densities of phytoplanktonic morpho-functional groups (a) and their respective relative abundances (b) identified during a one-year study (2009 and 2010). UMF=unicellular mixotrophic flagellates; CAF=colonial autotrophic flagellates; FCY=filamentous cyanobacteria; CCY=colonial cyanobacteria; CDI=centric diatoms; PDI=pennate diatoms; GCU=green coccoid and other unicellular species; GCC=green colonial and other colonial species.

of two MFGs: FCY and CDI. The main representatives of these groups were *Planktolyngbya* cf. *contorta* and *P. limnetica* for FCY, and *Aulacoseira* cf. *muzzanensis* for CDI, for which high densities have been reported previously in Merin lagoon (Pérez and Odebrecht, 2005). The MFG approach indicated that, despite the abundance of Cyanophyceae and Bacillariophyceae, these classes were not represented by colonial cyanobacteria or pennate diatoms. Zygnematophyceae and GCU were also abundant in May and September, and their temporal variation was explained mainly by *Closterium kuetzingii* and *Closterium aciculare*.

The principal monosaccharide components of the CDCHO found in Merin lagoon showed remarkable differences from those found by Dellamano-Oliveira *et al.* (2007) in the Barra Bonita reservoir (Brazil), as shown in the Tab. 3. Although the Barra Bonita reservoir also exhibited cyanobacteria and diatom abundance, the taxa responsible for these high phytoplanktonic densities were different from those found in Merin lagoon, since three well-known EPS producers were dominant on Barra Bonita reservoir (*Microcystis aeruginosa*, *Anabaena spiroides* and *Pseudanabaena mucicola*).

Additionally, the eutrophic condition of Barra Bonita reservoir resulted in higher phytoplanktonic densities and EPS concentrations compared to Merin lagoon. While in Merin lagoon, the total phytoplanktonic density was as high as 1500 ind. mL^{-1}, in the Barra Bonita reservoir the

Tab. 3. Comparison among values of dominant phytoplankton classes, species and density, and carbohydrate concentration of Merin lagoon, Barra Bonita and Lobo reservoir (Brazil).

	Merin lagoon	Barra Bonita reservoir	Lobo reservoir
Coordinates	32°52'S/52°46'W	22°29'S/48°10'W	22°10'S/47°54'W
Trophic state	-	Eutrophic	Oligotrophic
Phytoplankton density (ind. mL^{-1})	1500	5000	-
Carbohydrate concentration (mg L^{-1})	0.58	2.92	0.13-1.55
Dominant classes	Cyanophyceae and Bacillariophyceae	Cyanophyceae and Bacillariophyceae	Chlorophyceae, Bacillariophyceae and Cyanophyceae
Dominant species	*Planktolyngbya contorta*, *P. limnetica* and *Aulacoseira muzzanensis*	*Microcystis aeruginosa*, *Anabaena spiroides* and *Pseudanabaena mucicola*	-
References	-	Dellamano-Oliveira *et al*. (2007)	Striquer-Soares and Chevolot (1996)

S, south; W, west.

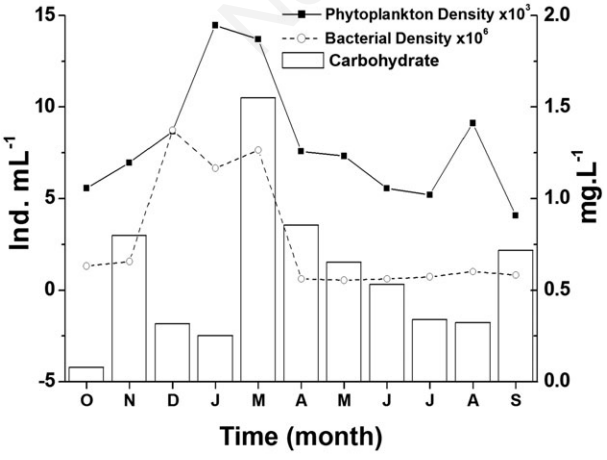

Fig. 8. Total dissolved carbohydrate concentration and phytoplanktonic and bacterioplanktonic total densities during 2009-2010.

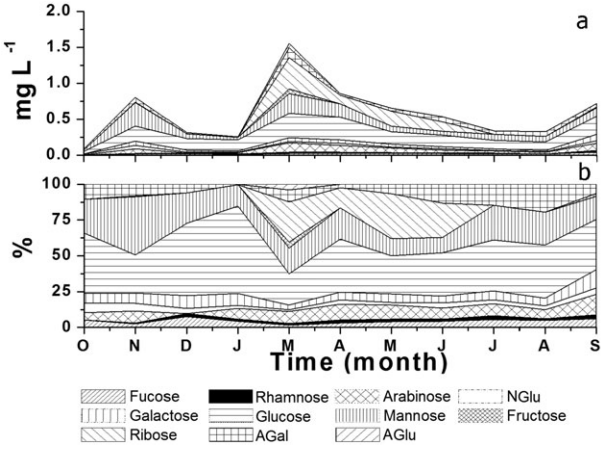

Fig. 9. Absolute (a) and relative (b) combined dissolved carbohydrates concentration and composition registered during 2009-2010. NGlu=N-acetyl-glucosamine; AGal=galacturonic acid; AGlu=glucuronic acid.

cyanobacteria density alone was 5000 ind. mL^{-1}. Such differences in the composition and abundance of taxa in these water bodies were decisive, not only for the higher carbohydrate concentration found in Barra Bonita reservoir (2.92 mg L^{-1} on average *vs* 0.58 mg L^{-1} in Merin lagoon), but also for its composition (Tab. 3). In contrast, the Lobo reservoir, which is an oligotrophic environment also located in São Paulo state, showed amounts of carbohydrates similar to Merin lagoon, a phytoplanktonic community represented mainly by Chlorophyceae and Bacillariophyceae (Striquer-Soares and Chevolot, 1996). Despite the few studies showing the relationships between phytoplankton and carbohydrate concentration in natural waters, this comparison indicated that trophic state and phytoplankton composition influence the dissolved carbohydrates in the planktonic environment.

The higher carbohydrate concentration observed in March was associated with high total bright sunshine duration, temperature, phosphate and specific conductance values, which could lead phytoplanktonic populations to produce an excess of photoassimilated carbon, promoting an increase in carbohydrate release (Fogg, 1983; Myklestad, 1995). Additionally, it is possible to observe growth stabilisation in January and March, similar to a stationary growth phase in cultures. Several authors have reported an increased EPS production by phytoplanktonic cells in the stationary growth phase. This happens because cell division decreases, while photosynthesis is still active, promoting the release of excess organic carbon in the form of EPS (Fogg, 1983; Giroldo and Vieira, 2005). Such strategy protects the photosynthetic apparatus from excess light and photo-oxidation (Smith and Underwood, 2000) and promotes bacterial growth supported by EPS as a substrate, contributing to the maintenance of nutrient levels through remineralisation of such compounds (Wood and Van Valen, 1990; Borsheim *et al.,* 2005; Giroldo *et al.,* 2007).

Based on data reported in the literature, it is possible to associate the high amounts of glucose and mannose/xylose detected to the abundance of cyanobacteria and diatoms. High percentages of glucose were found in several cyanobacterial cultured species, such as *Nostoc* (Huang *et al.,* 1998), *Raphidiopsis brookii* (Yunes *et al.,* 2009), *Anabaena spiroides* (Colombo *et al.,* 2004), and *Microcystis aeruginosa* (Raziuddin *et al.,* 1983). In Merin lagoon, two FCYs were abundant during the whole study, but no data on their carbohydrate compositions in culture were available in the literature. Several CDIs were also frequent throughout the study, and *Aulacoseira* cf. *muzzanensis* was abundant in all samples. Several studies have demonstrated the presence of high percentages of mannose and xylose in diatom EPS, including in other *Aulacoseira* species (Giroldo *et al.,* 2003; Giroldo and Vieira, 2005; Dellamano-Oliveira *et al.,* 2007). In contrast to the results found in Merin lagoon, diatoms and

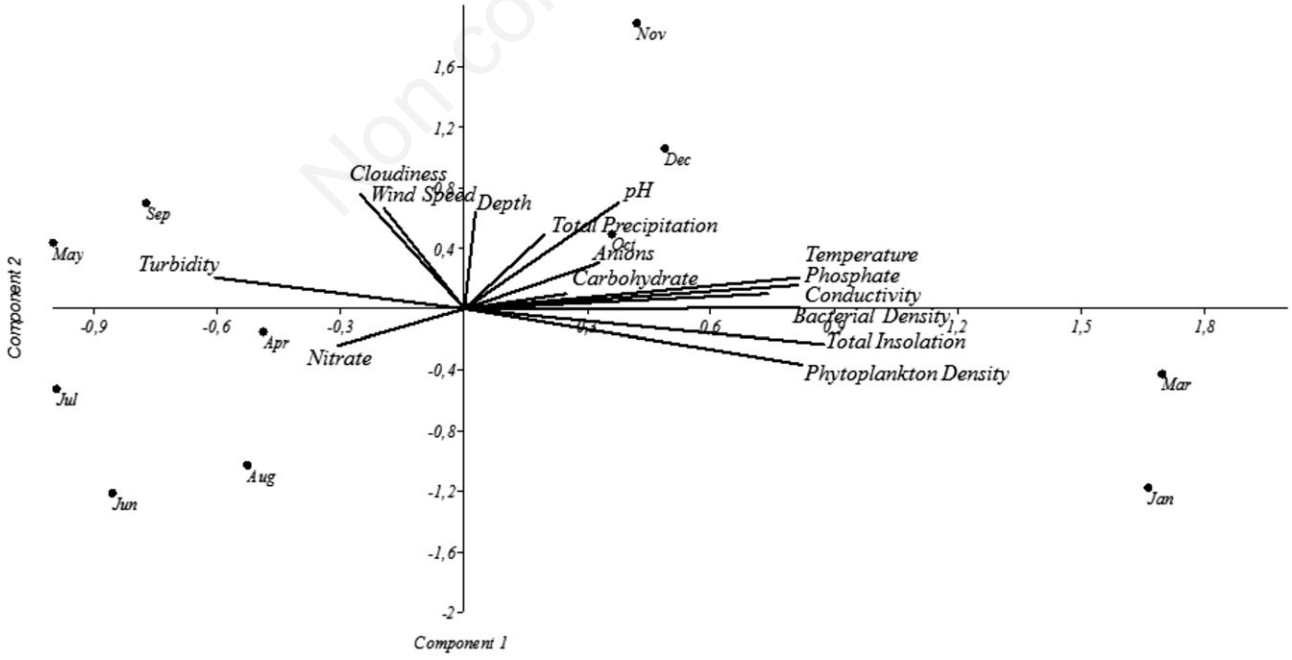

Fig. 10. Principal component analysis for physical and chemical parameters and meteorological data as well as total phytoplanktonic and bacterioplanktonic densities during 2009-2010.

cyanobacteria are frequently associated with high amounts of rhamnose (Colombo *et al.,* 2004).

Ribose increases only at the end of summer just after the main increase in phytoplankton and persists in the water column decreasing gradually until June. Ribose and fructose, which were also detected in this period, but at lower concentrations, were related to the decomposition of plant intracellular material by bacterial populations (Cowie and Hedges, 1984; Wicks *et al.,* 1991). In Merin lagoon, the ribose and fructose concentrations increased following higher phyto- and bacterioplankton densities, probably related to the decomposition of phytoplanktonic organic matter.

During the whole study, a similar pattern was observed between phytoplankton density and carbohydrates, except in December and January. This period also coincided with increases in temperature and total bright sunshine duration, contributing to both phytoplanktonic carbohydrate release and bacterial heterotrophic metabolism (Iriberri *et al.,* 1987). Additionally, the decomposition rates of different carbohydrate components are also an important parameter in such a discussion. Several authors have demonstrated that glucose, mannose and xylose, the main carbohydrate components in Merin lagoon in this period, could be quickly degraded, decreasing the residence time of these components during intense heterotrophic bacterial metabolism (Giroldo *et al.,* 2003, 2005b; Hanamachi *et al.,* 2008).

Phytoplanktonic exudates could be a significant source of organic matter to bacterioplankton. Barrera-Alba *et al.* (2008) demonstrated that 29-100% of the bacterial production may be potentially supported by the phytoplankton production in a tropical estuarine-lagoon system.

Our results supported the significance of dissolved carbohydrates in associations with algae and bacteria in the freshwater planktonic environment (Bell and Mitchell, 1972; Bell and Sakshaug, 1980; Giroldo *et al.,* 2003, 2005a). Fig. 11 proposes a conceptual model to explain the influence of phyto/bacterioplankton on the dissolved carbohydrate dynamics, based on the data obtained in this work. The increase of light, temperature and conductance promoted the phytoplankton growth and, consequently the release of glucose and mannose rich carbohydrates by phytoplankton (Step 1). As a consequence, in the followed months, bacterioplankton also increased and degraded this labile and rich carbohydrate source, disrupting the constant

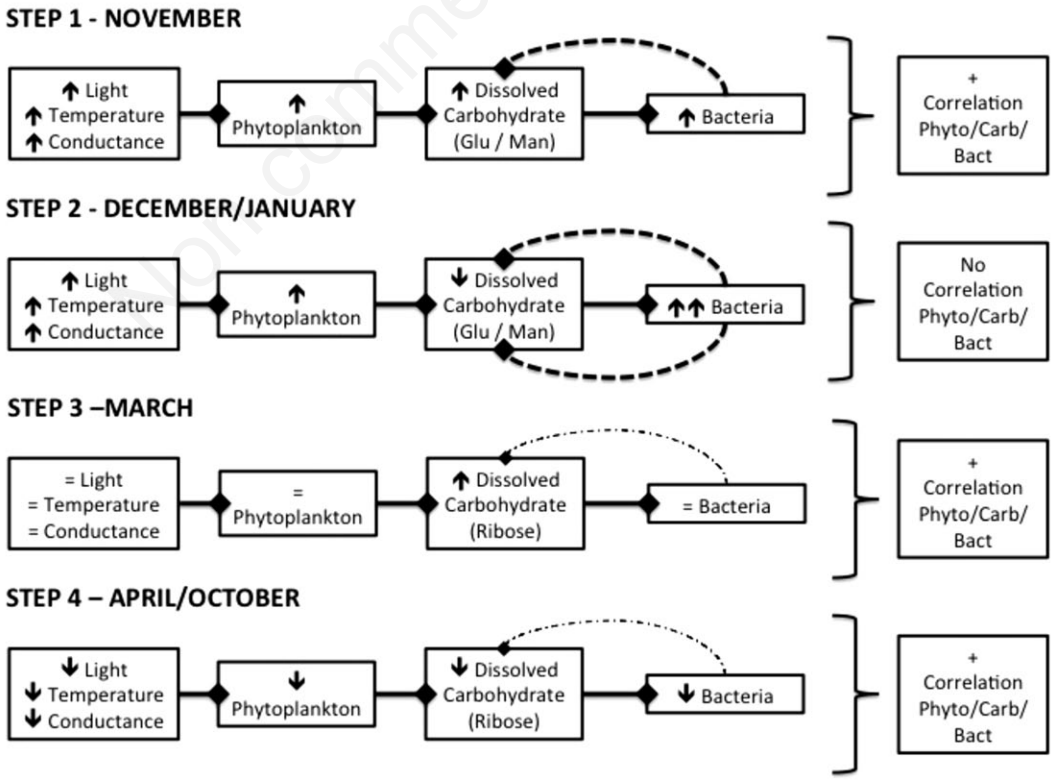

Fig. 11. Conceptual model describing the influence of phyto- and bacterioplankton on the dissolved carbohydrate dynamics in Merin lagoon during 2009-2010.

correlation among phytoplankton, bacterioplankton and dissolved carbohydrate (Step 2). Such decomposition activity on the phytoplankton-derived carbohydrates, coupled with the maintenance of the phytoplankton, bacterioplankton, conductance and temperature levels, increased the ribose content on the dissolved carbohydrate pool, reassuming the above-mentioned correlation (Step 3). The light, temperature, conductance and phytoplankton decrease in autumn and winter, dissolved carbohydrate and bacterioplankton, keeping such biological parameters all correlated (Step 4). Although such pattern requires more data in order to construct a mathematical model, our results supported the significance of dissolved carbohydrates in association with algae and bacteria in the freshwater planktonic environment.

CONCLUSIONS

The results presented in this paper indicate that carbohydrate concentration in Merin lagoon is related to the dynamics of the phyto- and bacterioplanktonic community, in addition to environmental parameters. Thus, the composition and density of phytoplankton and their physiological responses to environmental variables influence the variations of carbohydrates in natural environments. The phytoplankton community is mainly related to temperature, specific conductance, phosphate and total bright sunshine duration, and shows a strong positive correlation with the bacterial community. This reinforces the influence of phytoplankton community on the natural dissolved carbohydrate pool, besides the significance of such carbon source on the bacterial community dynamic.

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