Use of epilithic diatoms as bioindicators from lotic systems in southern Brazil, with special emphasis on eutrophication.

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ABSTRACT: Use of epilithic diatoms as bioindicators from lotic systems in southern brazil, with special emphasis on eutrophication. **Epilithic diatom biocenoses have been recommended by researchers from several countries as particularly suitable for water quality evaluation. However, saprobic systems using diatoms have been developed to assess water organic pollution, not taking into account the effects of eutrophication on the biological composition of the communities. Thus, the main aim of this study was to determine the tolerance of diatom species to eutrophication in three streams (Sampaio, Grande and Bonito) of the Mato Leitão countryside area, RS, Brazil, using multivariate analyses techniques. Results of physical, chemical and biological analyses obtained in studies carried on in the area between the years of 1993 to 1998 were used to develop the present study. Multivariate analyses of species composition data were applied in two different approaches. Firstly, species and sampling units were grouped using TWINSPAN (Two-way Indicator Species Analysis). Secondly, Canonical Correspondence Analysis (CCA) was applied in order to uncover the main gradients of changes in species composition, relating these changes to the eutrophication process. From the levels of tolerance to eutrophication determined for each diatom species, they were given an indicative value from 1 to 5, which corresponded, respectively, to very low, low, medium, high and very high tolerance levels, thus allowing the calculation of the Biological Water Quality Index (BWQI). These results are complementary to the saprobic system already proposed for use in Southern Brazilian rivers.**
Key-words: **diatoms, bioindicators, water quality assessment, organic pollution, eutrophication.**

RESUMO: Utilização de algas diatomáceas epilíticas como organismos bioindicadores de sistemas lóticos sul brasileiros, com especial enfoque à eutrofização**. Biocenoses de diatomáceas epilíticas têm sido recomendadas por pesquisadores em muitos países como particularmente adequadas para avaliar a qualidade da água. Contudo, os sistemas de sapróbios que utilizam diatomáceas têm sido desenvolvidos para avaliar a poluição orgânica da água, desconsiderando os efeitos da eutrofização na composição biológica das biocenoses. Assim, o objetivo principal deste trabalho foi determinar a tolerância de espécies de diatomáceas à eutrofização nos arroios Sampaio, Grande e Bonito, Município de Mato Leitão, RS, Brasil, utilizando análises multivariadas. Para a execução deste trabalho, foram utilizados os resultados físicos, químicos e biológicos obtidos em estudos desenvolvidos nestes arroios, entre os anos de 1993 a 1998. Análises multivariadas dos dados da composição de espécies foram aplicadas em duas formas distintas. Primeiro, as espécies e as estações de amostragem foram agrupados utilizando TWINSPAN (Análise de Espécies Indicadoras de Dupla Entrada). Segundo, Análise de Correspondência Canônica (CCA) foi aplicada para revelar os principais gradientes das mudanças na composição de espécies, relacionando esta mudança com o processo de eutrofização. A partir da determinação dos distintos graus de tolerância à eutrofização das espécies de diatomáceas,**

foram-lhes atribuídos valores indicativos de 1 a 5, correspondentes a níveis de tolerância muito baixa, baixa, média, alta e muito alta, respectivamente, possibilitando desta forma o cálculo do Índice Biológico de Qualidade da Água (IBQA). Estes resultados vêm complementar o sistema de sapróbios proposto para rios sul brasileiros.
Palavras-chaves: **diatomáceas, bioindicadores, avaliação qualidade da água, poluição orgânica, eutrofização.**

Introduction

Pollution of surface freshwaters is one of the greatest environmental issues in the world. Along these lines, the approaches to water quality evaluation can be basically divided into two categories. The first utilizes physical and chemical methods, and the second considers biological methods of evaluation (Lobo & Callegaro, 2000).

Regarding the first approach, physical and chemical methods allow only instantaneous measurements, therefore restraining the knowledge of water conditions to the moment when the measurements were taken. These limitations become more serious when the object of study is a lotic system (running waters, such as rivers and streams) where current promotes the continuous renewal of the water at each site. However, periodical measurements over a long period of time significantly enhance the information value of physical and chemical methods, since the discrete aspect of the data is reduced.

Considering the second approach, when biological methods are used to monitor water quality, long-term environmental effects can be detected, since these methods have the capacity of reflecting conditions which are not anymore present at the time of sampling and analysis, but were originated from the process of community development. Therefore, physical and chemical methods are complementary to biological methods. Together they constitute the basis to a correct assessment of the quality of running waters (Lobo & Callegaro, 2000).

Among the distinct aquatic communities used for the evaluation of freshwater quality, epilithic diatoms seem to be particularly suitable for this purpose (Kelly, 2002; Round, 1993; Schoeman & Haworth, 1986). In Brazil, however, the use of this group of organisms for water quality assessment has received little attention, and most of the investigations have been concentrated on the use of biotic indices. Hence, some studies related to this question have been published, especially in Southern Brazil (Lobo & Bender, 1998; Lobo & Ben da Costa, 1997; Oliveira et al., 2001; Rodrigues & Lobo, 2000; Lobo et al., 1995, 1996, 1999).

Lobo et al. (2002) published the first Brazilian saprobic system, characterizing 3 differential diatom groups: Group A (highly pollution-tolerant species), Group B (pollution-tolerant species) and Group C (less pollution-tolerant species). This new classification, including saprobic values characterizing each differential group, can be utilized to calculate biotic water quality indices. According to several authors (Lobo et al., 1995, 1996, 1999, 2002; Oliveira et al., 2001) the use of the Saprobic Index (SI) proposed by Pantle & Buck (1955) is desirable.

Although sampling size has been significantly increased between the original system proposed by Lobo et al. (1996) and the system of Lobo et al. (2002) – from 79 to 183 samples – the characterization of differential group C was flawed due to the low relative abundances of the observed species.

This fact can be explained considering that the saprobic systems which utilize diatoms was developed to assess water organic pollution, not considering the effects of eutrophication on the biological composition of the communities. Environmental monitoring in lotic systems of the region have indicated the presence of eutrophication processes (Lobo & Ben da Costa, 1997; Lobo & Bender, 1998; Lobo et al. 1996, 1999).

Biotic indices which consider the influence of eutrophication on the composition of epilithic diatoms biocenoses, usually referred to as Trophic Diatom Indices (TDI), have been developed mostly in Europe (Kelly & Whitton, 1995; Schiefele & Kohmann, 1993; Kwandrans et al. 1998; Eloranta & Soininen, 2002; Wu & Kow, 2002). Gomez & Licursi (2001) have developed the Pampean Diatom Index (PDI) for water quality evaluation in lotic systems of the Argentinean Pampa. Unlike the previous indices mentioned, which considered the epilithic community, the PDI, which integrates effects of organic enrichment and eutrophication, is based on the sensitivity of the epipelic diatom community.

In this context, thus, the main aim of this work was to determine the tolerance to eutrophication of diatom species in three streams (Sampaio, Grande and Bonito) of the Mato Leitão countryside area, RS, Brazil, in order to complement the saprobic system proposed by Lobo et al. (2002).

Material and methods

Physical, chemical and biological analyses data were obtained in the project "Water quality study on Sampaio, Grande and Bonito Streams, Mato Leitão, RS, Brazil" (Lobo & Kirst, 1994, 1999), carried out between 1993 and 1998 were utilized. To obtain these data, seasonal scientific trips were done to 12 sampling sites located along Sampaio, Grande and Bonito Streams, between co-ordinates 29°21'12"-29°36'37" S and 51°19'36"-51°58'53" W (Tab.I) (Fig. 1). Physical, chemical and biological variables were utilized as parameters for assessment of organic pollution and eutrophication. The following physical and chemical variables were used: water temperature (ºC), pH, conductivity (Cond), turbidity (Turb), dissolved oxygen (DO), biochemical oxygen demand (BOD_5), dissolved inorganic nitrogen (DIN), phosphates (PO_4), and total solids (TS) (Tab. II). Water temperature and oxygen were measured in situ, using a field multi-measurer YSI model 6.000. Samples for determination of the remaining variables were collected from the sub-surface in glass bottles (300 or 1000 ml). They were packed in ice and analysed one day after sampling. Sampling Protocols for physical and chemical analyses followed those described in American Public Health Association (1999).

Epilithic algal samples for the identification and counts of the diatom (Bacillariophyceae) community were taken every three months, representing the four seasons of the year. For qualitative and quantitative analyses, samples of epilithic diatoms were scrubbed off the upper surfaces of submerged stones of 10-15 cm in diameter using a toothbrush. Samples were fixed with formalin, following the method of Kobayasi and Mayama (1982). Diatom samples were cleaned with sulphuric and hydrochloric acid and mounted in Pleurax. All specimens found in a number of transects across the slides were identified and counted up to a minimum of 600 valves scored (Schoeman, 1979; Kobayasi and Mayama, 1982). For species identification, the following taxonomical sources were used: Krammer (1997, 2000, 2002, 2003), Krammer & Lange-Bertalot (1986, 1988, 1991*a*, *b*), Lange-Bertalot (1993, 1995, 1996a, b, 2001), Lange-Bertalot & Metzeltin (1996), Lange-Bertalot & Moser (1994), Lange-Bertalot et al. (2003), Metzeltin & Lange-Bertalot (1998, 2002), Rumrich (2000), and Simonsen (1987). Abundant species were indicated according to the criterion of Lobo and Leighton (1986). Voucher samples are stored in the Herbarium DIAT-UNISC, from University of Santa Cruz do Sul, RS.

For an integrated analysis of physical, chemical and biological data, multivariate analysis techniques were applied. The software used was PC-ORD version 4.0 for Windows (McCune & Mefford, 1999), with abiotic data transformation performed by the software FITOPAC (Shepherd, 1996).

Firstly, species and samples were grouped using TWINSPAN - Two-way Indicator Species Analysis (Hill, 1979). At this stage, data were not yet transformed. Percentages of occurrence of abundant species were used to build the matrix of Biological data, calculated according to Lobo & Leighton (1986). Program defaults were followed applied

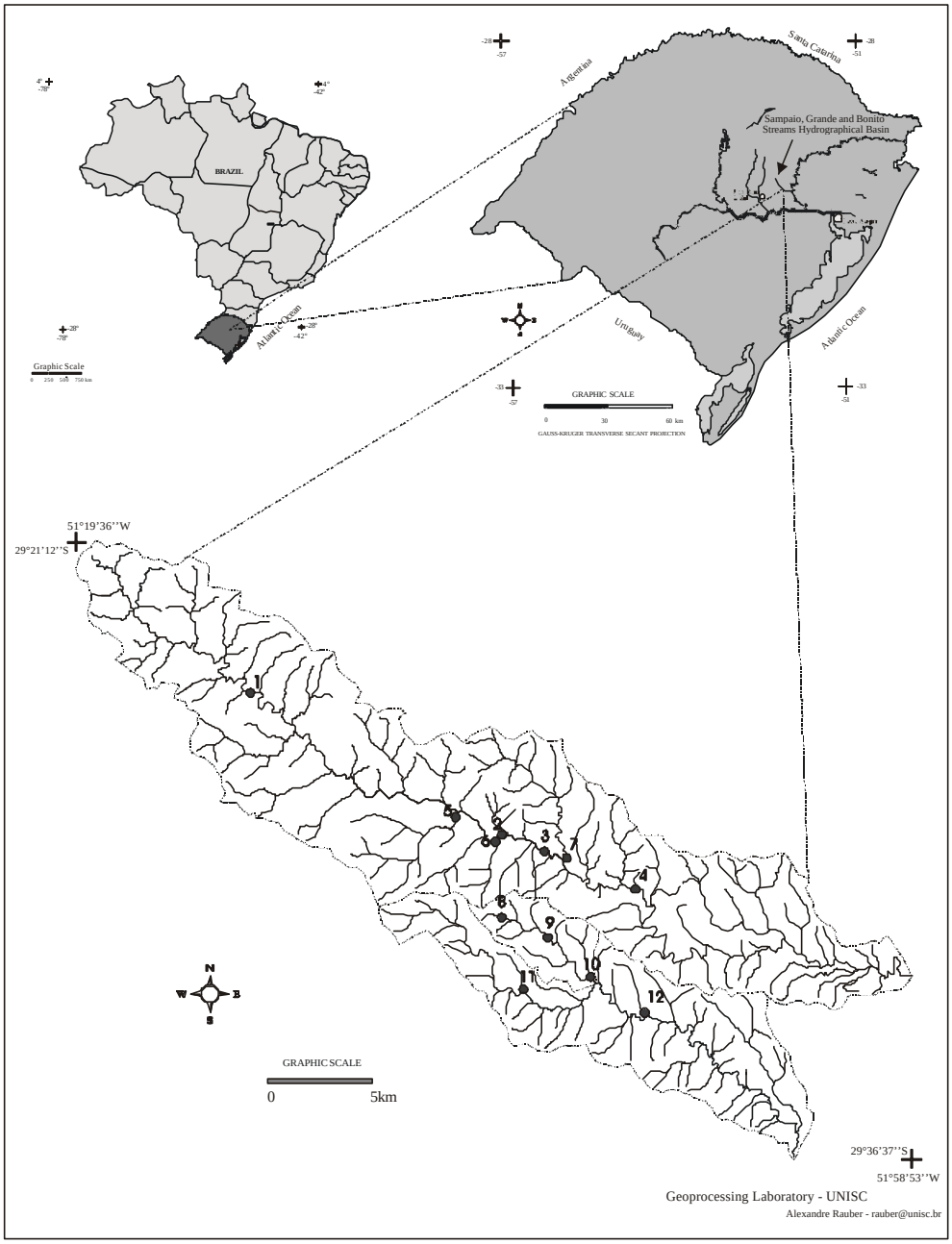

Figure 1: Map of the study area showing the location of Sampaio, Grande and Bonito streams, Mato Leitão, RS, Brazil, including sampling stations (1 to 12).

(Pseudospecies dichotomy levels: 0, 2, 5, 10 and 20; minimum group size for division: 5; maximum number of indicators per division: 5; highest number of species in the final table: 200; highest number of divisions: 6).

Canonical Correspondence Analysis (CCA), a direct multivariate gradient analysis, developed by Ter Braak (1986) was then applied in order to evaluate the relationship between the abiotic variables and diatom species composition patterns, within the groups defined by TWINSPAN, using the covariance matrices. A log transformation [log (x + 1)]

Table I. Location, year and season of 61 samples collected from Sampaio, Grande and Bonito Streams, countryside area of Mato Leitão, RS, Brazil, from 1993 to 1998.

SAMPLE	SAMPLING SITE	STREAM	SEASON	YEAR
E1	ML1	Sampaio	Winter	1993
E2	ML2	Sampaio	Winter	1993
E3	ML3	Sampaio	Winter	1993
E4	ML4	Sampaio	Winter	1993
E5	ML5	Sampaio	Winter	1993
E6	ML6	Sampaio	Winter	1993
E7	ML7	Sampaio	Winter	1993
E8	ML8	Bonito	Winter	1993
E9	ML9	Bonito	Winter	1993
E10	ML10	Bonito	Winter	1993
E11	ML1	Sampaio	Spring	1993
E12	ML2	Sampaio	Spring	1993
E13	ML3	Sampaio	Spring	1993
E14	ML4	Sampaio	Spring	1993
E15	ML5	Sampaio	Spring	1993
E16	ML6	Sampaio	Spring	1993
E17	ML7	Sampaio	Spring	1993
E18	ML8	Bonito	Spring	1993
E19	ML9	Bonito	Spring	1993
E20	ML10	Bonito	Spring	1993
E21	ML11	Grande	Spring	1993
E22	ML1	Sampaio	Summer	1994
E23	ML2	Sampaio	Summer	1994
E24	ML3	Sampaio	Summer	1994
E25	ML4	Sampaio	Summer	1994
E26	ML5	Sampaio	Summer	1994
E27	ML6	Sampaio	Summer	1994
E28	ML7	Sampaio	Summer	1994
E29	ML 8	Bonito	Summer	1994
E30	ML 9	Bonito	Summer	1994
E31	ML 10	Bonito	Summer	1994
E32	ML11	Grande	Summer	1994
E33	ML 12	Grande	Summer	1994
E34	ML1	Sampaio	Autumn	1994
E35	ML2	Sampaio	Autumn	1994
E36	ML3	Sampaio	Autumn	1994
E37	ML4	Sampaio	Autumn	1994
E38	ML5	Sampaio	Autumn	1994
E39	ML6	Sampaio	Autumn	1994
E40	ML7	Sampaio	Autumn	1994
E41	ML8	Bonito	Autumn	1994
E42	ML9	Bonito	Autumn	1994
E43	ML10	Bonito	Autumn	1994
E44	ML11	Grande	Autumn	1994
E45	ML12	Grande	Autumn	1994
E46	ML1	Sampaio	Winter	1994
E47	ML4	Sampaio	Winter	1994
E48	ML1	Sampaio	Summer	1995
E49	ML4	Sampaio	Summer	1995
E50	ML1	Sampaio	Winter	1995
E51	ML1	Sampaio	Spring	1995
E52	ML4	Sampaio	Spring	1995
E53	ML4	Sampaio	Summer	1996
E54	ML4	Sampaio	Summer	1997
E55	ML1	Sampaio	Autumn	1997
E56	ML1	Sampaio	Summer	1998
E57	ML4	Sampaio	Summer	1998
E58	ML1	Sampaio	Autumn	1998
E59	ML4	Sampaio	Autumn	1998
E60	ML1	Sampaio	Winter	1998
E61	ML4	Sampaio	Winter	1998

was applied to the abiotic data. Biotic data were transformed by rank adjustment, according to recommendation by McCune & Mefford (1999), due to a great number of zeroes found in the species data. Altogether, nine environmental variables were used - water temperature, pH, conductivity, turbidity, dissolved oxygen, biochemical oxygen demand, dissolved inorganic nitrogen, phosphates, and total solids - (Tab. II). Thirty five biological variables were analyzed, corresponding to the abundant species identified. A Monte Carlo test was applied in order to verify the probability of the eigenvalues of the ordination axes having been randomly attributed (999 iterations; $p < 0,05$) (McCune & Mefford, 1999).

Groups of sampling units based on species composition, given by the TWINSPAN analysis were determined from the sum of relative abundances within each group, divided by the number of sampling units in each group. Taxa were then associated with the group with where the resulting highest numeric value.

Results and discussion

Classification analysis using TWINSPAN (Tab. III; Fig. 2) resulted in 5 groups from 61 samples. Indicator species *Gomphonema cf. clevei* and *Cyclotella meneghiniana* were differential for group A, *Navicula rostellata* for groups B and C, and *Encyonema silesiacum* and *Gomphonema angustum* were differential for groups D and E (Fig. 2). Groups A, D and E were established on the second dichotomy level of the pseudospecies, whereas, groups B and C on the third level, because, in the first dichotomy (separating group A, with 14 samples, from the other group, with 21 samples) it was not possible to find a significant correlation. A new cut was therefore needed, separating groups B and C.

Canonical correspondence analysis (CCA) revealed low explicability (21.4%) of the total variability of the data on the first 3 axes (Tab. IV), which should be expected with ordination analyses of a large set of environmental data, according to Ter Braak & Prentice (1988), confirming the complexity of the factors determining community composition. However, the species-environment correlations for axis axes 1 ($r = 0.789$), 2 ($r = 0.748$) and 3 ($r = 0.740$) indicated a strong relationship between the species distribution and the environmental variables used for the ordination. Monte Carlo's permutation test revealed that the ordination on axes 1, 2 and 3 was statistically significant ($p<0.05$),

With regards to axis 1 (10.9 % of variation), the canonical coefficient showed that water temperature and total phosphate were the most important environmental variables most important to the ordination. These variables, which were, respectively, strongly correlated with the positive and negative ends of axis 1 (Fig. 3). Intra-set correlations added BOD_5, dissolved inorganic nitrogen and conductivity as significantly contributing variables, correlated with the positive end of axis 1 (Tab. V). In addition, intra-set correlations also showed phosphate as the most significant variable ($r = -0.635$), confirming its importance to the ordination of sampling units along axis 1, and characterising a specific eutrophication gradient.

On the second axis (6.7% variability explained) the canonical coefficient showed a high correlation of its positive end with the variables conductivity and turbidity. The negative end of axis 2 was mostly correlated with BOD_5 ($r = -0.636$) (Fig. 3). Intra-set correlations confirmed BOD_5 as the most significant variable ($r = -0.659$), and also included pH and water temperature, which had high correlations with the negative and positive ends of axis 2, respectively (Tab. V).

Water quality studies in the same streams carried out in 1997 and 1998 (Lobo et al., 1999), found that in all sampling sites, and seasons of the year, the phosphate concentrations in the water, were considered high, supporting this environmental characterisation. This, indeed, indicates that eutrophication processes are taking place.

BOD_5 was characterised by high correlation coefficients with both axes, reflecting the influence of organic pollution to the ordination of samples along the gradients detected, since this variable is directly related with to this kind of pollution (Hamm, 1969). These results, therefore, validate the hypothesis that the response of the diatom community in lotic systems in Southern Brazil is the result of the interaction of variables characterising processes of organic contamination as well as eutrophication.

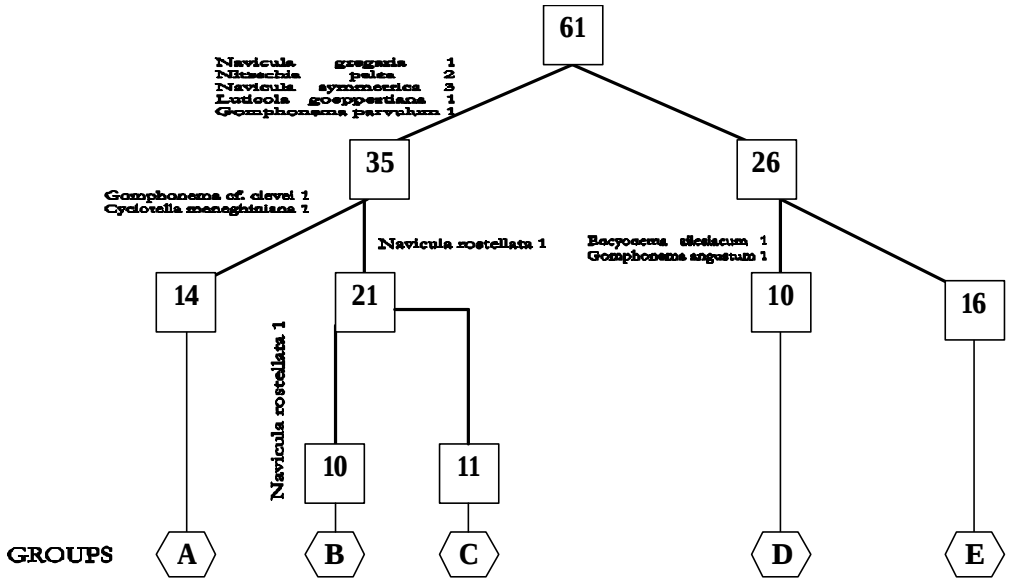

Figure 2: Indicator species of the main divisions from TWINSPAN classification, for 61 sampling sites. The numbers following the species names indicate the categories of relative abundance (1, 0-2%; 2, 2-4%; 3, 4-10%; 4, 10-20%; 5, >20%). Numbers of sampling sites, are given inside the squares.

Table II: Mean, standard deviation (s), coefficient of variation (C. V.), and ranges (lowest and highest value) of environmental variables.

	Mean	s (±)	C. V. (%)	Lowest	Highest
Temperature (ºC)	18.9	4.0	21.2	2.8	25.4
pH	7.3	0.3	4.0	6.7	8.1
Cond. (mS/cm)	0.1	0.1	65.4	0.0	0.5
Turb. (NTU)	26.1	25.1	96.2	4.0	139.0
DO (mg/L)	8.9	1.1	12.3	5.2	10.5
BOD-5 (mg/L)	14.8	15.2	102.6	0.8	54.8
DIN (mg/L)	2.4	2.7	115.3	0.2	21.1
PO_4 (mg/L)	0.115	0.127	110.459	0.018	0.600
TS	304.3	396.6	130.3	45.0	3135.0

In this context, for each diatom species from the 5 TWINSPAN groups distributed along the eutrophication gradient detected, was given and operational indicative values from 1 to 5, based on the tolerances to eutrophication, corresponding, respectively, to very low, low, medium, high and very high tolerance levels (Tab. VI). Using the indicative values for each species, the Biological Water Quality Index (BWQI) can be calculated, according to the equation in Wegl (1983), modified by the authors:

$$BWQI = \frac{\sum (s \times h \times vi)}{\sum (h \times vi)}$$

where *s* is the species saprobic value, according to the classification of Lobo *et al.* (2002); *h* is the percentage of occurrence (abundance) of each species in the sample and

Table III: Species versus samples, ordinated by TWINSPAN. Numbers indicate relative abundances: 1, 2, 3, 4 and 5 represent 0-2%, 2-4%, 4-10%, 10-20% and > 20%, respectively.

	SAMPLES 44223331211112333445555555 224 2 2223 11 11344454455335516 6 12890238157960567593678912347347625567190481293044680148253 01
SPECIES/GROUPS	
Rhopalodia gibberula	----------4----------
Amphora montana	----------123322--21----------
Frustulia cf. weinholdi	----------1111--3----------
Surirella angusta	----------4121223-11----------
Ulnaria ulna	----------1112123-12----------
Gomphonema parvulum	----------2423-53232334223-541----------
Navicula rostellata	----------2---4-33223112112--1----------
Fragilaria capucina v. rumpens	----------2----------
Luticola goeppertiana	--1-111112111--------33341422433-1-----11----------2
Navicula gregaria	3-444545554431--3-------32415313111----------
Melosira varians	---22241-1-111----------3-2-----1----------
Amphipleura lindheimeri	3211131----11----------
Cyclotella meneghiniana	--211124-1111----------
Cymbella affinis	-4-2211----------
Cymbella aff. hustedtii	--431-1111-1----------2----------
Nitzschia palea	235454345545423----44-332133233-12132111-11-1----3--------1--
Navicula symmetrica	-434454454444234434534324233334-1313322121223-4----------2
Nitzschia linearis	4222122----------2-------111122----21-1-----1----------
Encyonema silesiacum	-32131122234243-3-----333113134-3112222121314---3-----3---1--
Gomphonema angustatum	2-1-1---111212-------333-1----1----------4----------2
Geissleria aikenenses	--111111232221232---4-3235335--44--11-2131213---------3---22-
Navicula cryptotenella	-2112121113112-34-----222111-12-1-11111-11113-3----------1--
Adlafia bryophila	24252221111233----------411-1213----3--111113--23-------3----
Diadesmis contenta	--1-1-31-1-111----------24
Cocconeis placentula v. *euglypta*	---131133-1121---2----------533-------5-----
Sellaphora seminulum	3-11214114244555255535 44-535332534-32132114343555555555555254
Achnanthes exigua v. exigua	---111-1112122342---2---254211132-11111324313--2---334354442-
Achnanthidium minutissimum	--3-1-------23--------3------11--3-221121-2--------4----53543
Eolimna minima	----------3----------33
Nitzschia amphibia	-3-11-121111223333------2222121-55-13112214543-433--44----233
Gomphonema cf. clevei	5311-1122141224-------3-----------11--1--55-54-5--433-------
Planothidium rostratum	----------2-11-1--11----------2-
Cocconeis placentula v. *placentula*	----------1211132-1--4555555252-----35-4----2--
Gomphonema angustum	----------4443522441----------
Navicula atomus	----------22
GROUPS OF SAMPLES	AAAAAAAAAAAAAABBBBBBBBBBCCCCCCCCCCCDDDDDDDDDEEEEEEEEEEEEEEE

Table IV: Summary of CCA results.

		Axis 1	Axis 2	Axis 3
Eigenvalues (l)		**0.017**	**0.010**	**0.006**
Percentage of variance explained (%)		**10.9**	**6.7**	**3.7**
Cummulative variance (%)		**10.9**	**17.7**	**21.4**
Pearson correlation (species-environment)		**0.789**	**0.748**	**0.740**
Monte Carlo's test (p)	**Eigenvalues**	**0.001**	**0.001**	**0.001**
	Species environment correlations	**0.001**	**0.001**	**0.001**

Table V: Canonic coefficient and intra-set correlations of nine environmental variables with CCA axes 1, 2 and 3, with 35 biological variables.

	Canonic Coefficient			Intra-set correlation coefficient		
Variable	Axis 1	Axis 2	Axis 3	Axis 1	Axis 2	Axis 3
Temperature	**0.744**	**0.212**	**0.356**	**0.527**	**0.416**	**0.486**
PH	**0.241**	**-0.001**	**0.551**	**0.270**	**-0.575**	**0.012**
Conductivity	**0.125**	**0.475**	**-0.206**	**0.478**	**0.336**	**0.106**
Turbidity	**0.100**	**0.530**	**-0.031**	**-0.001**	**0.610**	**-0.467**
Dissolved Oxygen	**0.030**	**-0.028**	**-0.649**	**-0.169**	**-0.302**	**-0.711**
Biochemical Oxygen Demand	**0.200**	**-0.639**	**-0.039**	**0.557**	**-0.658**	**-0.201**
Dissolved Inorganic Nitrogen	**0.203**	**0.075**	**-0.369**	**0.494**	**-0.104**	**-0.607**
Phosphate	**-0.472**	**0.043**	**0.156**	**-0.635**	**0.213**	**0.362**
Total Solids	**-0.090**	**0.082**	**-0.294**	**0.155**	**0.125**	**-0.274**

Table VI: TWINSPAN groups with respective abundant species and indicative values related to eutrophication.

SPECIES	TWINSPAN GROUP	TOLERANCE TO EUTROPHICATION	INDICATIVE VALUE
Amphora montana **Grunow**	**C**	**Very Low**	**1**
Frustulia **cf.** ***weinholdii*** **Hustedt**	**(Plate 1)**		
Luticola goeppertiana **(Bleisch in Rabenhorst) D.G.Mann**			
Geissleria aikenenses **(Patrick) Torgan** ***et*** **Oliveira**			
Rhopalodia gibberula **(Ehrenberg) O.Muller**			
Surirella angusta **Kützing**			
Ulnaria ulna **(Nitzsch.) Compère**			
Cocconeis placentula **Ehrenberg var.** ***placentula***	**D**	**Low**	**2**
Encyonema silesiacum **(Bleisch) D.G.Mann**	**(Plate 2)**		
Gomphonema angustum **Agardh**			
Nitzschia amphibia **Grunow**			
Planothidium rostratum **(Oestrup) Lange- Bertalot**			
Adlafia bryophila **(Petersen) Moser Lange-Bertalot & Metzeltin**	**A (Plate 3)**	**Medium**	**3**
Amphipleura lindheimeri **Grunow**			
Cocconeis placentula **Ehrenberg var** ***euglypta*** **(Ehrenberg) Grunow**			
Cyclotella meneghiniana **Kützing**			
Cymbella affinis **Kützing**			
Cymbella **aff.** ***hustedii*** **Krasske**			
Diadesmis contenta **(Grunow ex Van Heurck) D.G.Mann**			
Gomphonema **cf.** ***clevei*** **Fricke**			
Melosira varians **Agardh**			
Navicula cryptotenella **Lange-Bertalot**			
Navicula gregaria **Donkin**			
Navicula symmetrica **Patrick**			
Nitzschia linearis **(Agardh) W.Smith**			
Nitzschia palea **(Kützing) W.Smith**			
Eolimna minima **(Grunow) Lange-Bertalot**	**B**	**High**	**4**
Fragilaria capucina **Desmazieres var.** ***rumpens*** **(Kützing) Lange-Bertalot**	**(Plate 4)**		
Gomphonema angustatum **(Kützing) Rabenhorst**			
Gomphonema parvulum **Kützing**			
Navicula rostellata **Kützing**			
Achnanthes exigua **Grunow var.** ***exigua***	**E**	**Very High**	**5**
Achnanthidium minutissimum **(Kützing) Czarnecki**	**(Plate 5)**		
Mayamea atomus **(Kützing) Lange-Bertalot**			
Sellaphora seminulum **(Grunow) D.G.Mann**			

vi is the species indicative value. Values of BWQI vary from 1 to 4 in aquatic environments: 0-0.9 (pollution absent), 1.0-1.4 (low pollution), 1.5-2.0 (moderate pollution), 2.1-2.7 (heavy pollution) and 2.8- 4.0 (very heavy pollution).

The results generally confirmed conclusions by others studies in streams and rivers in Europe. *Sellaphora seminulum* and *Mayamea atomus,* grouped among the species with high tolerance to eutrophication were described by Van Dam et al. (1994) as species characteristic of respectively eutrophic and hypereutrophic waters. The same authors described *Achnanthes exigua* var. *exigua* and *Achnantidium minutissimum* as species with broad tolerance ranges, occurring successfully from oligotrophic to eutrophic environments.

Kelly & Whitton (1995) assigned the indicative value 5 to *Gomphonema parvulum* and small (< 12 mm) species of the genera *Navicula* and *Sellaphora*, qualified as typical species of environments with phosphate values 0.3 mg/L. In the present study the same tendency was found, with these species also showing, high and very high tolerances to eutrophication. Kelly et al. (1996) reported *Cocconeis placentula* as dominant species in less eutrophic environments of the River Kennet, England a similar situation found in the Studied Hydrographic Basin, showing low tolerance to eutrophication.

The development of biological methods to indicate running waters trophic levels has been an important aim in diatom research in the past few years (Coring, 1999). Trophic indices have been developed by Schiefele and Kohmann (1993) and Kelly & Whitton (1995) in England. Both systems use weighted indicative values, which are related to the nutrient load. Since phosphate is one of the most important variables affecting running waters trophic levels, phosphate concentration is often used in the calibration of the trophic index.

The Trophic Diatom Index (TDI) proposed by Kelly & Whitton (1995) and modified by Kelly (1998) and Kelly & Harding (1999), has been widely used in the European Community, especially after the publication of the EU Water Framework Directive, in 1991. TDI uses the weighted average equations of Zelinka & Marvan (1961) to infer the epilithic diatom community structure, related to nutrient concentration in the river water. In this case, the relationship between species and environment was established from the analysis of the graphs which sum up the percentages of occurrence of each species, against the phosphate concentration in the water. Indicative values between 1 and 5 were given to each species, according to the concentration in which they were mostly abundant. TDI values vary from 1 (very low nutrient concentration) to 5 (very high nutrient concentration).

According to Kelly & Whitton (1995), a functional index requires a measure of eutrophication, as well as an indication of the proportion associated with organic pollution. TDI fulfils this requirement, since organic pollution indication is done from the percentage of valves of species characteristic of organically polluted waters, such as *Gomphonema parvulum*, *Navicula gregaria*, *Planothidium lanceolatum*, and small forms of the genera *Navicula*, *Sellaphora* and *Nitzschia*.

Compared to the use of trophic indices, the Biological Water Quality Index (BWQI) incorporates an integrate response of the epilithic diatom community to the eutrophication and organic contamination processes in Southern Brazilian rivers. Thus, system recommended here is the first attempt to assess water quality in surface freshwaters in Brazil, regarding both eutrophication and organic pollution.

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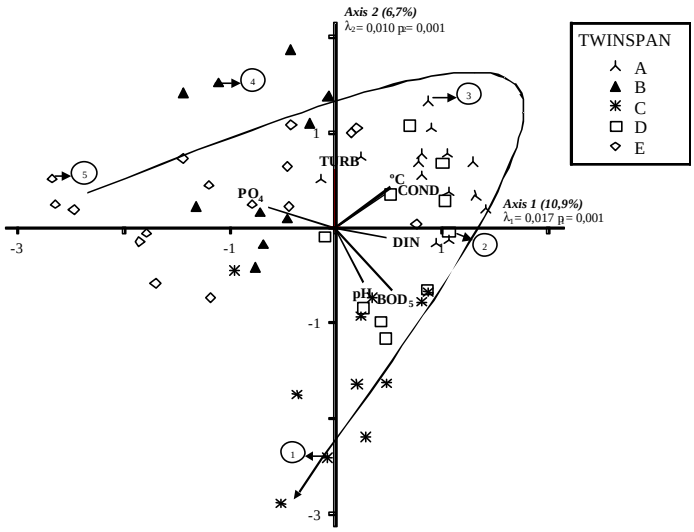

Figure 3: **Ordination graph based on Canonical Correspondence Analysis (CCA) of diatom samples (relative abundances) in 61 sampling sites correlated to the most important environmental variables in the ordination of axis 1 and 2 (**ºC: **temperature;** BOD: **biochemical oxygen demand;** COND: **conductivity;** DIN: **dissolved inorganic nitrogen;** pH: **hydrogen potential;** PO4: **phosphate;** TURB: **turbidity). Symbols represent sampling sites TWINSPAN groups. Arrow indicates the phosphate eutrophication gradient and numbers, within circles, the indicative values given to each group.**

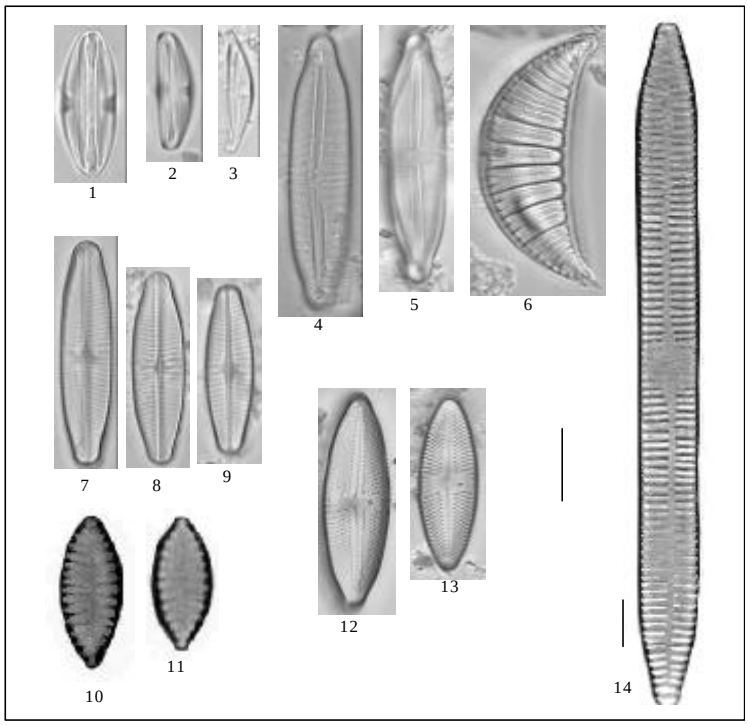

Plate 1. **Diatoms species with very low tolerance to eutrophication (Group C). Figs. 1-3.** ***Amphora montana;*** **Figs. 4-5.** ***Frustulia* cf. *weinholdii;*** **Fig. 6.** ***Rhopalodia gibberula*****; Figs. 7-9.** ***Geissleria aikenensis*****; Figs. 10-11.** ***Surirella ovata* var. *smithii*****; Figs. 12-13.** ***Luticola goeppertiana;*** **Fig. 14.** ***Ulnaria ulna.*** **Scale bars correspond to 10 m.**

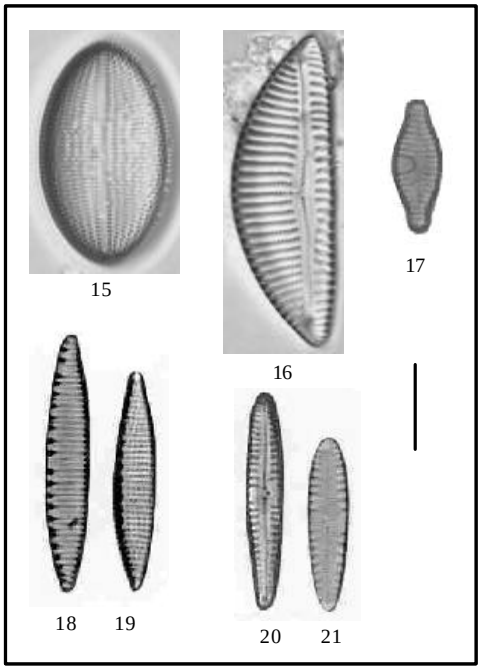

Plate 2: Diatom species with low tolerance to eutrophication (Group D). Fig. 15. *Cocconeis placentula* var. *placentula*; Fig. 16. *Encyonema silesiacum*; Fig. 17. *Planothidium rostratum*; Figs. 18-19. *Nitzschia amphibia*; Figs. 20-21. *Gomphonema angustum*. Scale bar correspond to 10 mm.

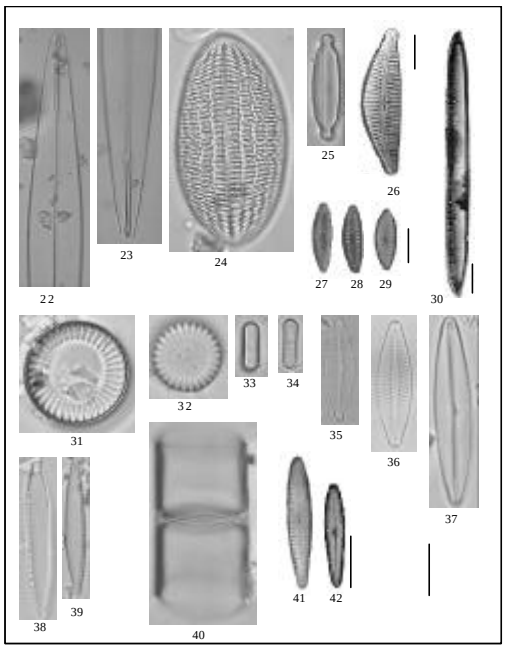

Plate 3: Diatom species with medium tolerance to eutrophication (Group A). Figs. 22-23. *Amphipleura lindheimeri*; Fig. 24. *Cocconeis placentula* var. *euglypta*; Fig. 25. *Adlafia bryophila*; Fig. 26. *Cymbella affinis*; Figs. 27-29. *Cymbella* aff. *hustedtii*; Fig. 30. *Nitzschia linearis*; Figs. 31-32. *Cyclotella meneghiniana*; Fig. 33-34. *Diadesmis contenta*; Fig. 35. *Navicula gregaria*; Fig. 36. *Navicula cryptotenella*; Fig. 37. *Navicula symmetrica* Figs. 38-39. *Nitszchia palea*; Fig. 40. *Melosira varians*; Figs. 41-42. *Gomphonema* cf. *clevei*. Scale bars correspond to 10 mm.

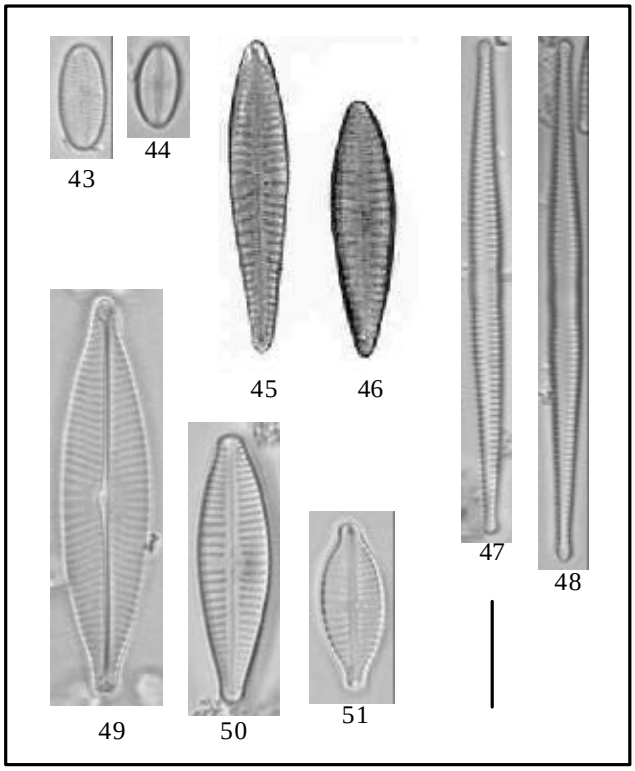

Plate 4. Diatom species with high tolerance to eutrophication (Group B). Figs. 43-44. *Eolimna minima*; Figs. 45-46. *Gomphonema angustatum;* Figs. 47-48. *Fragillaria capucina* var. *rumpens;* Fig. 49. *Navicula rostellata;* Figs. 50-51. *Gomphonema parvulum*. Scale bar correspond to 10 m.

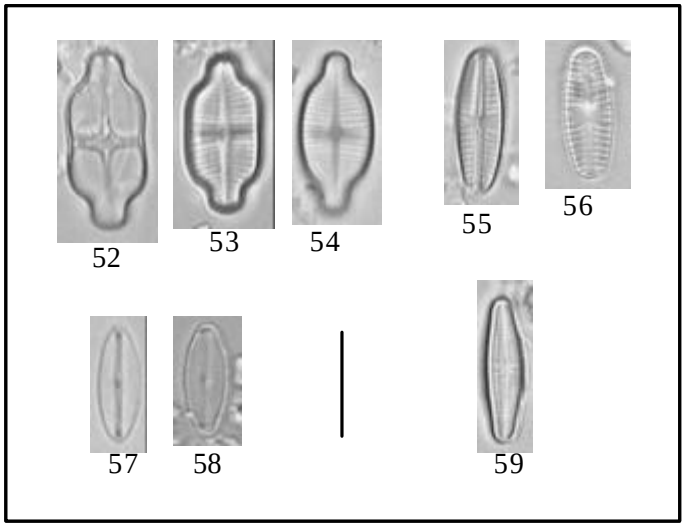

Plate 5. Diatom species with very high tolerance to eutrophication (Group E). Figs. 52-54. *Achnanthes exigua;* Figs. 55-56. *Sellaphora seminulum;* Figs. 57-58. *Mayamaea atomus;* Fig. 59. *Achnanthidium minutissimum*. Scale bar correspond to 10 m.

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