

Weighted average regression and calibration of conductivity and pH of benthic diatom assemblages in streams influenced by urban pollution – São Carlos/SP, Brazil

Média ponderada da regressão e calibração de condutividade e pH de comunidades de diatomáceas bentônicas em córregos influenciados pela poluição urbana - São Carlos/SP, Brasil

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Abstract: Aim: It was to assess the importance of conductivity and pH in structuring benthic diatom communities. **Methods:** Changes in diatom communities along an agricultural-to-urban conductivity and pH gradient were assessed during summer base flow period (September to October/08). Habitat assessment, diatom and water quality sampling was done at 10 sites. Weighted averaging regression and calibration were used to quantify relationships between individual diatom taxon's relative abundance and conductivity and pH. The predictive abilities of models were assessed in terms of correlation between observed and inferred values of conductivity and pH. **Results:** Frequently occurring diatoms were distributed continuously along gradient of conductivity and pH with the upstream, relatively low conductivity and slightly alkaline sites being characterised by such species as *Aulacoseira ambigua*, *Aulacoseira granulata* and *Cymbopleura naviculiformis*, while downstream, high conductivity and slightly acidic, sites were characterized by *Gomphonema parvulum*, *Nitzschia palea*, *Nupela praecipua*, *Rhoicosphenia abbreviata* and *Sellaphora pupula*. Conductivity and pH optima for these diatoms ranged from 25.96 to 324.76 $\mu\text{S}\cdot\text{cm}^{-1}$ and 6.61 to 7.54, respectively. **Conclusion:** the autecological information gained through this study augments previous works on diatom species-environmental relationships in streams in other regions and is a stepping stone towards further understanding of diatom ecology and the development of diatom biological monitoring protocol that is suitable for the study area.

Keywords: diatoms, weighted averaging, conductivity, pH.

Resumo: Objetivo: Avaliar a importância da condutividade e do pH na estruturação das comunidades de diatomáceas bentônicas. **Métodos:** Mudanças em comunidades de diatomáceas ao longo do gradiente de condutividade e de pH foram avaliadas durante o período do verão (setembro-outubro) de 2008. Amostragem de diatomáceas e avaliação de habitat, incluindo análise da qualidade da água, foram feitas em 10 locais. Regressões de média ponderada e calibração foram usadas na quantificação das relações entre a abundância relativa do táxon individual de diatomácea com os parâmetros condutividade e pH. A capacidade preditiva dos modelos foi avaliada em termos das correlações entre os valores observados e os valores inferidos destes parâmetros. **Resultados:** Diatomáceas de ocorrência mais freqüente foram coletadas a montante, em locais levemente alcalinos e de baixa condutividade caracterizados por espécies tais como *Aulacoseira ambigua*, *Aulacoseira granulata* e *Cymbopleura naviculiformis*, enquanto as espécies *Gomphonema parvulum*, *Nitzschia palea*, *Nupela praecipua*, *Rhoicosphenia abbreviata* e *Sellaphora pupula* foram coletadas em locais levemente ácidos e de elevada condutividade a jusante. Os valores ótimos dos parâmetros para essas espécies de diatomáceas de ocorrência mais freqüente foram encontrados em intervalos de condutividade entre 25,96 e 324,76 $\mu\text{S}\cdot\text{cm}^{-1}$ e pH entre 6,61 e 7,54. **Conclusões:** A informação ecológica obtida através deste estudo enriquece trabalhos anteriores sobre as relações entre as espécies de diatomáceas e parâmetros físicos e químicos do meio circundante em cursos de água de outras regiões e constitui um trampolim rumo à melhor compreensão da ecologia de diatomáceas e o desenvolvimento de protocolos de monitoramento biológico adequados para uma dada área de estudo.

Palavras-chave: diatomáceas, média ponderada, condutividade, pH.

1. Introduction

Diatom assemblages have important implications for ecosystem processes in lotic environments (Rocha, 1992; Patrick and Hendrickson, 1993; Mann, 1999; Biggs and Kilroy, 2000; Lobo et al., 2006; Doung et al., 2007). The maintenance of proper community structure and functioning of diatom assemblages in lotic systems in the face of encroaching human development and climate change, among other threats, is therefore, important in river health management. To this effect therefore there is need to understand the factors that govern the distribution patterns of these communities.

Diatoms are cosmopolitan, with others being endemic to specific regions (Kelly et al., 1998; Potapova and Charles, 2003). Their assemblage structures in streams are controlled by multiple factors prevailing at different temporal and spatial scales (Biggs, 1995; Stevenson, 1997; Pan et al., 1996). These factors include water chemistry (particularly pH, ionic strength and nutrient concentrations), substrate, current velocity, light (degree of shading) grazing and temperature (which also correlate strongly with latitude and altitude) (Patrick and Reimer, 1966a; Round, 1991; Pan et al., 1996; Potapova and Charles, 2002; Necchi-Júnior et al., 2003). Most of these factors depend strongly on climate, geology, topography, land-use and other landscape characteristics, and therefore diatom communities are similar within ecological regions defined by these characteristics (Pan et al., 1996).

Conductivity and pH are among the most important determinants of diatom assemblage structure in rivers (Potapova and Charls, 2002; Walker and Pan, 2006). Several work has been carried out on the effects of pH and conductivity on diatoms (e.g. Lobo et al., 1996; Pan et al., 1996; Sonneman et al., 2001; Oliveira et al., 2001; Potapova and Charles, 2002, 2003; Lobo et al., 2004; Billinger et al., 2006; Salomoni et al., 2006). Most of this work has been carried out in lentic systems with few studies concentrating on lotic systems. Monitoring the changes in pH and conductivity is carried out by simple observation of shifts in the dominant taxa or by inference using reported optima and some numerical procedures such as weighted average (WA) regression and calibration as in the case of this study.

Weighted averaging provides a quantitative evaluation of diatom autecology (Hall and Smol, 1992; Pan et al., 1996). The weighted average of a diatom taxon with respect to a give environmental variable provides a computationally simple and reliable estimate of the taxon's optimum (Biggs, 1990). This is based on the assumption that a taxon with a particular optimum for a given environmental variable will be most abundant in sites where the variable is close to the optimum (Ter Braak and van Dam, 1989). This computational process is referred to as WA regression and

it involves taking the average of the values of environmental variable over those sites where the species is present weighted by the abundance of the species. WA calibration performs the reverse function; it uses the optima and relative abundances of diatoms to estimate the environmental variable at a given site.

Weight averaging is just as efficient as regression methods for estimating optimum when a species is rare and has narrow ecological amplitude and when the distribution of the environmental variable among the sites is reasonably homogeneous over the whole range of occurrences of the species along the environmental variables (Ter Braak and Juggins, 1993). The major disadvantage of weighted averaging is that it depends on the distribution of environmental variables; highly uneven distributions can scramble the order of WAs of different species (Ter Brack, 1995). However, weighted averaging remains a simple and useful tool to show up structure in data tables by rearranging species and sites on the basis of an exploratory variable. The weighted averaging method was proposed as a biotic index for vascular plants (Ellenberg, 1948; Whittaker, 1956), algae (Zalinka and Marvan, 1961) and faunal communities in streams and rivers (Chutter, 1972).

Despite their ecological importance, practical usefulness, and previous studies by taxonomists and ecologists elsewhere, current knowledge of diatom autecology is incomplete in the study area, gleaned from studies which are not specifically designed to determine the environmental requirements of common species. As a consequent, species environmental lists are incomplete and inconsistent and autecological information about common species in the study area is lacking. For this cause, there is lack of capacity in the form of data for management of aquatic systems. This study was designed to asses the importance of conductivity and pH in structuring benthic diatom communities.

2. Material and Methods

2.1. Study area

The area under study (Figure 1) is bound by latitudes 22° 00' and 33° 30' S, and longitude 47° 30' and 48° 00' E. Headwaters of the study streams (Monjolinho, Gregório and Água Quente) fall within mainly agricultural area. Apart from agricultural practices in the headwaters, the study area is predominantly urban. The city of São Carlos covers a total area of 1143.9 km². The area is characterized by 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.

In 2008, the population of São Carlos was estimated at 218,080 inhabitants by the Instituto Brasileiro de Geografia e Estatística (IBGE, 2008). The expansion of the city at the moment does not meet the technical standards that should

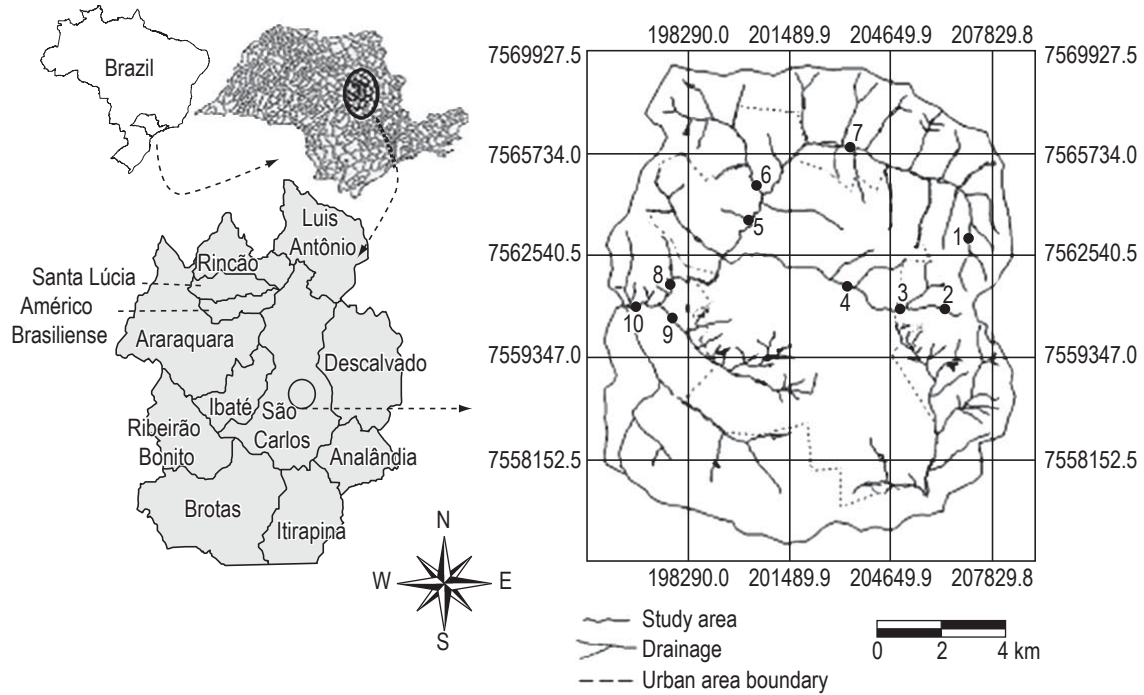


Figure 1. The location of the sampling sites in the study area.

go with it in terms of streets, sewage treatment and collection of garbage, urban drainage, water supply, road system and recreational area. The council also does not have an adequate system of sorting and disposal of waste. 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 the city of São Carlos. The city also expanded without taking into account environmental, geological and topographical factors leading to deforestation, erosion, and siltation. This disorderly growth of São Carlos promoted: a) deterioration of stream health; b) erosion of soil; c) flooding; d) loss of the remaining primary vegetation; and e) eutrophication and contamination of surface and underground aquifers.

A total of 10 sites were established in three stream systems; 3 sites in the relatively less impacted headwaters to act as references, 4 sites in the urban area, and 3 sites downstream after the urban area. The rationale for choosing the sampling sites was to obtain a pollution gradient of all the stream systems from relatively unpolluted headwaters to highly polluted downstream sites.

2.2. Data collection

Diatom and water quality sampling was done during summer season when flow was stable (September to October/08) at 10 sites. Dry season was selected to avoid variable effects of rainy season like great variations in water level and velocity, floods and inundations, which affect diatom development, especially growth rate and relative abundance of different species (Duong et al., 2006). 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).

The depth and current velocity were maintained relatively uniform among all the sites (10-30 cm and 1.5-2.0 m.s⁻¹ respectively). The percentage riparian vegetation cover was estimated at each site. The percentage embeddedness was also estimated along each stretch and rated on a 0-5 scale following Platts et al. (1983). Altitude was determined at each site using a GPS (Northport Systems, Inc. Toronto, Canada).

Where possible, epilithic (growing on stones), epiphytic (growing on macrophytes), epipellic (growing on mud) and epipsammic (growing on sand) diatoms were sampled separately at each site. Some of the dead cells in these microhabitats undoubtedly originated from microhabitats other than the sampled ones and these allochthonous cells could not be distinguished from autochthonous cells. Mixing of microhabitats was avoided.

Epilithic diatoms were sampled by brushing stones with a tooth brush following Kelly et al. (1998). Prior to sampling of epilithic surfaces, all substrata were washed with water ejected from a syringe or shaken in the stream to remove any loosely attached sediments and non-epilithic diatoms. At least five pebble-to-cobble (5-15 cm, total sampled area was approximately 100 cm²) sized stones were randomly collected along each sampling stretch, brushed and the resulting diatom suspensions were pooled to form a single sample which was then put in a labeled plastic bottle. Epiphytic diatoms were sampled after Fisher and Dunbar (2007); the sampled area was approximately 100 cm².

Epipelagic and epipsammic diatoms were sampled by pressing petri dish lid (area = 17 cm²) into the top layer of sand or silt/clay to a depth of 5-7 mm followed by sliding the spatula blade under the petri dish to isolate the contents in the dish which were then gently brought to the surfaces. The contents were then emptied into a labelled container. Samples from 6 locations in each sampling reach were pooled into a single sample; the total area sampled was 102 cm². Sampling effort for epilithic, epiphytic, epipelagic and epipsammic diatoms were standardized for all sites. No preservatives were added to all diatom samples.

2.3. Laboratory analysis

Sub-samples of the diatom suspensions were cleaned from organic material using wet combustion with concentrated sulphuric acid and mounted in Naphrax (Northern Biological supplies Ltd. UK. RI = 1.74) following Biggs and Kilroy (2000). Three replicate slides were prepared for each sample. A total of 250-600 frustules per sample (depending on the abundance of diatoms) were identified and counted using the phase contrast light microscope (1000 X). The diatoms were identified to species level based on studies by Mizuno (1964), Patrick and Reimer (1966a,b), Bourrelly (1981), John (2000), Biggs and Kilroy (2000), Wehr and Sheath (2003) and Bicudo and Menezes (2006) and the following website: <http://diatom.acnatsci.org>.

2.4. Data analysis

Diatom counts from each site were expressed as relative abundances. Input for numerical analysis included the diatom taxa that were present in a minimum of two samples and had a relative abundance of ≥5% in at least one sample. Shannon's diversity and equitability (uniformity of abundance in an assemblage of species which is greatest when species are equally abundant) indices were calculated according to the Shannon (1948). The significance of the differences in diversity and equitability among the sites was assessed using ANOVA using STATISTICA software package (Release 7, Stat Soft. Inc., United States of America).

Weighted averaging regression and calibration were used to quantify relationships between individual diatom taxon's relative abundance and conductivity and pH. The taxon's optima or indicator values were calculated as the mean of measured environmental variables (conductivity and pH) weighted by the abundance of this taxon in all sites according to WA regression formula (Equation 1):

$$U_k^* = \sum_i y_{ik} x_i / y + k \quad (1)$$

where U_k^* = weighted average (estimate of species optima); y_{ik} = abundances of taxon k at site i ; x_i = value of the environmental variable at site i .

Tolerance values were calculated as the weighted standard deviation of the taxon abundance in all the sites (Weilhoefer and Pan, 2008). Conductivity and pH were

calibrated at each site according to Ter Braak and Juggins (1993) WA calibration formula (Equation 2):

$$xi^* = \frac{\sum_{k=1}^m y_{ik} U_k^*}{\sum_{k=1}^m y_{ik}} \quad (2)$$

Weighted average and weighted average tolerance models were developed for conductivity and pH. The models were cross-validated using all the 10 sampling sites. The predictive abilities of models were assessed in terms of correlation between observed and inferred values of conductivity and pH. The correlation coefficients (r^2) for the relationship between observed and inferred environmental variable were used to evaluate the precision of WA indicators and to test for statistically significant relationships between observed and inferred values. Classical and inverse regression was used for dreshrinking.

3. Results

3.1. Water quality

The values of physical and chemical variables measured in the study area during the study period are shown in 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 effluent as well as other diffuse sources on pollution from the city. The pH values did not show significant differences among sites, although they present values slightly inferior to neutral pH. Temperature, conductivity and turbidity tended to increase downstream while dissolve oxygen and altitude tended to decrease downstream.

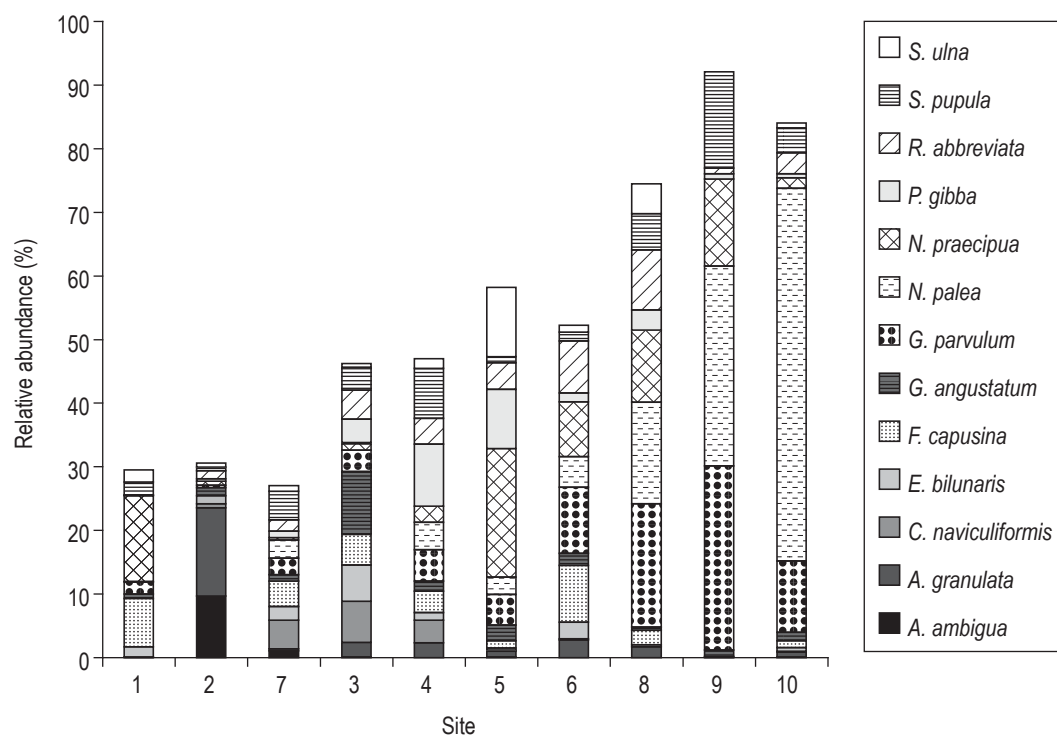
3.2. Diatom distribution

A total of 198 diatom species belonging to 71 genera that are distributed among the families Achnanthesiaceae, Achnanthesaceae, Bacillariaceae, Eunotiaceae, Cymbellaceae, Gomphonemataceae, Fragilariaceae, Melosiraceae, Naviculaceae, Rhoicospheniaceae, Rhopalodiaceae and Surirellaceae were recorded in all the diatom samples collected. Of the 198 species observed, 13 species were considered to be the most frequently occurring in the study area (≥5% occurrence and present in at least 2 samples).

The most frequently occurring diatom species had a generally wide spread distribution, occurring in almost all the sites sampled (Figure 2). The upstream, relatively low conductivity and slightly alkaline (less impacted), sites (1, 2, 3, 7) were characterised by such species as *Aulacoseira ambigua* (Grunow) Simonsen, *Aulacoseira granulata* (Ehrenberg) Simonsen and *Cymbopleura naviculiformis* (Auerswald) Krammer, while downstream, high conductivity and slightly acidic (highly impacted), sites were characterised by *Gomphonema parvulum* (Kützing) Cleve, *Nitzschia palea* (Kützing) Smith, *Nupela praecipua* (Reichardt) Reichardt, *Rhoicosphenia abbreviata* (Agardh) Lange-

Table 1. The values of physical and chemical variables measured on all the sites. (Emb = Embeddedness; Cond = conductivity, Turb = turbidity).

Site	Temp (°C)	Cond ($\mu\text{S}\cdot\text{cm}^{-1}$)	DO ($\text{mg}\cdot\text{L}^{-1}$)	pH	Turb (NTU)	TDS ($\text{g}\cdot\text{L}^{-1}$)	Altitude (m)	Canopy %	Emb	Mean depth (m)
Al	18.31	45	2.24	7.41	0.0	29.39	761	80	0	0.11
2	20.93	20	9.77	7.08	0.0	13.40	837	95	0	0.34
3	20.56	53	7.07	6.15	0.0	22.61	831	60	1	0.35
4	21.16	89	4.92	7.02	0.0	57.36	794	50	3	0.38
5	21.24	103	4.53	7.35	0.0	66.47	745	4	1	0.26
6	20.39	30	4.30	6.88	0.0	19.34	761	45	1	0.39
7	24.04	28	2.96	6.68	0.0	18.09	774	20	2	0.24
8	24.79	715	3.36	7.31	0.1	457.75	724	20	5	0.50
9	22.95	322	3.16	7.63	0.1	206.11	630	50	1	0.25
10	21.30	283	1.75	7.54	0.1	181.74	627	5	4	0.34

**Figure 2.** The relative abundances of most frequently occurring diatom. The arrow indicates a general increase in electrical conductivity and a decrease in pH.

Bertalot and *Sellaphora pupula* (Kützing) Mereschkowsky. Moderately impacted sites were dominated by *Eunotia bilunaris* (Ehrenberg) Mills, *Fragilaria capucina* Desmazière, *Gomphonema angustatum* (Kützing) Rabenhorst, *Pinnularia gibba* (Ehrenberg) Grunow, and *Synedra ulna* (Nitzsch) Ehrenberg. The distribution of the dominant taxa among the sites is shown in Figure 2.

Species diversity differed significantly (ANOVA, $p < 0.05$) among sampling stations, tending to be higher in relatively unpolluted sites (3, 4, 5, 6, 7) compared to the polluted sites (8, 9 and 10) being highest at 7 and lowest at 9 (Table 2). Site 1 had low diversity despite being relatively less impacted. This could be due to low habitat diversity

encountered. There were no significant differences in diversity (ANOVA, $p > 0.05$), among the habitats at the same site. Species equitability was significantly higher (ANOVA, $p < 0.05$) in relatively less polluted sites compared to the polluted sites being highest a site 4 and lowest at site 10 (Table 2).

3.3. WA regression and calibration

The weighted averages and tolerances of frequently occurring species with respect to conductivity and pH are shown in Table 3. Conductivity optima for these diatoms ranged from 25.96 to 324.76 $\mu\text{S}\cdot\text{cm}^{-1}$ (observed values of conductivity ranged from 20 to 715 $\mu\text{S}\cdot\text{cm}^{-1}$) and pH

Table 2. Shannon's diversity indices (H') and Shannon's Equitability indices (EH) for the sites sampled.

Site	1	2	3	4	5	6	7	8	9	10
H'	2.41	3.10	3.26	2.94	2.75	2.87	3.58	2.50	1.43	1.51
EH	0.75	0.90	0.88	0.91	0.80	0.87	0.86	0.78	0.58	0.46

Table 3. Conductivity and pH weighted averages and tolerances of 13 most frequently occurring diatom taxa.

Taxon	Conductivity ($\mu\text{S}\cdot\text{cm}^{-1}$)		pH	
	WA	Tolerance	WA	Tolerance
<i>A. ambigua</i>	25.96	1.36	7.06	0.06
<i>A. granulata</i>	102.83	16.10	7.07	0.15
<i>C. naviculiformis</i>	54.23	6.16	6.61	0.03
<i>E. bilunaris</i>	72.58	14.31	6.72	0.10
<i>F. capucina</i>	107.07	27.13	6.93	0.11
<i>G. angustatum</i>	109.51	12.55	6.76	0.14
<i>G. parvulum</i>	324.76	52.78	7.33	0.05
<i>N. palea</i>	321.32	49.76	7.54	0.20
<i>N. praecipua</i>	241.18	64.55	7.33	0.04
<i>P. gibba</i>	162.37	25.35	7.09	0.06
<i>R. abbreviata</i>	256.26	71.63	7.07	0.01
<i>S. pupula</i>	257.30	25.88	7.24	0.04
<i>S. ulna</i>	227.49	88.74	7.25	0.06

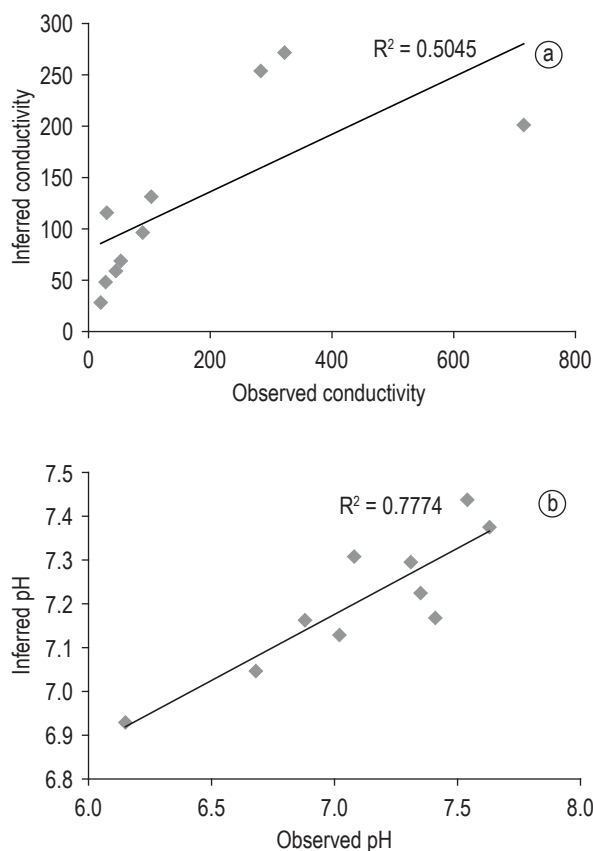
optima ranged from 6.61 to 7.54 (observed values of pH ranged from 6.15 to 7.54).

The estimated (calibration) values of conductivity and pH at each site are shown in Table 4. For conductivity models, the correlation between the observed and diatom inferred values was weak ($r^2 = 0.50$) (Figure 3a). The estimated values tended to be higher than observed values in relatively less impacted upstream sites and lower than observed values in highly impacted downstream sites. For pH models, the correlation between the observed and diatom inferred values was high ($r^2 = 0.78$) (Figure 3b).

4. Discussion

The results demonstrated that diatom assemblages are distributed continuously along gradient of conductivity. The positions of frequently occurring species along conductivity gradient in this study generally correspond to the affinities reported by others (Round, 1991; Leland, 1995; Potapova and Charles, 2003; Duong et al., 2006). As conductivity increased, low conductivity tolerant species such as *A. ambigua*, *A. granulata* and *C. naviculiformis* were replaced by high conductivity tolerant species such as *G. parvulum*, *N. palea*, *N. praecipua*, *R. abbreviata* and *S. pupula*.

The latter group of species has been reported to be associated with waters of relatively high ionic strength and high conductivity, and is known to be resistant to organic and heavy metal pollution (Round, 1991; Leland, 1995;

**Figure 3.** Relationship between observed and inferred; a) conductivity and b) pH.**Table 4.** Observed and inferred values of conductivity and pH on the 10 sites.

Site	Conductivity ($\mu\text{S}\cdot\text{cm}^{-1}$)		pH	
	Observed	Inferred	Observed	Inferred
1	45	58.87	7.17	7.41
2	20	28.20	7.08	7.31
3	53	68.80	6.15	6.93
4	89	96.40	7.02	7.13
5	103	131.00	7.35	7.22
6	30	116.00	6.88	7.16
7	28	48.20	6.68	7.05
8	715	201.00	7.31	7.30
9	322	272.00	7.63	7.38
10	283	254.00	7.54	7.44

Biggs and Kilroy, 2000; Potapova and Charles, 2003; Duong et al., 2006). These species have also been frequently recorded in waters that are nutrient rich and poorly oxygenated (Takamura et al., 1990; Round, 1991) that accompanied the downstream conductivity gradient in this study. Increase in conductivity may be accompanied by elevated dissolved nutrients in streams (Leland, 1995; Walker and Pan, 2006). Conductivity has therefore been used as an easy and conservative surrogate for nutrient enrichment

(Biggs, 1990; 1995). Welch et al. (1998) suggested that conductivity might be a surrogate of urban development in the Pacific Northwest. Conductivity correlates well with near-stream urban land use (Walker and Pan, 2006).

A weak correlation was observed between observed field values of conductivity and inferred (calibration) values of conductivity. The sampling design of this study, and all water quality assessment protocols using physical and chemical variables, allows for only instantaneous measurements, therefore restricting the knowledge of water conditions to the period when measurements were taken. The chemistry at any given time is, therefore, a snapshot of water quality at the time of sampling ignoring temporal variation of water quality variables that is usually high in lotic environments (Rocha, 1992). Since the biological response of diatoms is to the integrated chemical environment to which the organism has been exposed for some time (Schoemann, 1979; Round, 1991), it is not surprising that the observed values of conductivity do not correlate with inferred values. This emphasises the importance of biological monitoring oriented approach relative to physical and chemical approach in river health management. A large sample size is likely to improve the predictive power of models developed in this study.

Diatom assemblages have also been shown to be distributed continuously along gradient of pH. WA pH models had relatively high predictive power. This agrees with other previous works (e.g. Ter Braak and van Dam, 1989; Kovács et al., 2006; Weilhoefer and Pan, 2008). The relationship between diatoms and pH is strong because pH exerts a direct physiological stress on diatoms (Gensemer, 1991), and also strongly influences other water chemistry variables (Stumm and Morgen, 1981).

The pH optima for the 13 most commonly occurring taxa were circumneutral to alkaline (Van Dam, 1994) agreeing with WA optima developed for Swedish and Hungarian streams (Kovács et al., 2006). Weighted pH optima of *A. ambigua*, *E. bilunaris*, *F. capucina*, *G. parvulum*, *N. pelea* and *S. ulna* generally compared favourably with previously proposed autecological classification (e.g. Van Dam et al., 1994).

Quantitative autecological characteristics derived from small scale regional datasets like this can not only be used to generate calibration functions that are specific to the region of application (Davis and Smol, 1986) but can possibly also be used with literature data on pH and conductivity optima and tolerance of diatoms, provided that comparable taxonomical and chemical methods have been used (Ter Braak and van Dam, 1989). However, as they are dependent on the restricted range and distribution of environmental variables in the dataset, their application to areas with different water chemistry must be treated with caution.

5. Conclusion

The auto-ecological information gained through this study augments previous works on diatom species-environmental relationships in streams in other regions and is a stepping stone towards further understanding of diatom ecology and the development of diatom biological monitoring protocol that is suitable for the study area. Using the weighted averaging method and models developed in this study, prediction of pH was made possible. However, this must be interpreted with caution as the study involved a single sample with no statistical degrees of freedom. WA averaging could be used in conjunction with other models (e.g. generalised additive model, change point regression analysis, partial least squares regression) to provide better insight into interactions between diatoms and environmental variables. Further investigations are needed to increase our knowledge of ecological requirements of diatom taxa in this region.

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