Changes in diatom associations with altitudinal gradient and land use in Itajaí-Mirim River, Southern Brazil

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ABSTRACT – The present study aimed to describe the floristic composition and the changes of diatom associations in the Itajai-Mirim River along its altitudinal gradient and with different land uses. The study was based on quarterly sampling of epilithon at six stations between 2004 and 2006. A total of 79 taxa, distributed in 24 families and 36 genera, were identified. The families with the highest species richness were *Naviculaceae*, *Gomphonemataceae*, *Cymbellaceae*, and *Bacillariaceae*. These families along with the *Achnanthidiaceae* had the greatest relative abundances. Changes in diatom associations along the altitudinal gradient of the Itajaí-Mirim River were more evident in lowland areas, with anthropogenic activities such as sand extraction and discharge of domestic and industrial effluents. The important species, as provided by the Important Species Index, reflected the environmental conditions.

Key-words: epilithon, Important Species Idex, subtropical region

RESUMO – **Mudanças nas associações de diatomáceas relacionadas ao gradiente altitudinal e uso do solo no rio Itajaí-Mirim, Sul do Brasil.** O presente estudo teve como objetivo descrever a composição florística e as mudanças nas associações de diatomáceas ao longo do gradiente altitudinal do rio Itajaí-Mirim e com diferentes usos do solo. O estudo baseou-se em coletas trimestrais do epilíton, em seis pontos, entre 2004 e 2006. Um total de 79 táxons foram identificados, distribuídos entre 24 famílias e 36 gêneros. As famílias mais representativas em relação à riqueza específica foram *Naviculaceae*, *Gomphonematacea, Cymbellaceae*, e *Bacillariaceae*, que também foram as mais abundantes, juntamente com *Achnanthidiaceae*. Mudanças nas associações de diatomáceas ao longo do gradiente altitudinal do rio Itajaí-Mirim foram mais evidentes nas áreas de menor altitude com atividades antropogênicas, como extração de areia e descarga de efluentes domésticos e industriais. As espécies importantes fornecidas pelo índice ISI refletiram as condições ambientais.

Palavras-chaves: epilíton, ISI índice, região subtropical

INTRODUCTION

Periphytic algae are considered a group with the highest contribution to primary productivity in many aquatic environments. The species respond quickly to environmental changes due to the high turnover as well as having numerous opportunistic competitive strategies, characteristics that ensure success in the establishment of these organisms in lotic systems (Stevenson 1996). The epilithic communities, specially the diatoms species that show ecological tolerances are used for assessing water quality and the land use in several countries (e.g. Coste *et al.* 2009, Fetscher *et al*. 2014, Gallo *et al.* 2013, Lobo *et al.* 2002, Tan *et al*. 2013). Recent *revisions* of the periphytic diatoms as bioindicator are presented in Rimet (2012) and Lobo (2013).

The Itajai-Mirim River has an importance in the socio-*economic* and *regional context, but* in some places the land use is inappropriate (Minatti-Ferreira & Beaumord 2006). Although pristine areas are observed in many sites of the river, sand mining, industrial and domestic effluents discharges, deforestation, erosion of soil, and farm lands where pesticides are used, can also be found in other sites (Conceição 2004, Minatti-Ferreira & Beaumord 2006).

The periphytic diatom communities in the Itajaí-Mirim River have not been well studied to date. The first study carried out with epilithic diatoms was conducted only in the spring of 2003, where 22 species were reported as well as the spatial variation in some populations within the river (Burliga *et al*. 2005). In this context, the current research aimed to study the composition of the diatom flora, highlighting their richness and abundance pattern along the river during two years, and their relation with the altitudinal gradient and land use.

MATERIAL AND METHODS

Study Area

The Itajai-Mirim River Basin is located in the Itajai Valley, Santa Catarina State, between 26 °53’17 “and 26 ° 56’05” South 48 ° 40’57 “and 48 ° 44’12” West (Fig.1). Climate is humid subtropical mesothermal with hot summer (Cfa) according to the Köppen classification (Köppen & Geiger 1928). Six sampling points were distributed in the Medium-High Itajai-Mirim region, covering the cities of Brusque, Botuverá and Vidal Ramos (Fig. 1), with elevation ranging between 16 and 404 meters above sea level (Fig. 2). Sampling site A is located downstream of Brusque downtown in a semi-urban area, where domestic and industrial effluents discharge, as well as mining of sand were registred. Sampling site B is located in downtown Brusque with intense urban occupation where both domestic and industrial discharges were also registred. Sampling site C is located downstream of downtown Botuverá, showing a mosaic of grassland and dense vegetation. Site D is located in the rural area of Vidal Ramos, next to the mouth of Areia stream. Above this sampling site the river drains a large densely forested area. Site E is also located in the Vidal Ramos County, with pristine areas, although tobacco farmlands are found in some stretches upstream. Sampling site F is located upstream of the urban center of Vidal Ramos, in a tributary of the Itajaí-Mirim River with preserved marginal vegetation but also under the influence of food crops and tobacco.

Sampling and Analysis

Fieldwork was conducted seasonally between May 2004 and February 2005, and May 2005 to February 2006 covering the four seasons. Dissolved oxygen and temperature of surface water were measured using a portable Digimed DM4P meter, pH using a Digimed DM-2P meter, and electrical conductivity using Thermo Orion 162-A meter. Suspended particulate matter (SPM) was determined according to APHA (1995). The rainfall data for the region were obtained from Agência Nacional de Águas (2006). For qualitative and quantitative diatom analysis, samples were obtained by scraping off the upper surface (15 to 20 cm in diameter) of twelve submerged stones using a toothbrush. The material was preserved with 4% formalin and stored in polyethylene bottles of 250 mL (adapted from Kelly *et al.* 1998). The diatom samples were oxidized following the method described by Simonsen (1974), and mounted on permanent slides with Entellan®.

Identification of species was based on Frenguelli (1923), Patrick & Reimer (1966), Krammer & Lange-Bertalot (1986, 1988, 1991), Souza (2002) and Van de Vijver *et al.* (2013). The classification was based on Medlin & Kaczmarska (2004) and Round *et al.* (1990). The relative abundance of diatom valves was determined by counting transects on permanent slides, reaching 80% of sample sufficiency (Pappas & Stoermer 1996).

To verify the significant differences between the average abundance and species richness among the samples, the one-way ANOVA test was applied using

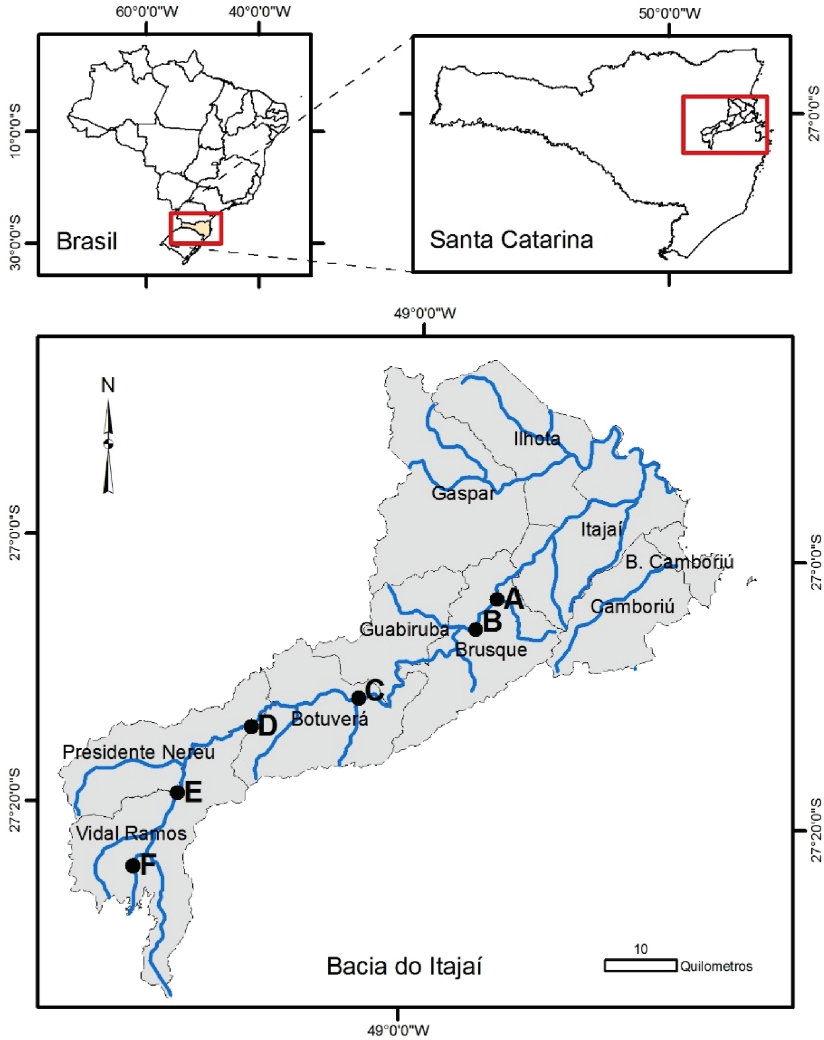

Fig. 1. Location of Itajai-Mirim River Basin, in Santa Catarina State. Position of the sampling sites is indicated by letters (A-F).

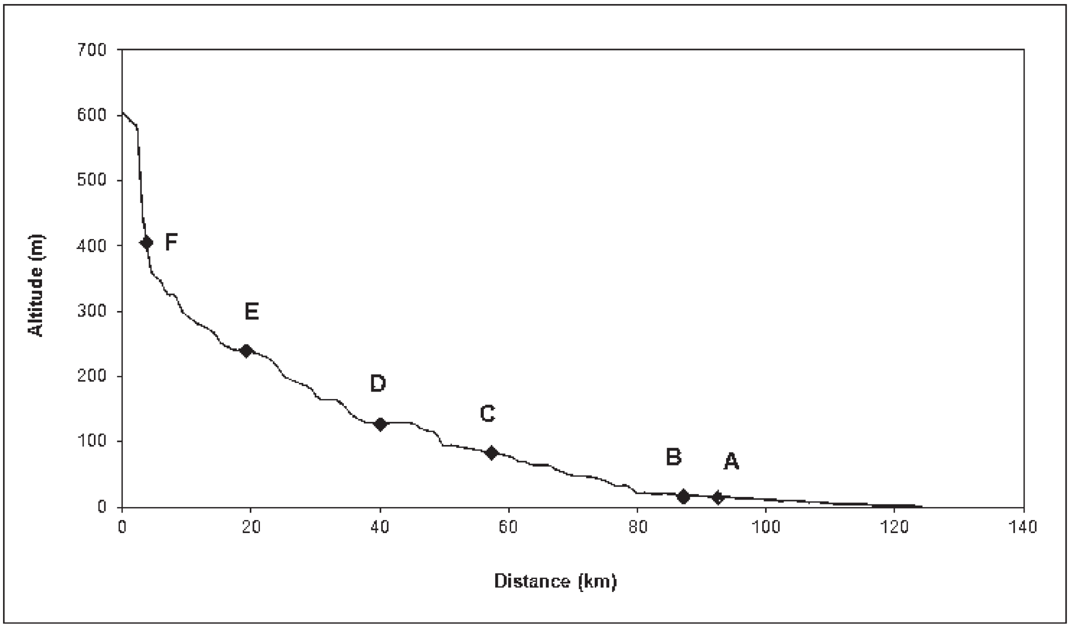

Fig. 2. Altimetric profile of the sampling sites (A - F), of the Itajai-Mirim River, Santa Catarina State, Brazil.

the software PAST version 1.95 (Hammer *et al.* 2001). The statistical requirements to use ANOVA (homogeneity of variance, residuals and normal distribution) were proved.

Important Species Index ISI (Ross & Rushforth 1980, Sgro *et al*. 2007) was used, where index values greater than or equal to 1.0 pointed out the most important species. Sample sufficiency was tested using species accumulation curves and the index Chao 1 (Colwell *et al.* 2004, Colwell 2005).

Cluster analysis was conducted on the matrix of species abundance with log transformed data, using Ward as linking method, available in PC-ORD, version 5.10 (McCune & Mefford 2006). Discriminant analysis (SAS Institute 2000) was also performed to test the hypotheses that there are no differences in the diatom assemblages between the periods (2004-2005 and 2005-2006) (periods I and II), temporal differences in the associations between campaigns (May, August, November and February) and spatial differences in the assemblages of diatoms among the six sampling sites (A, B, C, D, E and F). In this analysis we used data matrix of relative abundance Ln (x+1) transformed, based on 36 species which comprised 97.5% of total relative abundance of the dataset.

RESULTS AND DISCUSSION

Environmental conditions

During the first study period (between May 2004 and January 2005) the rainfall pattern followed that of historical average amounts, and during the second period (May 2005 and February 2006) the rainfall pattern changed considerably, with a drier late summer; autumn, winter and early spring were wettest and the late spring and early summer were drier than the historical average (Fig. 3).

In general, physical and chemical factors differed along the altitudinal gradient of the river, with some variation between the two annual cycles (Table 1). Conductivity increased at some high sites (E, F) and lower sites (A, D), while dissolved oxygen concentrations tended to decrease toward the lower sampling sites. The lowest values of dissolved oxygen at sites A and B are possibly related to the contribution of domestic and industrial effluents in the lower river. The pH ranged from 5.9 (site F) to 8.0 (sites D and E), while the average was 6.9, a value which is also referred to the Camboriú River (Antunes *et al.* 2007). Concentrations of suspended particulate matter levels were high at sites with soils exposed to the activity of sand mining and agricultural activities (sites A, E, F). Other studies in Itajaí-Mirim River also found critical conditions of water quality in the Middle-Lower river, with high concentrations of nitrate, ammonium, organic phosphorus, total phosphorus, biochemistry oxygen demand and suspended particulate matter Coelho 2004, Pereira-Filho *et al.* 2010).

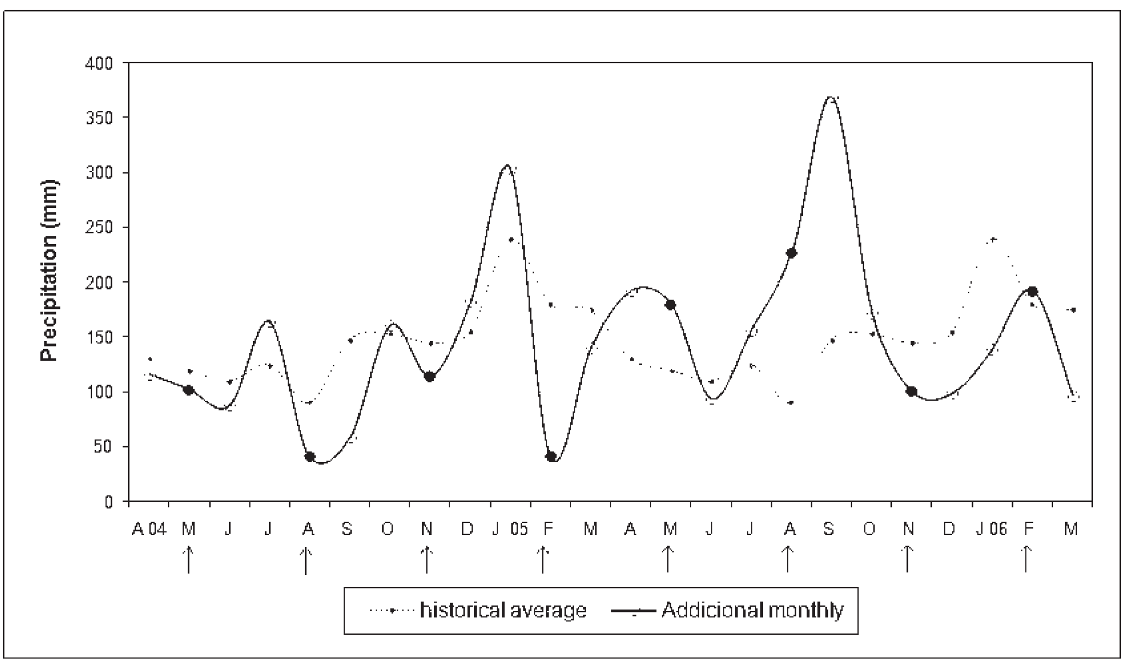

Fig. 3. Rainfall in the Itajai region during the study period. Dark circles indicate the sampling months.

Table 1. Average and standart deviation of the physical and chemical factors of surface water sampling conditions at the six sampling sites on the Itajai-Mirim River, during May 2004 to February 2005 (I) and May 2005 to February 2006 (II), at different altitudes and land use conditions (TEMP = Temperature; COND = Condutividade; DO = Dissolved Oxygen; SPM = Suspended Particulate Matter.

Variables/Sampling sites	A	B	C	D	E	F
Latitude	70°98'66" N	70°69'84" N	69°09'08" N	67°61'20" N	66°61'43" N	66°01'82" N
Longitude	70°05'053" E	70°00'791" E	69°90'950" E	69°86'705" E	69°77'301" E	69°67'068" E
Altitude (m)	16	18	76	127	240	404
I						
TEMP (ºC)	17,3 (1,0)	17,1 (0,9)	16,1 (1,4)	16,2 (1,8)	15,4 (1,9)	14,6 (2,4)
pH	6,7 (0,04)	6,7 (0,26)	7,2 (0,23)	7,4 (0,33)	6,9 (0,58)	6,6 (0,69)
COND (mS/cm)	0,27 (0,02)	0,11 (0,01)	0,06 (0,01)	0,04 (0,01)	0,05 (0,01)	0,47 (0,41)
SPM (mg L^{-1})	63,7 (14,7)	24,2 (15,2)	52,5 (36,7)	17,5 (8,5)	151,0 (135,0)	49,5 (41,5)
DO (mg L^{-1})	6,68 (0,73)	7,40 (0,13)	9,39 (0,79)	8,84 (0,94)	10,38 (1,12)	9,96 (1,53)
II						
TEMP (ºC)	23,0 (2,0)	22,7(1,7)	22,7 (1,7)	22,5 (1,5)	21,5 (1,5)	19,5 (1,5)
pH	6,7 (0,3)	7,0 (0,5)	7,2 (0,2)	7,4 (0,6)	7,2 (0,7)	7,0 (0,5)
COND (mS/cm)	0,70 (0,55)	1,20 (0,05)	0,35 (0,20)	0,22 (0,07)	0,37 (0,27)	0,45 (0,30)
SPM (mg L^{-1})	30,5 (10,50)	14,5 (5,50)	7,57 (7,42)	8,3 (0,05)	7,65 (7,35)	0,35 (0,05)
DO (mg L^{-1})	5,10 (0,40)	5,15 (0,35)	5,75 (0,75)	5,80 (0,20)	5,80 (0,20)	5,05 (0,55)
Land use	sand extraction domestic sewage industrial wastewater	sand extraction domestic sewage industrial wastewater	farm plantations forest area	farm plantations forest area	tobacco plantations farm plantations forest area	tobacco plantations farm plantations forest area

Floristic composition

The floristic composition of diatoms was represented by 79 species, distributed in 24 families and 36 genera according to Table 2.

The families with higher species richness were *Naviculaceae* (13 spp.), *Gomphonemataceae* (9 spp.), *Cymbellaceae* (9 spp.), and *Bacillariaceae* (7 spp.). With respect to abundance, these families, along with *Achnanthaceae*, also had the highest relative abundances (Fig. 4).

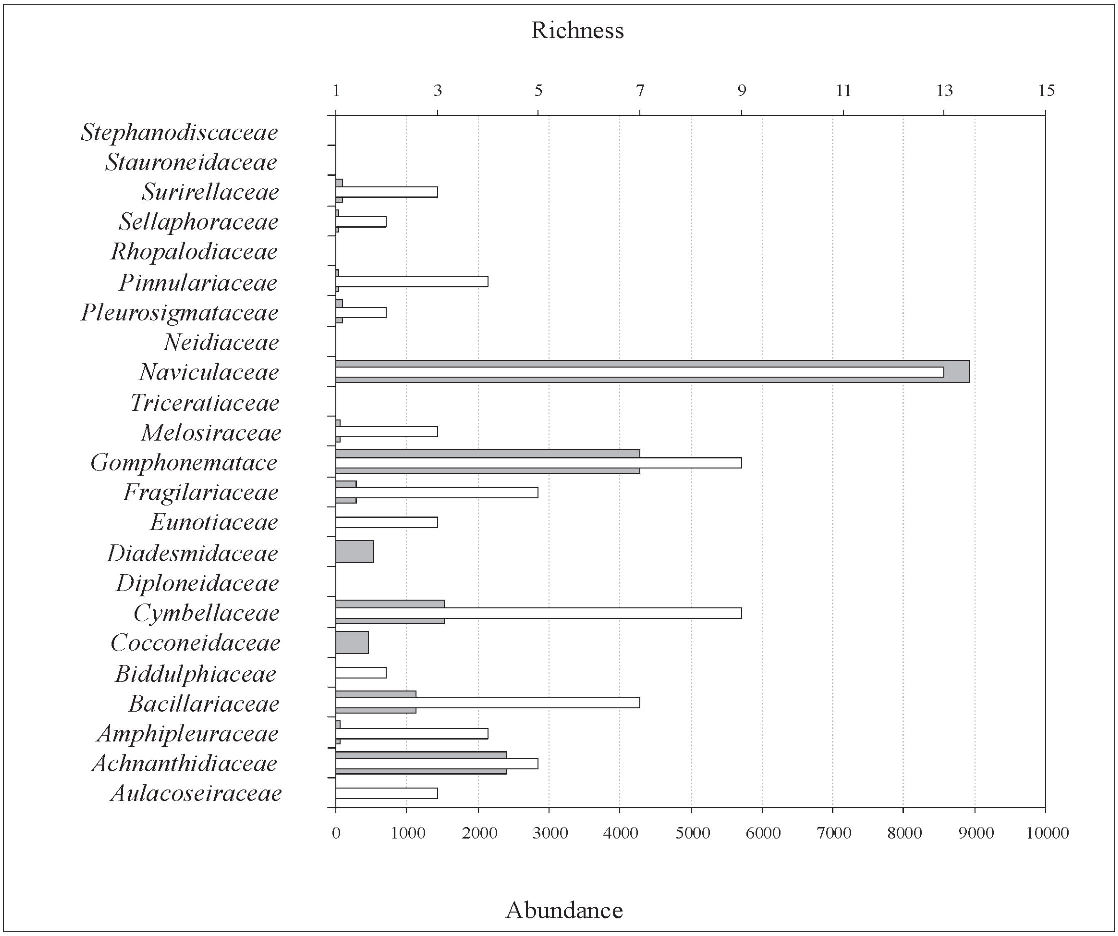

Fig. 4. Relative abundance of the diatom families (light bars) and species richness (black bars) in Itajaí-Mirim River, during the study period.

The genera *Navicula* Bory, *Gomphonema* Eherenberg, *Nitzschia* Hassal and *Encyonema* Kützing had the greatest number of species. These genera were also representative in lentic system (Lobo & Torgan 1988, Salomoni & Torgan 2008), and in lotic systems in the subtropical region of Brazil (Rodrigues & Bicudo 2001, Oliveira *et al.* 2001, Lobo *et al.* 2002 2004b, Hermany *et al* 2006, Salomoni *et al.* 2006, 2011, Schneck *et al.* 2010, Bere 2010, Moresco *et al.* 2011).

Table 2. List of the diatoms observed in in the Itajai-Mirim River between May 2004 and February 2006.

Division *Bacillariophyta*
Subdivision *Bacillariophytina*
Class *Bacillariophyceae*
Order *Achnanthales*
Family *Achnanthidiaceae*
- *Achnanthes coarctata* (Brébisson *ex* W.Smith) Grunow
- *Achnanthes hungarica* (Grunow) Grunow
- *Achnanthidium exiguum* (Grunow) Czamecki
- *A. minutissimum* (Kützing) D.B. Czarne
- *Planothidium lanceolatum* (Brébisson *ex* Kützing) F.E. Round & L. Bukhtiyarova cki

Family *Cocconeidaceae*
- *Cocconeis placentula* Ehrenberg

Order *Bacillariales*
Family *Bacillariaceae*
- *Hantzschia amphioxys* (Ehrenberg) Grunow
- *N. amphibia* Grunow
- *Nitzschia clausii* Hantzsh
- *N. gracilis* Hantzsch
- *N.* cf. *inconspicua* Grunow
- *N. palea* (Kützing) W.Smith
- *N. sigma* (Kützing) W.Smith
- *Tryblionella levidensis* W.Smith

Order *Cymbellales*
Family *Cymbellaceae*
- *Cymbella affinis* Kützing
- *C. tumida* (Brébisson) Van Heurck
- *Encyonema mesianum* (Cholnoky) D.G.Mann
- *E. minutum* (Hilse) D.G.Mann
- *E. neomesianum* Krammer
- *E. perpusillum* (A. Cleve) D.G.Mann
- *E. silesiacum* (Bleisch ex Rabenhorst) D.G.Mann
- *E. ventricosum* (Agardh) Grunow
- *Placoneis disparilis* (Hustedt) Metzeltin & Lange-Bertalot

Family *Gomphonemataceae*
- *Gomphonema affine* Kützing
- *G. angustatum* (Kützing) Rabenhorst
- *G. brasiliense* Grunow
- *G.* cf. *clevei* Fricke
- *G. gracile* Eherenberg emend. Van Heurck
- *G. lanceolatum* C.A. Agardh
- *G. parvulum* (Kützing) Van Heurck
- *G. subclavatum* Grunow
- *G. truncatum* Ehrenberg

Order *Eunotiales*
Family *Eunotiaceae*
- *Eunotia exigua* (Brébisson ex Kützing) Rabenhorst
- *E.* cf. *sudetica* O.F. Müller
- *Eunotia sp.*

Order *Naviculales*
Family *Amphipleuraceae*
- *Amphipleura lindheimeri* Grunow
- *Frustulia rhomboides* (Ehrenberg) deToni
- *F. vulgaris* (Thwaites) deToni

Family *Diploneidaceae*
- *Diploneis subovalis* Cleve

Family *Diadesmidaceae*
- *Luticola geoppertiana* (Bleisch) D.G.Mann

Family *Naviculaceae*
- *Geissleria aikenensis* (Patrick) Torgan & Oliveira
- *Geissleria sp.*
- *Hippodonta hungarica* (Grunow) Lange-Bertalot, Metzeltin &Witkowski
- *Diadesmis contenta* (Grun. *ex* Van Heurck) Mann
- *Mayamaeae atomus* (Kützing) Grunow
- *Navicula capitatoradiata / N. rostellata*
- *N. cryptocephala* Kützing
- *N. cryptotenella* Lange-Bertalot
- *N. exilis* Kützing
- *N. minima* (Grunow) Van Heurck
- *N. symmetrica* R.M.Patrick
- *Navicula* sp.

Family *Neidiaceae*
- *Neidium* sp.

Family *Pinnulariaceae*
- *Pinnularia mesolepta* (Ehrenberg) W.Smith
- *P. microstauron* (Ehrenberg) Cleve
- *Pinnularia* sp1
- *Pinnularia* sp2

Family *Pleurosigmataceae*
- *Gyrosigma acuminatum* (Kützing) Rabenhorst
- *G. scalproides* (Rabenhorst) Cleve

Family *Sellaphoraceae*
- *Sellaphora pupula* (Kützing) Mereschk.
- *S. rectangularis* (Gregory) Lange-Bertalot & Metzeltin

Family *Stauroneidaceae*
- *Caloneis westii* (W.Smith) Hendey
- *Capartogramma crucicula* (Grunow) R. Ross

Order *Fragilariales*
Family *Fragilariaceae*
- *Fragilaria capucina* Desmazière
- *F. goulardii* (Brébisson) Lange-Bertalot
- *Synedra* sp.
- *Ulnaria ulna* (Nitzsch) Compère

Order *Rhopaloidales*
Family *Rhopalodiaceae*
- *Rhopalodia gibberula* Ehrenberg

Order *Surirellales*
Family *Surirellaceae*
- *Surirella angusta* Kützing
- *Surirella* sp1
- *Surirella* sp2

Classe *Mediophyceae*
Ordem *Biddulphiales*
Family *Biddulphiaceae*
- *Terpsinoe musica* Ehrenberg
- *Hydrosera whampoensis* (Schwarz) Deby

Subdivision *Coscinodiscophytina*
Class *Coscinodiscophyceae*
Order *Aulacoseirales*
Family *Aulacoseiraceae*
- *A. ambigua* (Grunow) Simonsen
- *A. distans* (Ehrenberg) Simonsen
- *Aulacoseira granulata* (Ehrenberg) Simonsen

Order *Melosirales*
Family *Melosiraceae*
- *Melosira varians* C.A. Agardh

Class *Mediophyceae*
Order *Thalassiosiraceae*
Family *Stephanodiscaceae*
- *Cyclotella meneghiniana* Kützing

Order *Triceratiales*
Family *Triceraticeae*
- *Pleurosira laevis* (Ehrenberg) Compére

The species accumulation curve showed the observed value (79 species) versus the expected values calculated from the Chao index (76, 74-87), indicating satisfactory, sufficient sampling of the assemblage (Fig. 5).

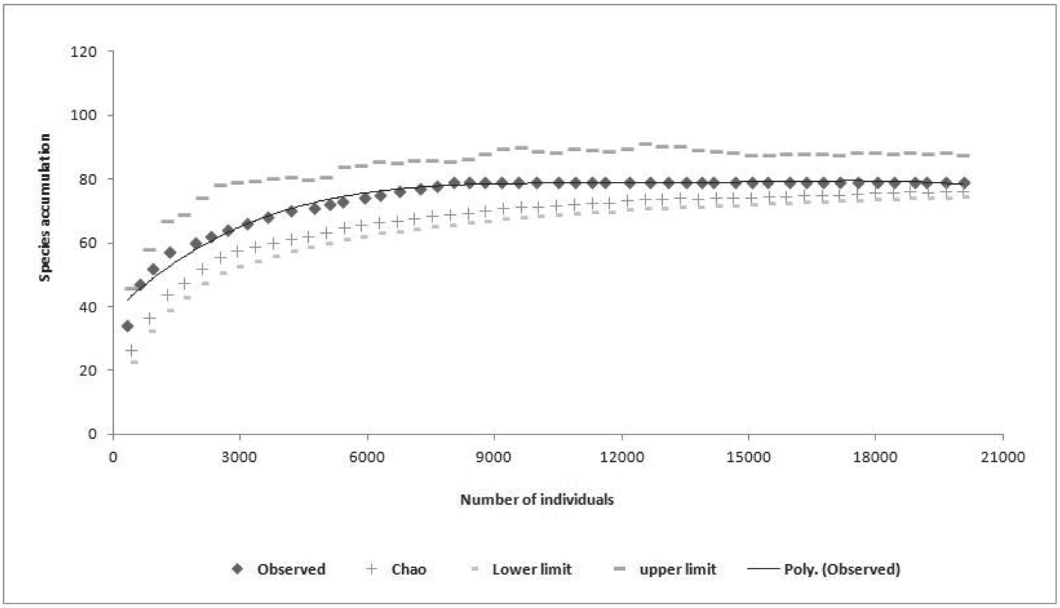

Fig. 5. Species accumulation curve as a function of the observed abundance compared with the expected curve and their limits simulated by the model using the Chao 1 index.

Richness and Abundance

Species richness ranged from 10 species (D) to 34 species (B and D), with an average of 21 species (Fig. 6). The means of relative abundance and species richness were not significantly different between the sample sites (p=0.16 and p=0.32).

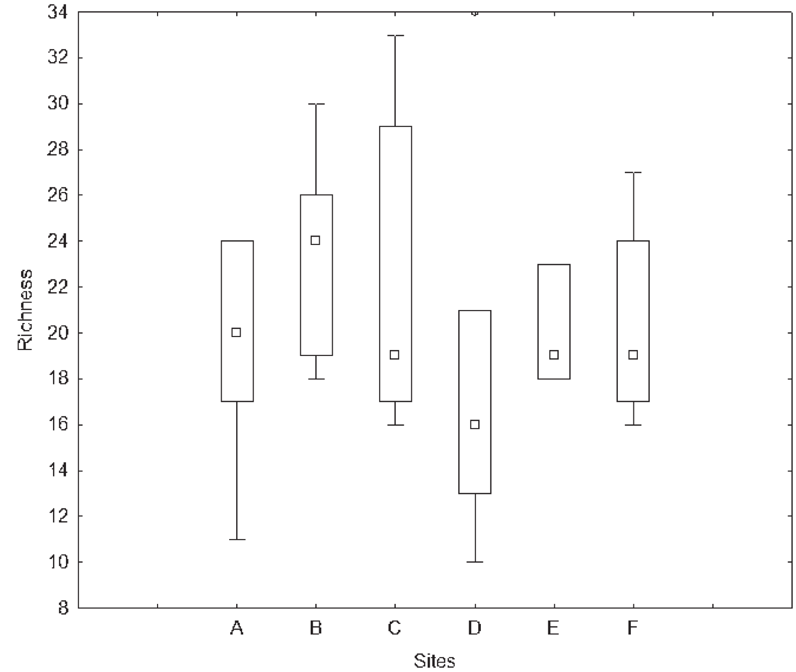

Fig. 6. Boxplot showing the variation in diatoms species richness from Itajaí-Mirim River during the study period.

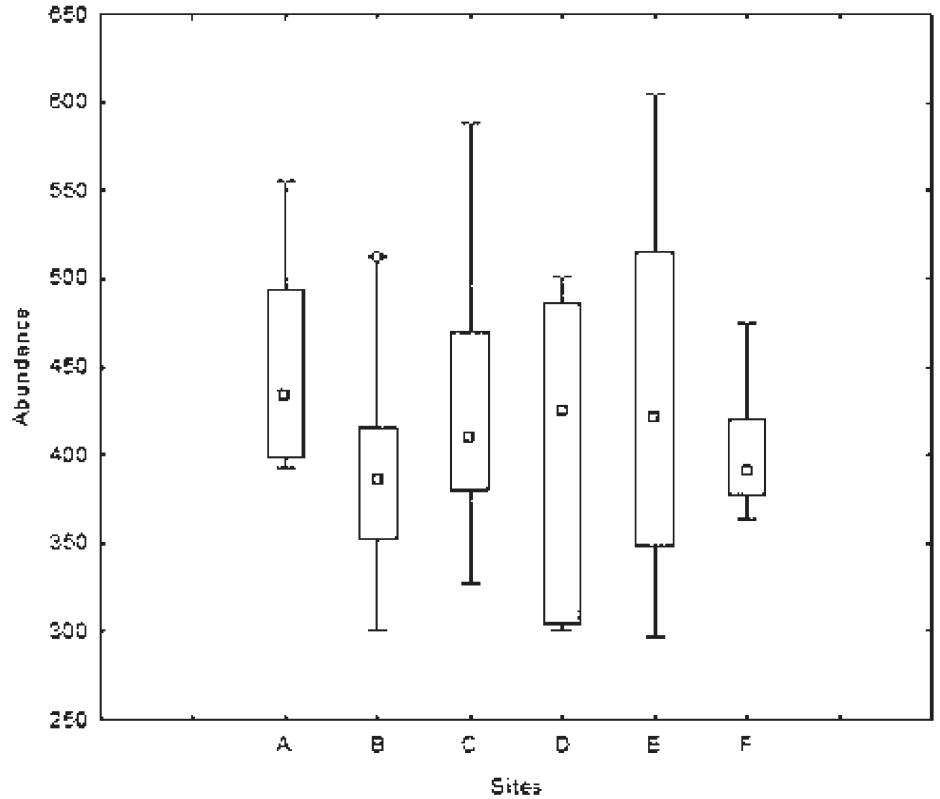

Fig. 7. Boxplot showing the variation in diatoms species richness from Itajaí-Mirim River during the study period.

Time-space variation of diatom associations

In the cluster analysis, based on species relative abundance data, we identified two main groups, reflecting temporal variation (Fig. 8). Group 'A' is comprised of samples taken in the period 2004-2005, and group 'B' is comprised of samples taken during the sampling campaigns of 2005-2006. The species *Gomphonema parvulum* and *Mayamea atomus* had higher relative abundances in the years 2004-2005, which also recorded higher values of suspended particulate matter and dissolved oxygen, when campaigns 2004-2005 (Group A) and 2005-2006 (Group B) are compared separately (Table 1).

The species *Encyonema minutum* (Hilse), *Navicula cryptocephala,Planothidium lanceolatum* and *Nitzschia palea* had highest relative abundances in collections (2005-2006 period, group B), which also had the highest values of temperature, pH, and conductivity.

In discriminant analysis, all hypotheses were rejected, indicating that there were highly significant differences (Wilks' Lambda = 0.0095, F = 31.99, GL = 36, P-value <0.0001) in diatoms associations between the two collection periods (2004-2005 and 2005-2006). There were also significant differences (Wilks' Lambda = 0.0015, F =2.02, Degrees of freedom (DF) = 108, P-value = 0.0175) in diatoms associations between months (May, August, November and February). These results suggest differences in the diatom community structure due to the hydrological cycle between sampling years (Fig. 9).

Highly significant differences were found (Wilks' Lambda = 0.0001, F = 2.81, DF =180, P-value <0.0001) in the associations between the diatom sampling points, showing spatial variation of the assemblages diatoms along the altitudinal gradient of the river. The position of sites A and B on the graph coordinates, separated by the other sites, where these sites are located in stretches of low altitude and at higher impact on land use (Fig. 9).

Important species index (ISI)

Fourteen species were identified by Important Species Index (ISI). They are: *Gomphonema parvulum* (ISI = 14.35), *Navicula symmetrica* (ISI =

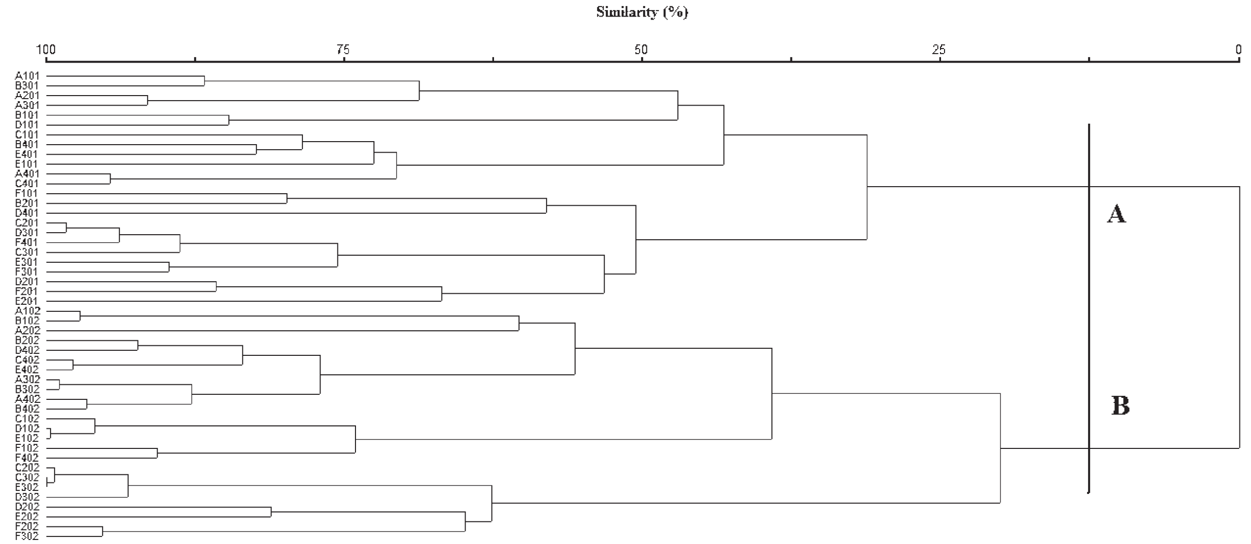

Fig. 8. Cluster analysis of sampling sites, based on diatom relative abundances found in Itajaí-Mirim River.

10.91), *N. cryptocephala* (ISI = 5.57), *Planothidium lanceolatum* (ISI = 4.58), *Diadesmis contenta* (ISI = 4.39), *Nitzschia palea* (ISI = 3.77), *Cocconeis placentula* (SI = 2,01), *Achnanthidium minutissimum* (ISI = 14.52), *Mayamea atomus* (ISI = 1.91), *Encyonema minutum* (ISI = 1.88), *Achnanthidium exiguum* (ISI = 1.47), *Geissleria aikenensis* (ISI = 1.47), *Encyonema perpusillum* (ISI = 1.38) and *Gomphonema affine* (ISI = 1.14). These species are considered tolerant to organic pollution, according to studies from South America (Gomez & Licursi 2001, Licursi & Gomez 2003, Lobo *et al.* 2002, 2004 a, b, c, Salomoni *et al.* 2006). The figure 10 shows the spatial variation of the nineteen more abundant species (that contributed above 2% of the total abundance) in each site along the altitudinal gradient of the river, including ISI species.

Gomphonema parvulum occurred in all the sites and its relative abundance increased downstream. On the other hand, *Achnanthidium minutissimum, Diadesmis contenta* and *Planothidium lanceolatum* occurred in highest altitude sites (C, D, E, F) while

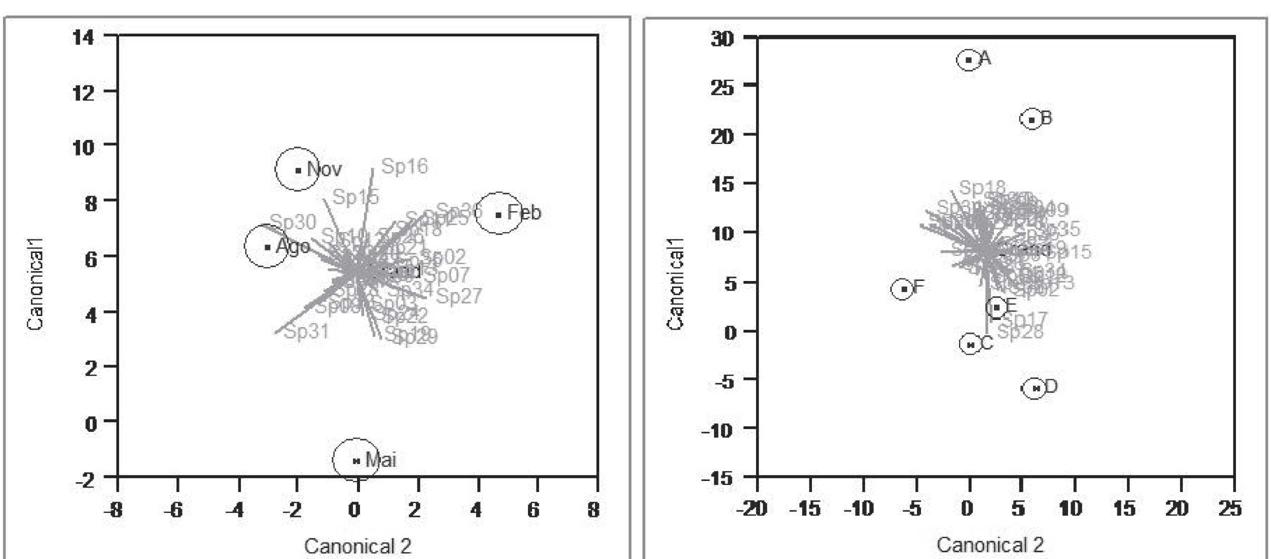

Fig. 9. Results of discriminant analysis showing the centroids of the groups tested species and vectors, projected on the two canonical axes, to distinguish seasonal (left) and spatial (right) of the associations of diatoms from Itajaí-Mirim River in the study periodo.

Cocconeis placentula was exclusive to the highest sites (F). In these places there is less anthropogenic pressure in relation to sand mining, evidenced by the gradual reduction of suspended particulate material when compared to low sites (A and B), especially in between the years 2005-2006. *Mayamaea atomus, Nitzschia palea, Geissleria aikenensis, Encyonema minutum, Gomphonema affine, G. angustatum* and *Luticola goeppertiana* were only found at sites A and B, which are more disturbed in relation to land use.

Many *studies have* also *demonstrated* the spatial and temporal changes of periphytic algae assemblages in streams are influenced by anthropogenic activities (Lobo *et al.* 2004 a, b, c, Dela-Cruz *et al.* 2006, Walker & Pan 2006, Hermany *et al.* 2006, Sgro *et al.* 2007, Salomoni *et al.* 2011). This study further on to show the influence of land use on the spatial and temporal changes of the diatom associations could also show that the ISI index were able to reflect the environmental conditions.

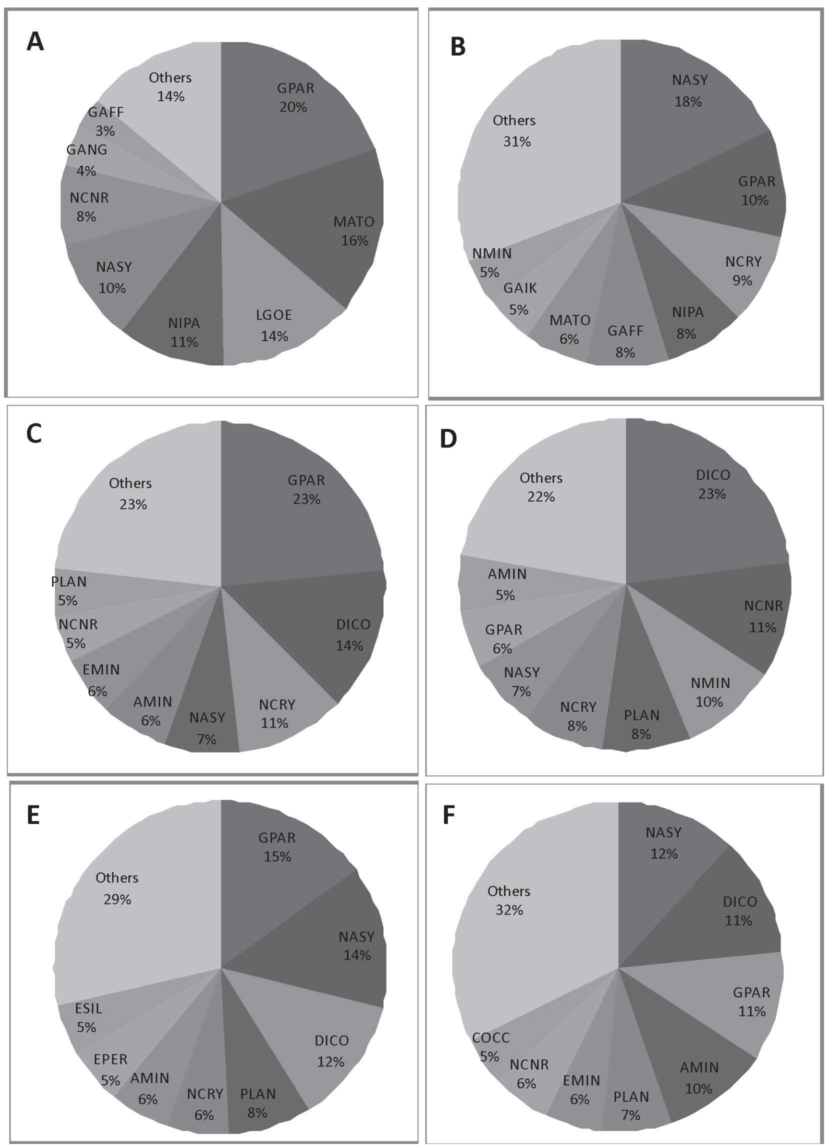

Fig. 10. Spatial variation of diatom relative abundances (%) in six sites from Itajai-Mirim River during the study period. Legend: *Achnanthidium minutissimum* (AMIN); *Cocconeis placentula* (COCC); *Diadesmis contenta* (DICO); *Encyonema minutum* (EMIN); *E. perpusillum* (EPER); *Geissleria aikenensis* (GAIK); *Encyonema silesiacum* (ESIL); *Gomphonema affine* (GAFF); G. *angustatum* (GANG); *G. gracile* (GGRA); *G. parvulum* (GPAR); *Luticola goeppertiana* (LGOE); *Mayamea atomus* (MATO); *Navicula capitatoradiata/N. rostellata* (NCNR); *N. cryptocephala* (NCRY); *N. minima* (NMIN); *N. symmetrica* (NASY); *Nitzschia palea* (NIPA); *Planothidium lanceolatum* (PLAN).

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