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Ecological preferences of benthic diatoms in a tropical river system in São Carlos-SP, Brazil

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Abstract: The objective of the study was to assess ecological preferences of diatoms in tropical river system, São Carlos-SP, Brazil. Benthic diatom communities, nutrients, ions, organic pollution, metal levels and other variables were assessed for sampling sites established along the river system. Canonical correspondence analysis (CCA) was used to identify the environmental variables governing the diatom assemblages. Weighted averaging was used to estimate optima and tolerances of some diatom taxa for the most influential variables. Upstream sites with significantly low nutrients, ions, organic pollution and metal levels were dominated by species belonging to the genera *Neidium, Diadesmis, Eunotia, Aulacoseira, Stauroneis* and *Surirella,* and the species *Cymbopleura naviculiformis*, *Orthoseira dentroteres*, and *Thalassiosira weissflogii*. Downstream sites with significantly high nutrients, ions, organic pollution and metal levels were dominated by such species as *Nitzschia palea*, *Achnanthes lanceolata*, *Fallacia monoculata*, *Sellaphora pupula*, *Cyclotella meneghiniana* and *Luticola goeppertiana*. Diatom community structure and dynamics were important indicators of ecological conditions in the tropical lotic system.

Resumen: El objetivo del estudio fue evaluar las preferencias ecológicas de diatomeas en un sistema fluvial tropical, São Carlos - SP, Brasil. Las comunidades de diatomeas bentónicas junto con los nutrimentos, los iones, la contaminación orgánica, los niveles de metales y otras variables fueron evaluados en sitios de muestreo establecidos a lo largo del sistema fluvial. Se usó un Análisis Canónico de Correspondencias para identificar las variables ambientales que rigen los ensambles de diatomeas. Se usó una Promediación ponderada para estimar óptimos y tolerancias de algunos taxones de diatomeas para las variables más influyentes. Los sitios ubicados corriente arriba con valores significativamente bajos de nutrimentos, iones, contaminación orgánica y niveles de metales estuvieron dominados por especies pertenecientes a los géneros *Neidium, Diadesmis, Eunotia, Aulacoseira, Stauroneis* y *Surirella*, y por las especies *Cymbopleura naviculiformis, Orthoseira dentroteres* y *Thalassiosira weissflogii.* Los sitios corriente abajo con valores significativamente altos de nutrimentos, iones, contaminación orgánica y niveles de metales estuvieron dominados por especies tales como *Nitzschia palea, Achnanthes lanceolata, Fallacia monoculata, Sellaphora pupula, Cyclotella meneghiniana* y *Luticola goeppertiana.* La estructura y la dinámica de la comunidad de diatomeas fueron indicadores importantes de condiciones ecológicas en el sistema lótico tropical.

Resumo: O objetivo do estudo foi avaliar as preferências ecológicas de diatomáceas num sistema fluvial tropical, São Carlos-SP, Brasil. As comunidades de diatomáceas bênticas, nutrientes, iões, poluição orgânica, níveis de metais e outras variáveis foram avaliadas por amostra gensestabelecidas ao longo do sistema fluvial. A análise de correspondência canónica (CCA) foi utilizada para identificar as variáveis ambientais governando as assemblagens de

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diatomáceas. Para estimar o óptimo e as tolerâncias de alguns táxons de diatomáceas para as variáveis mais influentesfoi utilizada a média ponderada. Locais, a montante,com teores significativamente baixos de nutrientes, iões, poluição orgânica e níveis de metais foram dominados por espécies pertencentes aos géneros *Neidium, Diadesmis, Eunotia, Aulacoseira Stauroneis* e *Surirella*, e pelas espécies *Cymbopleura naviculiformis, Orthoseira dentroteres*, e *Thalassiosira weissflogii*. Os locais a jusante, com teores significativamente elevados emnutrientes, iões, poluição orgânica e níveis de metais foram dominados por espécies como *Nitzschia palea, Achnanthes lanceolata, Fallacia monoculata, Sellaphora pupula, Cyclotella meneghiniana* e *Luticola goeppertiana*. A estrutura da comunidade e a dinâmica das Diatomáceas foram indicadores importantes das condições ecológicas no sistema tropical lótico.

Key words: Bacillariophyta, bioindication, pollution, tropical lotic systems.

Introduction

Diatom communities are responsive to the nature of the physical and chemical characteristics of lotic systems. Therefore, the integrity of these communities provides a direct, holistic and integrated measure of the integrity of the lotic systems. For that reason, pollution control and monitoring programmes routinely include the examination of diatoms to investigate the ecological status of lotic systems (Bere & Tundisi 2011a,b; Lobo *et al.* 2002; Pan *et al.* 1996; Potapova & Charles 2003; Round 1991).

Understanding the relation of geographical and environmental factors to diatom distribution is important for developing diatom-based water quality indicators. Multiple factors prevailing at different temporal and spatial scales play an important role in structuring benthic diatom communities in lotic systems (Potapova & Charles 2003). Some of the factors most often found to be important in shaping the distribution patterns of benthic diatoms in lotic systems are water chemistry (particularly pH, ionic strength and nutrient concentrations), substrate, current velocity, light (degree of shading), grazing, temperature (which also correlates strongly with latitude and altitude) (Pan *et al.* 1996; Potapova & Charles 2005; Nautiyal *et al.* 2000; Round 1991). Most of these factors depend strongly on climate, geology, topography, land-use and other landscape characteristics, and, therefore, diatom communities are similar in ecological regions defined by these characteristics (Pan *et al.* 1996).

Despite their ecological importance, practical usefulness, and previous studies by taxonomists and ecologists elsewhere, current knowledge of diatom autecology is incomplete in the tropical region, in this case Brazil. In most cases, the knowledge is gleaned from studies, which are not specifically designed to determine the environmental requirements of common species. Consequently, autecological information about common species in the tropics is lacking. This study, therefore, was designed to expand the ecological information base available on aquatic resources in the tropics in order to support rational management of these systems. Specifically, the study aimed at determining the ecological preferences of diatoms in Monjolinho River system, São Carlos-SP, Brazil.

Material and methods

Study area

The study area is shown in Fig. 1. The area is characterised by a rugged topography and an average annual temperature of around 19.5 °C, with mean monthly maximum of around 21.9 °C recorded in January and February and the mean monthly minimum of around 15.9 °C recorded in July. Headwaters of the Monjolinho River and the tributaries studied fall mainly within an agricultural area. From the agricultural area, the streams pass through urban area of the city of São Carlos, which covers a total area of 1,143.9 km^2. Current expansion of the city does not meet the technical standards required for systems such as sewage treatment, collection of garbage, urban drainage and so on. Streams in the study area, therefore, receive untreated or semi-treated effluent from various domestic and industrial sources as well as other diffuse sources as they pass through

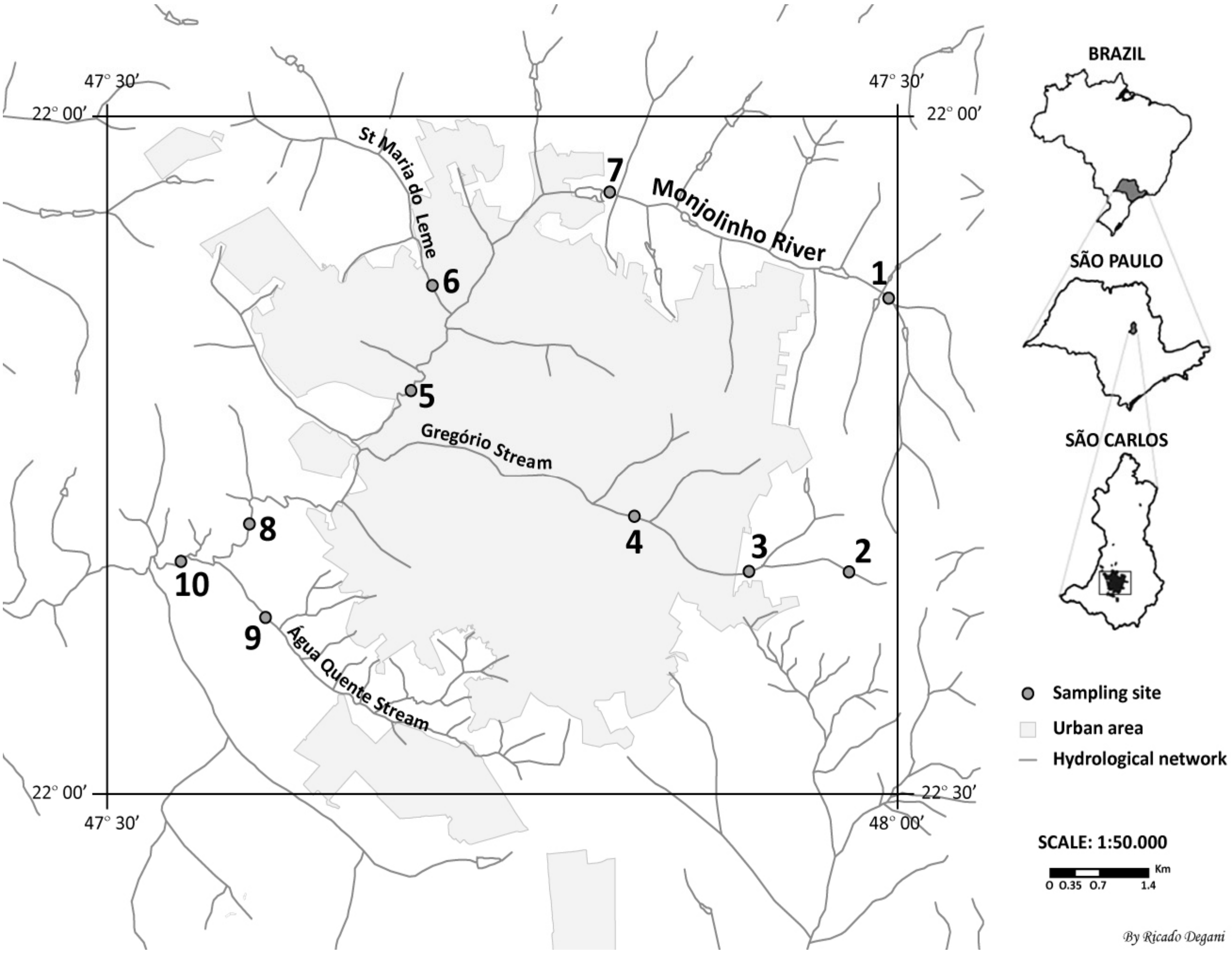

Fig. 1. The location of the study area and sampling sites.

the city. This disorderly growth of the city results in the deterioration of stream health, loss of the remaining primary vegetation, organic pollution and eutrophication amongst other problems.

Study design

Ten sites were established along Monjolinho river and its tributaries: four sites (1, 2, 3 and 7) in the relatively less impacted agricultural and forested headwaters to act as reference sites; 3 sites (4, 5 and 6) in the moderately polluted urban area; and 3 sites (8, 9 and 10) in highly polluted downstream area after the urban area (Fig. 1). The rational for choosing the sampling sites was to obtain a pollution gradient of all the stream systems from relatively unpolluted agricultural headwaters to highly polluted urban downstream sites.

Sampling for diatom and water quality was done during the dry season when flow was stable. Four samples were collected, two in September and October 2008 and two in May and June 2009. Sampling was done during the dry season to avoid the immediate effects of rainy season like great variation in water level and velocity, floods and inundation which affect diatom development, especially growth rate and relative abundance of different species (Duong *et al*. 2006).

Environmental variables

At each site, dissolved oxygen (DO), electrical conductivity, temperature, pH, concentration of total dissolved solids (TDS) and turbidity were measured using a Horiba U-23 and W-23XD Water Quality Meter (Horiba Ltd, Japan). Depth and current velocity were measured at each site with an FP 201 global flow probe (Global Water Instrumentation, AK, USA). The percentage riparian vegetation cover was visually estimated at each site. Altitude was determined at each site using a GPS (Northport Systems, Inc. Toronto, Canada). The percentage embeddedness (the degree to which fine sediments surround coarse substrates on the surface of a streambed) was also estimated along each stretch and rated on a 0 - 5 scale following Platts *et al.* (1983). The following physical substrate characteristics were visually estimated following USGS NAWQA protocol (Fitzpatrick *et al.* 1998): percentage of silt-clay size particles, sand size particles, gravel size particles, cobble size particles, boulder size particles; ratio of silt-gravel size particles, gravel-cobble size particles, and cobble-boulder size particles.

Water samples for the analysis of metals, ions, total nitrogen (TN) and total phosphorus (TP) were also collected at each site near the middle of the stream in a flowing water section into acid-cleaned polyethylene containers (Valderrama 1981). Sediments for TN and TP analysis were also collected at the same time. Water samples for biological oxygen demand (BOD_5) and chemical oxygen demand (COD) were also collected following APHA (1988).

In the laboratory, the concentrations of TN and TP in the water samples were determined following Golterman *et al.* (1978) and Valderrama (1981), respectively. TN and TP levels in sediments were determined following the micro-Kjeldahl and the ignition method, respectively as in APHA (1988). Biological oxygen demand after five days (BOD_5) and chemical oxygen demand (COD) were determined following standard methods (APHA 1988). The concentrations of fluoride (F^-), chloride (Cl^-), nitrite (NO_2^-), nitrate (NO_3^-), phosphate (PO_4^{3-}), sulphate (SO_4^{2-}), sodium (Na^+), ammonium (NH_4^+), potassium (K^+), calcium (Ca^{2+}) and magnesium (Mg^{2+}) were analysed by isocratic ion analysis using suppressed conductivity detection ion chromatography method using Dionex DX-80 Ion Analyzer (DX-80, USA). The monovalent-divalent cations ratios (M:D), shown to be an important factor in structuring benthic diatom communities (Potapova & Charles 2003), were calculated.

Total levels of the following metals were analysed in water samples using Flame Atomic Absorption Spectrometry Analytical Methods (Varian Australia Pty Ltd, Victoria, Australia): aluminium (Al), cadmium (Cd), lead (Pb), Zinc (Zn), chromium (Cr), cobalt (Co), copper (Cu), iron (Fe) manganese (Mn) and nickel, (Ni). Water samples were processed following standard methods (APHA 1988) before analysis. Metal levels at a given site are likely to have additive effects even at chronic concentration (Guasch *et al.* 2009). A measure of total metal concentration in water, the cumulative criterion unit (CCU) which has already been used to analyse the response of different organisms to metals in streams (Guasch *et al.* 2009) was, therefore, used.

Biological elements

At each site, epilithic diatom samples were collected as outlined in Bere & Tundisi (2011b). Dead wood was used as a substrate in the absence of boulders as suggested by Kelly *et al.* (1995). In the laboratory, sub-samples of the diatom suspensions were cleaned of organic material using wet combustion with concentrated sulphuric acid and mounted in Naphrax (Northern Biological supplies Ltd., UK, RI=1.74), following Biggs & Kilroy (2000). Three replicate slides were prepared for each sample. A total of 250 - 600 valves per sample based on counting efficiency determination method by Pappas & Stoermer (1996) were identified and counted using a phase-contrast light microscope (×1,000; Leica Microsystems, Wetzlar GmbH, Type -020-519.503 LB30T, Germany). The diatoms were identified to species level based on studies by Bicudo & Menezes (2006), Metzeltin *et al.* (2005), and Metzeltin & Lange-Bertalot (1998, 2007).

Data analysis

Environmental variables that were not normally distributed (Shapiro-Wilk, $P \leq 0.05$) or had no equal variance (Levene's test, $P \leq 0.05$) were transformed as follows: (a) log transformation for BOD_5, conductivity, TDS, TN, TP, Cu and Fe; (b) arcsin transformation for DO, turbidity, Cr, Co, Pb and Zn. Two-way Analysis of Variance (Two-way ANOVA) was used to compare means of environmental variables among the four sampling periods and among the three sites categories.

Preliminary detrended correspondence analysis (DCA) was applied on diatom data set to determine the length of the gradient. This DCA revealed that the gradient was greater than three standard deviation units (4.2) justifying the use of unimodial ordination techniques (Ter Braak & Verdonschot 1995). Thus, Canonical Correspondence Analysis (CCA) was used to investigate relationships between predictor variables and benthic diatom communities from different sites.

The CCA process also involved down-weighting of rare species. A forward selection procedure was performed on the set of environmental variables. A Monte Carlo test using 999 unrestricted permutations ($P \leq 0.05$) was performed to test for the significance of the correlations between the environmental factors and the species distributions. Only the significant variables were included in the model (Ter Braak & Verdonschot 1995).

Weighted averaging (WA) was used to estimate optima and tolerances of BOD_5, TP levels in sediments, CCU, M:D, pH and percentage canopy cover for the taxa that stood out in the CCA. These variables were chosen because they were retained by forward selection of environmental variables by CCA. Since taxa had unequal occurrences, the recommendations of Birks *et al.* (1990) were used and the number of occurrences was used to adjust the tolerance assigned to each taxon. The taxon's optima or indicator values were calculated as the mean of measured environmental variables (BOD_5, TP levels in sediments, CCU, M:D, pH and percentage canopy cover) weighted by the abundance of the concerned taxon in all sites.

Shapiro-Wilk test, Levene's test and Two-way ANOVA were performed using Palaeontological statistics (PAST) software version 2.01 (Hammer *et al.* 2009). CCA and DCA were performed using CANOCO version 4.5 (Ter Braak & Šmilauer 2002). For all statistical results, a probability of $P < 0.05$ was considered significant.

Results

Physical and chemical variables

The values of physical and chemical variables measured in the study area during the study period are shown in Appendix Table 1. The water quality generally tended to deteriorate downstream as the streams pass through the urban area due to discharge of treated and untreated domestic and industrial effluent as well as other diffuse sources of pollution from the city. The pH increased slightly down the agricultural to urban gradient being slightly acidic at upstream sites and slightly alkaline/neutral at downstream sites. However, the difference in pH among the three site categories was not statistically significant (ANOVA, $P > 0.05$). Temperature increased downstream, but as in the case of pH, the increase was not significant (ANOVA, $P > 0.05$). On the other hand, conductivity, BOD_5, COD, TDS, turbidity, TN, TP, and embeddedness increased significantly downstream (ANOVA, $P < 0.05$) while percentage of fine particles, DO and percentage riparian vegetation cover decreased significantly downstream (ANOVA, $P < 0.05$).

The concentrations of all the ions in water increased significantly downstream (ANOVA, $P < 0.05$). Chloride and alkaline earth metals especially Na^+ and Ca^{2+} were the dominant ions in the study area, while PO_4^{3-} and Fe were low at upstream sites and high at highly polluted downstream sites. Metal levels were generally low in the study area. Metal concentrations in water generally increased significantly (ANOVA, $P < 0.05$) downstream along the agricultural to urban gradient due to urban pollution (Appendix Table 1). An exception to this was the concentration of Fe which was high at site 1 (3.04 mg L^{-1}) that was situated in agricultural area which was expected to be relatively less polluted. This exceeded U.S. EPA guideline on critical concentration (2 mg L^{-1}) and contributed highly to CCU values at this site. This is probably due to the geology of the site. Besides this site, however, the concentration of Fe followed the general trend of other metals, tending to increase downstream exceeding the criterion unit value at site 9. Cd, Al and Pb were not detected at some sites but in all sites where they were detected they exceeded U. S. EPA guidelines on critical concentrations and also contributing highly to CCU values at these sites. Cu exceeded U. S. EPA guidelines on critical concentrations at sites 8 and 10 substantially contributing to CCU values. Zn, Ni, Mn and Cr had generally high concentrations at most of the sites, but their contribution to the CCU was considerably smaller than that of Cd, Cu, Fe and Al at some sites. Heavy metal concentrations, expressed as CCU values showed a general tendency of increasing along agricultural to urban gradient though sites 5 and 6 situated in the urban area had the lowest values (3.35 and 0.85 respectively).

Community structure

A total of 208 diatom species was recorded in all the diatom samples collected. Thirty-five genera made up 85.58 % of the overall diatom community (Appendix Table 2). The CCA ordination results of all the variables are shown in Fig. 2. Forward selection of environmental variables retained seven significant variables, which influenced

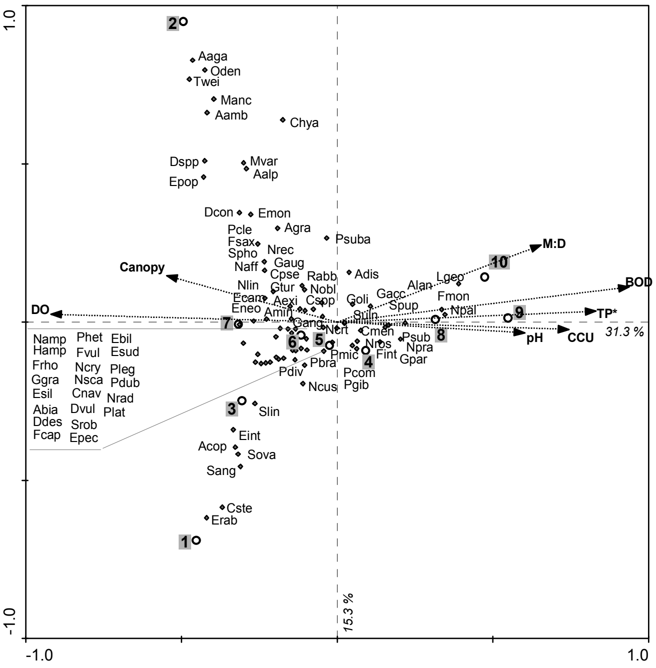

Fig. 2. Canonical correspondence analysis (CCA) diagram showing selected environmental variables and most frequently occurring diatom taxa in the ordination space of the first and second axes. Taxa codes correspond to those in Appendix Table 2. (* = sediment levels).

diatom distribution (in decreasing importance): BOD$_5$, DO, TP levels in sediments, CCU, M : D ratio and percentage canopy cover. These seven environmental variables explained 46.6 % of the total variation of the diatom assemblages, that is, CCA axis 1 = 31.3 % and axis 2 = 15.3 %. Monte Carlo unrestricted permutation test indicated that axis 1 (999 permutations) and axis 2 (999 permutations of axis 2 with axis 1 as a covariable) were statistically significant ($P \leq 0.05$). Of the 208 species observed, 82 species stood out in the CCA (Appendix Table 2).

The CCA separated highly polluted sites 8, 9 and 10 from the rest of the sites. Diatom species associated with highly polluted sites were small adnate species such as *Nitzschia palea*, *Gomphonema parvulum*, *Achnanthes lanceolata*, *Fallacia monoculata*, *Sellaphora pupula*, *Gomphonema accuminatum*, *Cyclotella meneghiniana, Luticola goeppertiana*, *Pinnularia subcapitata*, and *Nupela praecipua*. These species were thus, dominant at highly polluted, low altitude, open and hypereutophic sites 8, 9 and 10. On the other hand, the rest of the species, mostly of the genera *Neidium, Eunotia, Aulacoseira, Surirella, Orthoseira, Thalassiosira,* and *Pinnularia,* which are characteristic of moderately and less polluted environments were negatively associated with metal and organic pollution, ionic strength and eutrophication tending to favour relatively clean-forested upstream sites.

Ecological preferences

Appendix Table 3 shows the optima and tolerances estimated for the taxa that stood out in the CCA, for BOD$_5$, CCU, M:D, TP levels in sediments, DO, pH and percentage canopy cover, which were also the variables that stood out in the CCA. It is possible to separate highly pollution tolerant species from less pollution tolerant species, as already observed in the CCA diagram (Fig. 2). Most of the species belonging to the genera *Neidium, Diadesmis, Eunotia, Aulacoseira* and *Surirella* and the species C*ymbopleura naviculiformis, Orthoseira dentroteres, Stauroneis phoenicenteron*, and *Thalassiosira weissflogii* presented low optima for BOD$_5$ (1.93 to 6.70 mg L^{-1}), CCU (1.73 to 9.83), M:D (0.54 to 0.95) and TP level in sediments (25.62 to 45.14 mg kg^{-1} sed.). These species also presented high optima for DO (6.16 to 8.06 mg L^{-1}) and percentage canopy cover (42.75 to 91.09 %). On the other hand, *N. palea*, *G. parvulum*, *A. lanceolata*, *F. monoculata*, *S. pupula*, *G. accuminatum*, *L. goeppertiana*, *P. subcapitata*, and *N. praecipua* presented high optima for BOD$_5$ (12.04 to 19.34 mg L^{-1}), CCU (13.60 to 24.83), M:D (0.91 to 1.77) and TP level in sediments (81.70 to 116.83 mg kg^{-1} sed.). These species also recorded low optima for DO (2.11 to 4.82 mg L^{-1}) and percentage canopy cover (11.04 to 41.93 %). All species had pH optima between 6.40 and 7.13.

Discussion

Community structure in relation to environments variables

Based on metal and organic pollution, eutrophication, ionic strength and other environmental variables, a gradient along the streams from agricultural to urban area was observed. This gradient is characterised by oligotrophic, low metal and low organic pollution, low ionic strength and high percentage canopy cover upstream sites and hypereutrophic, high metal, high ionic strength and high organic pollution and low percentage cover downstream sites. Diatom community structure and composition in the investigated streams are a result of interplay of metal and organic pollution, eutrophication, ionic strength and other environmental variables.

Different species of diatoms responded differently to metal and organic pollution and eutrophication, ionic strength and other environmental variables because of differences in tolerance. As pollution increased, low or moderate pollution tolerant species of the genera *Neidium, Eunotia, Aulacoseira,* and *Surirella,* and the species *C. naviculiformis, O. dentroteres, S. phoenicenteron,* and *T. weissflogii* were replaced by highly pollution tolerant species such as *N. palea, G. parvulum, A. lanceolata, F. monoculata, S. pupula, G. accuminatum, L. goeppertiana, P. subcapitata,* and *N. praecipua.* The latter group of species is known to be resistant to organic pollution, which was the major driver of diatom community structure in this study, and heavy metal pollution (Biggs & Kilroy 2000; Duong *et al.* 2006; Potapova & Charles 2003; Round 1991). These species have also been frequently recorded in waters that are nutrient rich and poorly oxygenated with high electrical conductivity (Bere & Tundisi 2009, 2011 a, b; Lobo *et al.* 2002; Round 1991).

In this study, metal levels were generally lower than expected for streams receiving effluent from industrial areas. This could be due to the type of industrial activities. Despite the low levels and importance of metals, the cumulative effect of these metals (represented by CCU) was important in structuring benthic diatom communities in this study (Fig. 2). Therefore, use of the CCU values is an option worth considering in assessment of toxicity effects of metals on benthic diatom communities in low metal areas. Small sized adnate diatoms like *N. palea, G. parvulum, A. lanceolata, F. monoculata, S. pupula, G. accuminatum, L. goeppertiana, P. subcapitata,* and *N. praecipua* dominated in metal-polluted downstream sites. Canopy cover was also found to be important in structuring benthic diatom communities in the study area as it was retained in final CCA. This is because of the importance of light for diatom photosynthesis (Pan *et al.* 1996; Potapova & Charles 2003; Round 1991).

Ecological preferences

The BOD_5, CCU, M:D, TP levels in sediments, DO, pH and percentage canopy cover estimated optima and tolerances are in agreement with the species distribution in the CCA diagram. Due to the lack of literature on the optimal values of these variables for diatoms in tropical systems, it is difficult to compare the results obtained in this study. The difficulty in the interpretation and reliability of the estimated optima and tolerances is a common problem in ecology. In some cases, a species is characterised by an individual behaviour in one system, while in another aquatic system it behaves differently, thus leading to different optima and tolerances. These differences are probably a consequence of the existence, within several species, of two or more biotypes with different tolerances and environmental requirements. This has obviously important consequences also on the reliability of the indicator value of species in different environments. As an example, *Aulacoseira distans* (Ehrenberg) Simonsen, is usually described as a less pollution tolerant species. However, in this study the optimum and tolerance values estimated for this species are typically similar to values from moderately and highly polluted waters.

Certain taxa associated with the gradient extremes for BOD_5, CCU, M:D, TP levels in sediments, DO, pH and percentage canopy cover may be used in future studies as potential indicator species for changes in these variables. For example, species such as those of the genera *Neidium, Eunotia, Aulacoseira,* and *Surirella,* and the species *C. naviculiformis, O. dentroteres, S. phoenicenteron,* and *T. weissflogii* were found to be less pollution tolerant. On the other hand, species such as *N. palea, G. parvulum, A. lanceolata, F. monoculata, S. pupula, G. accuminatum, L. goeppertiana, P. subcapitata*, and *N. praecipua* dominated in highly polluted sites. These species can be subjected to further experiments to confirm their status as indicator species.

Conclusions

The autecological data presented in this study further contribute to our understanding of how diatoms are distributed in tropical lotic systems with respect to metal and organic pollution, eutrophication, ionic strength and other environmental variables. This is useful for effective diatom-based water quality assessment and general assessment of ecological conditions of lotic systems and contributes to further understanding of the ecology of diatoms in tropical systems.

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Appendix Table 1. The mean values of physical and chemical variables measured at 10 sites during 4 sampling periods.

Site	1	2	3	4	5	6	7	8	9	10
Temperature °C	19.00	19.74	19.41	20.34	20.81	20.07	21.19	23.33	22.94	21.95
DO (mg L^{-1})	7.12	8.34	7.16	6.34	6.41	6.44	6.15	3.59	2.83	1.39
pH	6.6	6.4	6.3	6.8	7.2	6.8	6.7	7.2	7.2	7.1
Turbidity(NTU)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.10	0.10	0.10
TDS (g L^{-1})	0.02	0.02	0.02	0.18	0.07	0.02	0.02	0.19	0.20	0.17
Conductivity (μScm^{-1})	23.76	11.51	31.52	42.74	48.81	17.77	15.27	303.09	216.42	234.39
Altitude (m)	761	837	831	794	745	761	774	724	630	627
Canopy cover (%)	80	95	60	50	4	45	20	20	50	5
Depth (m)	0.41	0.15	0.15	0.18	0.32	0.31	0.22	0.49	13.4	0.53
Width (m)	0.76	0.82	1.43	1.63	6	1.03	3.48	13	3.7	9
Velocity (m s^{-1})	1.35	1.62	1.51	1.63	0.92	1.92	1.29	1.91	1.61	1.47
BOD_5 (mg L^{-1})	1.04	1.93	2.37	7.01	3.58	7.47	1.96	20.79	24.25	26.61
COD (mg L^{-1})	3.90	6.53	10.53	63.11	16.43	11.65	6.70	75.05	106.66	84.97
TN (mg L^{-1})	0.46	0.48	0.21	3.18	0.87	0.90	0.60	19.28	12.93	10.50
TN (mg kg^{-1} sed.)	330.14	341.47	292.43	522.72	1297.54	393.77	677.48	931.21	391.71	1565.10
TP (mg L^{-1})	0.02	0.03	0.05	0.40	0.10	0.06	0.03	1.77	1.22	0.82
TP (mg kg^{-1} sed.)	29.07	27.42	28.56	63.49	41.41	25.29	28.91	96.51	203.72	85.20
Fluoride (μg L^{-1})	38.38	45.85	67.53	88.78	95.66	124.68	43.73	287.55	305.43	262.89
Chloride (mg L^{-1})	2.01	4.94	4.12	15.49	7.32	6.83	2.81	19.80	21.71	29.96
Nitrite (μg L^{-1})	0.00	0.00	0.00	423.89	42.38	24.28	0.00	884.95	2036.58	3164.94
Nitrate (μg L^{-1})	51.86	195.62	469.98	714.78	819.55	745.00	98.17	1141.22	176.52	441.10
Phosphate (μg L^{-1})	14.99	0.00	2.44	136.24	26.70	7.70	0.00	21.40	132.53	190.77
Sulphate (mg L^{-1})	0.13	1.53	1.06	8.32	4.86	3.13	0.34	3.75	15.08	9.53
Sodium (mg L^{-1})	1.96	2.34	2.38	7.49	4.14	4.07	2.15	12.42	15.28	19.65
Ammonium (μg L^{-1})	11.75	116.09	11.93	858.92	418.79	141.40	15.01	1361.61	4610.85	3310.54
Potassium (mg L^{-1})	1.03	0.58	1.03	2.27	1.27	0.95	0.71	2.43	4.33	3.82
Magnesium (mg L^{-1})	0.67	0.56	0.78	1.17	1.18	1.05	0.69	1.66	2.54	1.62
Calcium (mg L^{-1})	1.38	1.89	1.29	3.92	5.66	4.51	2.38	7.38	11.87	8.24
M:D	0.74	0.99	0.73	1.27	0.63	0.52	0.57	0.82	1.30	1.97
Cr (mg L^{-1})	0.01	0.01	0.01	0.02	0.01	0.01	0.01	0.02	0.02	0.02
Cu (mg L^{-1})	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.01	0.01
Mn (mg L^{-1})	0.05	0.06	0.07	0.09	0.03	0.02	0.03	0.05	0.21	0.08
Fe (mg L^{-1})	1.01	0.12	0.29	0.23	0.24	0.13	0.18	0.34	0.74	0.74
Co (mg L^{-1})	0.00	0.00	0.01	0.01	0.00	0.00	0.01	0.04	0.04	0.04
Ni (mg L^{-1})	0.00	0.00	0.00	0.06	0.00	0.00	0.01	0.03	0.03	0.03
Cd (mg L^{-1})	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.01
Pb (mg L^{-1})	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.06	0.07	0.08
Zn (mg L^{-1})	0.02	0.01	0.02	0.03	0.01	0.02	0.01	0.09	0.03	0.03
Al (mg L^{-1})	0	0	0	0	0.07	0	0	0	0.17	0
CCU	7.7	5.2	17.66	13.74	3.35	0.85	9.86	14.44	32.95	27.48
Embeddedness	0	0	1	3	1	1	2	5	1	4

Contd...

Appendix Table 1. Continued.

Site	1	2	3	4	5	6	7	8	9	10
Silt-Clay (%)	95	95	10	15	10	50	30	5	10	10
Sand (%)	2	0	90	50	90	80	60	10	90	10
Gravel (%)	0	2	3	5	5	5	40	5	3	15
Cobble (%)	0	2	4	20	5	5	10	5	5	50
Bolders (%)	0	3	0	5	2	2	0	10	0	60
Silt-gravel (%)	95	97	95	80	83	85	85	35	95	40
Gravel-Cobble (%)	0	2	5	15	10	10	10	50	5	50
Cobble-Bolder (%)	0	1	0	10	7	5	5	60	0	70

Appendix Table 2. List of the most abundant diatom species found during the study and their relative abundances at ten sampling sites (*: 0-10 %, ** : > 10-30 %, *** : > 30 %).

Species	Code	1	2	3	4	5	6	7	8	9	10
Achnanthes exigua Grunow	Aexi	*	*	*	*	*	*	*	*	*	*
Achnanthes lanceolata (Brébisson & Kützing) Grunow	Alan	*	*	*	*	*	*	*	*	*	*
Achnanthidium biasolettianum (Grunow) Lange-Bertalot	Abia	*	*	*	*	*	*	*	*	*	*
Achnanthidium minutissimum (Kützing) Czarnecki	Amin	*	*	*	*	*	*	*	*	*	*
Amphora copulata (Kützing) Schoeman and Archibald	Acop	*	*	*	*	*	*	*	*	*	
Aulacoseira agassizii (Hustedt) Simonsen	Aaga		*	*	*	*	*	*			
Aulacoseira alpigena (Grunow) Krammer	Aalp		*	*	*	*	*	*			
Aulacoseira ambigua (Grunow) Simonsen	Aamb	*	*	*		*	*	*			
Aulacoseira distans (Ehrenberg) Simonsen	Adis		*	*		*	*	*	*	*	
Aulacoseira granulata (Ehrenberg) Simonsen	Agra	*	*	*	*	*	*	*		*	
Caloneis hyaline Hustedt	Chya	*	*					*			*
Cyclotella meneghiniana Kützing	Cmen		*		*	*	*	*	*	*	*
Cyclotella pseudostelligera Hustedt	Cpse		*	*		*	*	*	*		
Cyclotella spp.	Cspp	*	*	*	*	*	*	*	*	*	*
Cyclotella stelligera (Cleve and Grunow) Van Heurck	Cste	*					*	*			*
Cymbopleura naviculiformis (Auerswald) Krammer	Cnav	*	*	*	*	*	*	*			
Diadesmis contenta (Grunow) Mann	Dcon	*	*	*	*	*	*	*	*		*
Diadesmis dissimilis Moser, Lange-Bertalot & Metzeltin	Ddes	*		*			*				
Diatoma spp.	Dspp	*	*	*				*			
Diatoma vulgare Bory	Dvul	*	*	*	*	*	*	*	*	*	*
Encyonema neomesianum Krammer	Eneo	*	*	*	*	*	*	*			
Encyonema silesiacum (Bleisch) Mann	Esil	*	*	*	*	*	*	*	*		
Eunotia bilunaris (Ehrenberg) Mills	Ebil	*	*	**	*	*	*	*			
Eunotia camelus Ehrenberg	Ecam	*	*	*	*	*	*	*			
Eunotia intermedia (Hustedt) Nörpel and Lange-Bertalot	Eint	**	*	*	*	*	*	*			
Eunotia monodon Ehrenberg	Emon	*	*	*		*	*	*			
Eunotia pectinalis (Kützing) Rabh	Epec	**	*	*	*	*	*	*			
Eunotia papillo (Ehrenberg) Hustedt	Epop	*	*			*	*	*			
Eunotia rabenhorstii Cleve & Grunow	Erab	*		*			*	*			

Contd...

Appendix Table 2. Continued.

Species	Code	1	2	3	4	5	6	7	8	9	10
Eunotia sudetica Müller	Esud		*	*	*	*		*			
Fallacia monoculata (Hust) Mann	Fmon				*	*	*	*	*	*	*
Fragilaria capucina Desmazières	Fcap	*	*	*	*	*	*	*	*	*	*
Fragilaria intermedia Grunow	Fint	*	*	*	*	*	*	*	*	*	*
Frustulia rhomboides (Rabenhorst) de Toni	Frho	*	*	*	*	*	**	*	*	*	
Frustulia saxonica Rabenhorst	Fsax	*	*	*	*	*	*	*	*	*	*
Frustulia vulgaris (Twaithes) de Toni	Fvul	*	*	*	*	*	*	*	*	*	*
Gomphonema accuminatum Ehrenberg	Gacc	*	*	*	*	*	*	*	*	*	*
Gomphonema angustatum (Kützing) Rabenhorst	Gang	*	*	*	*	*	*	*	*	*	*
Gomphonema augur (Ehrenberg) Lange-Bertalot	Gaug	*	*	*	*	*	*	*	*	*	*
Gomphonema gracile Ehrenberg	Ggra	*	*	*	*	*	*	*	*	*	*
Gomphonema olivaceum (Hornemann) Brébisson	Goli	*	*	*	*	*	*	*	*	*	*
Gomphonema parvulum (Kützing) Kützing	Gpar	*	*	*	*	*	*	*	*	**	*
Gomphonema turris Ehrenberg	Gtur	*	*			*		*	*		
Hantzschia amphioxys (Ehrenberg) Grunow	Hamp	*	*	*	*	*	*	*	*		*
Luticola goeppertiana (Bleisch) Mann	Lgeo	*	*	*	*	*	*	*	*	*	**
Melosira varians Agardh	Mvar		*	*	*	*	*	*			
Meridion anceps (Ehrenberg) Williams	Manc	*	*	*	*	*		*	*		
Navicula cryptocephala (Grunow) Cleve	Ncry	*	*	*	*	*	*	*	*	*	*
Navicula cryptotenella Lange-Bertalot	Ncrt	*	*	*	*	*	*	*	*	*	*
Navicula cuspidata Kützing	Ncus	*		*					*		
Navicula oblonga Kützing	Nobl	*	*	*	*	*	*	*	*	*	*
Navicula radiosa Kützing	Nrad	*	*	*	*	*	*	*	*	*	*
Navicula rostellata Kützing	Nros			*	*	*	*	*	*	*	*
Neidium affine (Ehrenberg) Pfitzer	Naff	*	*	*	*	*	*	*	*		*
Neidium ampliatum (Ehrenberg) Krammer	Namp						*	*			
Nitzschia linearis (Agardh) Smith	Nlin	*	*	*	*	*	*	*	*	*	*
Nitzschia palea (Kützing) Smith	Npal		*	*	**	*	**	*	***	**	***
Nitzschia recta Hantzsch ex Rabenhorst	Nrec	*	*	*	*	*	*	*	*	*	*
Nitzschia scalaris (Kütz) Grunow	Nsca	*		*	*	*	*	*	*		
Nupela praecipua (Reichardt) Reichardt.	Npra	*	*	*	*	**	*	*	*	**	*
Orthoseira dentroteres (Ehrenberg) Crawford	Oden	*	*	*							*
Pinnularia braunii (Grunow) Cleve	Pbra	*		*	*	*	*	*	*	*	*
Pinnularia divergens Krammer	Pdiv	*		*	*	*	*	*	*	*	*
Pinnularia gibba Ehrenberg	Pgib	*	*	*	*	*	*	*	*	*	*
Pinnularia lata (Brébisson) Rabenhorst	Plat	*		*	*	*	*	*	*	*	*
Pinnularia legumen Ehrenberg	Pleg			*	*		*	*	*		
Pinnularia microstauron (Ehrenberg) Cleve	Pmic	*			*	*	*	*	*		
Pinnularia subcapitata Gregory	Psub				*	*	*	*	*	*	*
Placoneis clementis (Grunow) Cox	Pcle		*	*	*	*	*	*	*		
Planothidium dubium (Grunow) Round and Bukhtiyarova	Pdub	*	*	*	*	*	*	*	*		*
Planotidium heteroideum	Phet		*	*	*	*	*	*	*		

Contd...

Appendix Table 2. Continued.

Species	Code	1	2	3	4	5	6	7	8	9	10
Pleurosigma compactum Greville	Pcom				*		*	*			
Psammothidium subatomoides (Hustedt) Bukhtiyarova	Psuba	*	*	*	*	*	*	*	*	*	*
Rhoicosphenia abbreviata (Agardh) Lange-Bertalot	Rabb	*	*	*	*	*	*	*	*	*	*
Sellaphora pupula (Kützing) Mereschkowsky	Spup	*	*	*	*	*	*	*	*	*	*
Stauroneis phoenicenteron (Nitzsch) Ehrenberg	Spho	*	*	*	*	*	*	*	*		
Surirella angusta Kützing	Sang	*		*	*	*	*	*	*		
Surirella linearis Smith	Slin	*	*	*	*	*	*	*			
Surirella ovata Kutzing	Sova	*		*		*	*				
Surirella robusta Enrenburg	Srob	*		*	*		*	*			
Synedra ulna (Nitzsch) Ehrenberg	Suln	*	*	*	*	*	*	*	*		*
Thalassiosira weissflogii (Grunow) Fryxell and Hasle	Twei	*	*	*							

Appendix Table 3. Optima ($\hat{u}_k$) and tolerances (t_k) for BOD$_5$ (mg L^{-1}), metal cumulative criterion unit (CCU), monovalent:divalent ratio (M:D), TP levels in sediments (mg kg^{-1} sed.), DO (mg L^{-1}), pH and percentage canopy cover. Taxa codes correspond to those in Table 2.

Code	BOD		CCU		M:D		TP		DO		pH		Canopy	
	$\hat{u}_k$	t_k	$\hat{u}_k$	t_k	$\hat{u}_k$	t_k	$\hat{u}_k$	t_k	$\hat{u}_k$	t_k	$\hat{u}_k$	t_k	$\hat{u}_k$	t_k
Aexi	5.40	1.41	11.11	0.62	0.77	0.08	48.07	7.51	5.96	0.09	6.81	0.04	28.65	3.79
Alan	12.84	1.77	13.75	2.02	0.91	0.07	88.08	6.50	4.82	0.31	7.13	0.04	22.50	4.79
Abia	8.02	1.90	13.97	0.88	0.87	0.09	54.88	8.60	5.64	0.18	6.76	0.04	37.99	4.58
Amin	3.67	3.23	10.37	1.18	0.74	0.27	36.42	14.19	6.40	0.23	6.67	0.01	40.20	28.16
Acop	2.87	0.52	8.25	0.16	0.76	0.01	36.35	2.10	6.75	0.10	6.70	0.03	61.70	4.62
Aaga	2.05	0.17	5.75	0.66	0.95	0.05	28.02	0.78	8.06	0.33	6.44	0.05	86.16	10.67
Aalp	4.36	1.55	8.42	1.55	0.93	0.00	37.90	6.26	7.03	0.62	6.62	0.11	61.35	14.28
Aamb	2.68	0.82	6.86	1.40	0.91	0.05	29.44	2.08	7.60	0.61	6.50	0.08	74.17	16.18
Adis	11.83	0.83	10.26	0.00	0.91	0.03	59.19	5.23	5.27	0.15	6.97	0.06	30.94	3.00
Agra	6.32	4.38	7.84	4.49	0.85	0.02	40.07	17.65	6.54	1.56	6.72	0.25	50.91	32.29
Chya	9.87	0.86	12.59	0.83	1.29	0.03	46.10	2.03	6.00	0.27	6.64	0.03	62.71	4.04
Cmen	7.46	4.78	6.37	3.89	0.74	0.16	51.10	11.91	5.85	0.69	7.12	0.11	14.85	14.38
Cpse	5.46	0.28	3.46	0.73	0.63	0.02	35.80	0.59	6.47	0.01	6.95	0.06	28.91	5.41
Cspp	6.17	1.45	7.94	2.57	0.82	0.10	44.64	2.22	6.14	0.17	6.95	0.13	26.88	12.03
Cste	3.18	0.81	8.99	0.48	0.82	0.03	33.08	1.51	6.65	0.18	6.65	0.02	71.47	3.29
Cnav	3.52	1.39	14.31	5.18	0.83	0.11	36.83	9.02	6.80	0.46	6.52	0.31	50.53	12.77
Dcon	4.46	0.36	9.10	0.48	0.94	0.00	34.81	1.04	6.97	0.17	6.58	0.02	65.04	3.58
Ddes	6.92	0.15	1.73	0.25	0.54	0.01	25.62	0.09	6.50	0.02	6.77	0.01	47.67	0.73
Dspp	2.01	0.02	8.64	0.94	0.86	0.04	27.93	0.15	7.71	0.19	6.43	0.01	75.35	5.81
Dvul	5.32	0.52	11.92	0.31	0.79	0.01	42.86	2.31	6.55	0.10	6.63	0.04	56.80	1.84
Eneo	4.14	0.58	9.08	0.05	0.79	0.05	35.92	1.61	6.50	0.02	6.72	0.01	42.75	3.61
Esil	4.39	1.05	8.46	0.06	0.68	0.08	34.49	3.99	6.33	0.08	6.74	0.02	34.25	6.81
Ebil	3.38	3.60	11.20	8.26	0.79	0.10	32.14	11.42	6.97	0.86	6.53	0.44	60.48	15.67
Ecam	6.70	0.17	11.82	0.16	0.95	0.00	45.14	0.92	6.16	0.05	6.74	0.00	45.85	0.12
Eint	2.98	7.24	8.96	4.32	0.79	0.20	33.30	16.25	6.84	1.11	6.63	0.15	64.92	53.58
Emon	5.09	0.35	6.34	0.36	0.77	0.01	33.37	1.24	6.92	0.15	6.64	0.02	58.96	3.30

Contd...

Appendix Table 3. Continued.

Code	BOD		CCU		M:D		TP		DO		pH		Canopy	
	$\hat{u}_k$	t_k	$\hat{u}_k$	t_k	$\hat{u}_k$	t_k	$\hat{u}_k$	t_k	$\hat{u}_k$	t_k	$\hat{u}_k$	t_k	$\hat{u}_k$	t_k
Epec	3.82	3.99	9.40	1.07	0.81	0.09	37.01	10.81	6.78	0.69	6.68	0.13	54.45	22.51
Epop	2.39	0.36	5.31	0.00	0.87	0.08	27.89	0.32	7.78	0.36	6.51	0.07	82.41	8.85
Erab	1.65	0.43	7.22	0.33	0.72	0.02	28.72	0.25	7.05	0.05	6.62	0.01	75.96	2.96
Esud	3.91	0.01	13.65	1.08	0.93	0.01	40.76	0.34	6.97	0.02	6.54	0.04	56.97	0.53
Fmon	19.34	3.22	21.77	3.38	1.34	0.22	105.58	5.58	3.32	0.76	7.12	0.02	20.57	2.82
Fcap	6.04	2.25	11.98	0.44	0.84	0.08	42.88	7.33	6.18	0.34	6.69	0.10	45.88	2.30
Fint	11.88	0.86	14.64	0.34	1.00	0.01	71.69	2.26	5.16	0.09	6.88	0.02	40.71	0.06
Frho	6.41	4.87	3.13	10.87	0.57	0.25	27.74	12.46	6.44	0.06	6.77	0.12	42.50	9.92
Fsax	4.39	0.43	6.47	1.71	0.76	0.00	35.83	4.60	6.91	0.28	6.72	0.03	52.00	5.37
Fvul	5.09	1.00	9.29	0.91	0.79	0.07	37.38	3.51	6.32	0.11	6.75	0.00	42.04	1.22
Gacc	13.18	4.51	17.11	6.13	0.97	0.12	96.56	43.73	4.94	0.86	6.94	0.12	41.93	2.45
Gang	9.00	2.62	13.64	1.37	0.94	0.08	54.51	13.50	5.75	0.55	6.76	0.18	41.83	5.25
Gaug	8.95	0.25	11.73	0.01	0.81	0.03	51.16	1.68	5.66	0.09	6.79	0.02	37.36	2.48
Ggra	6.38	0.20	8.45	0.84	0.78	0.03	41.95	2.41	6.25	0.05	6.79	0.00	42.29	0.43
Goli	11.93	2.06	14.26	0.54	1.00	0.02	66.12	3.79	5.09	0.44	6.90	0.07	33.74	6.16
Gpar	13.91	15.72	15.42	4.39	0.97	0.11	84.84	68.32	4.79	2.68	6.97	0.49	36.91	20.76
Gtur	7.87	0.19	8.86	0.08	0.76	0.00	51.73	0.68	5.95	0.04	6.88	0.01	39.31	0.38
Hamp	4.99	0.33	11.03	0.63	0.98	0.08	45.80	4.15	6.73	0.04	6.67	0.02	58.61	0.58
Lgeo	23.85	16.74	24.85	15.75	1.77	1.17	81.70	21.64	2.11	4.33	7.06	0.23	11.04	36.65
Mvar	4.28	1.25	6.59	0.90	0.85	0.06	36.61	5.23	7.29	0.54	6.62	0.11	65.95	14.24
Manc	2.72	0.83	7.03	2.00	1.00	0.01	32.81	5.64	7.93	0.44	6.47	0.07	85.16	10.91
Ncry	3.82	3.62	9.74	0.40	0.74	0.21	35.52	12.20	6.49	0.01	6.69	0.05	44.42	14.66
Ncrt	9.37	1.44	10.71	1.01	0.80	0.12	57.42	5.33	5.47	0.39	6.95	0.14	26.42	13.00
Ncus	8.40	0.39	15.88	0.21	0.76	0.00	51.18	1.52	5.97	0.08	6.62	0.02	48.13	0.62
Nobl	7.00	0.32	12.25	0.03	1.00	0.03	53.26	0.62	6.08	0.09	6.80	0.00	40.33	0.82
Nrad	4.09	0.41	8.83	0.26	0.75	0.03	32.31	2.24	6.77	0.10	6.63	0.03	55.85	3.25
Nros	11.16	0.61	12.37	0.73	0.95	0.01	65.96	3.21	5.28	0.17	6.91	0.02	35.17	1.73
Naff	4.18	0.42	6.97	0.54	0.73	0.04	34.37	1.36	6.62	0.00	6.77	0.02	38.96	3.80
Namp	2.06	0.04	9.70	0.06	0.57	0.00	28.85	0.02	6.15	0.00	6.70	0.00	20.44	0.17
Nlin	4.24	0.71	8.37	0.16	0.74	0.04	36.50	3.23	6.61	0.00	6.71	0.01	44.10	3.39
Npal	19.76	48.72	22.36	69.30	1.28	1.62	116.83	415.16	3.50	9.93	7.08	0.97	32.07	20.34
Nrec	7.93	0.03	10.49	0.11	0.91	0.00	51.36	0.08	6.01	0.03	6.80	0.00	45.41	0.67
Nsca	5.62	0.81	5.09	2.07	0.66	0.06	33.97	3.51	6.45	0.01	6.83	0.00	37.14	2.00
Npra	13.86	2.84	16.73	5.91	0.97	0.01	106.26	63.06	4.69	0.67	7.12	0.31	31.13	13.72
Oden	3.49	0.90	7.08	1.10	1.04	0.03	31.23	2.20	7.81	0.31	6.45	0.03	87.62	4.30
Pbra	7.64	0.43	11.96	0.06	0.93	0.02	49.41	0.10	5.89	0.13	6.79	0.01	38.19	1.65
Pdiv	5.54	1.00	11.33	0.44	0.70	0.01	44.03	5.07	6.29	0.22	6.74	0.06	36.57	2.25
Pgib	8.79	3.04	12.49	0.25	0.98	0.26	60.99	1.09	5.80	0.80	6.89	0.05	36.29	10.45
Plat	7.18	0.39	9.26	0.48	0.81	0.03	43.42	1.89	5.88	0.08	6.83	0.01	37.41	0.53
Pleg	4.95	0.12	12.32	0.11	0.94	0.02	45.80	1.25	6.42	0.01	6.70	0.01	42.94	0.21
Pmic	7.48	0.22	6.88	0.50	0.74	0.01	47.67	1.15	5.99	0.05	6.96	0.01	28.46	0.92
Psub	12.04	0.36	13.60	0.33	1.12	0.00	67.59	1.48	4.88	0.06	7.04	0.00	21.59	0.62
Pcle	4.80	0.25	9.70	0.12	0.78	0.01	39.22	0.97	6.57	0.02	6.66	0.01	47.06	0.21
Pdub	6.75	0.38	13.22	0.34	0.88	0.02	40.71	1.22	5.96	0.07	6.68	0.03	39.61	0.67

Contd...

Appendix Table 3. Continued.

Code	BOD		CCU		M:D		TP		DO		pH		Canopy	
	$\hat{u}_k$	t_k	$\hat{u}_k$	t_k	$\hat{u}_k$	t_k	$\hat{u}_k$	t_k	$\hat{u}_k$	t_k	$\hat{u}_k$	t_k	$\hat{u}_k$	t_k
Phet	5.19	0.02	9.60	0.09	0.77	0.00	38.26	0.20	6.44	0.01	6.70	0.00	42.23	0.31
Pcom	6.75	0.11	13.12	0.28	1.21	0.03	60.42	1.38	6.33	0.00	6.79	0.00	48.22	0.79
Psuba	11.07	0.12	12.19	0.57	0.90	0.01	72.20	2.04	5.66	0.16	6.84	0.03	50.23	4.23
Rabb	9.34	0.22	11.41	3.01	0.93	0.08	51.10	11.86	5.73	0.20	6.80	0.03	41.47	1.75
Spup	16.18	8.60	20.31	10.27	1.17	0.29	100.72	65.20	4.17	1.70	6.98	0.19	34.51	0.99
Spho	3.87	0.13	9.83	0.02	0.95	0.05	40.87	1.27	7.01	0.06	6.63	0.01	60.67	2.36
Sang	2.74	0.90	7.98	0.20	0.71	0.01	33.26	2.15	6.84	0.15	6.70	0.04	59.69	9.16
Slin	4.09	0.37	8.22	0.17	0.68	0.00	33.41	1.29	6.66	0.07	6.68	0.02	53.79	3.21
Sova	2.70	0.07	8.25	0.01	0.69	0.00	29.62	0.05	6.94	0.01	6.63	0.00	61.61	0.90
Srob	5.56	0.88	3.72	1.33	0.56	0.02	26.86	0.80	6.46	0.02	6.75	0.02	44.09	0.17
Suln	9.98	0.63	10.56	0.71	0.89	0.05	56.68	4.29	5.48	0.15	6.96	0.10	29.75	7.31
Twei	1.93	0.00	6.47	0.67	0.95	0.02	27.60	0.10	8.17	0.09	6.40	0.00	91.09	2.09