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Full Length Article

Diatom Assemblages in the Mnweni River System in KwaZulu-Natal, South Africa

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Abstract

Microalgae of benthic origins are abundant in aquatic ecosystems which have been affected by anthropogenic factors and provide a valuable aid in determining water quality. This study was initiated to understand the changes that occurred in the microphytobenthos diatom community in the pristine Mnweni river system in the KwaZulu-Natal Drakensberg region. Diatom frustules were examined under an Olympus BX41TF light microscope with Differential Interference Contrast (DIC) optics. The PRIMER v5 computer software package was utilised to perform multivariate data analyses which included clustering, ordination and k-dominance curves. The diatom *Cymbella turgidula* was present in all five sites whilst *Encyonopsis stafsholtii* was abundant in sites three and four. A SIMPER analysis of the data showed an average dissimilarity between the sites. Sites with higher total dissolved solids (TDS), phosphorous and nitrogen showed suppressed pH values although all values were above seven. The diatom composition together with environmental gradients in the catchment suggests that river chemistry, environment structure, current pattern and terrestrial use had important impacts on the integrity of the system. The diatom species that were found in the study sites suggested that the water in the Mnweni catchment was clean or mildly enriched.

Keywords: Diatoms; Drakensberg; Mnweni catchment; Water quality

Introduction

Diatoms are a diverse group of microalgae that commonly occur in nearly all types of aquatic ecosystems (Stevenson and Pan, 1999). These microalgae form an important food resource in freshwater ecosystems (Stoermer and Smol, 1999). The distribution and abundance of riverine diatoms are often correlated with aquatic chemistry, substrata, flow velocity, light and grazing (Patrick and Reimer, 1966; Round, 1981; Stevenson *et al*., 1996). Most of these factors depend strongly on weather conditions, geology, land-use and other site characteristics and therefore, are similar within ecological zones defined by these parameters (Stevenson and Pan, 1999; Steele *et al*., 2014).

The uses of benthic diatoms (microphytobenthos) in South African aquatic systems have been researched extensively since the early 1950's (Cholnoky, 1953; 1960; 1968; Archibald, 1983; van der Molen, 2000; Bate *et al*., 2004a, b). Attempts were made previously to relate diatom species to water quality (Archibald, 1983; Schoeman, 1979; Schoeman and Archibald, 1986). The occurrence of microphytobenthos in an aquatic system is as a consequence of interactions between the water quality, hydrological and biotic interactions. Short term differences in the community composition are affected by the movement of cells and the differences between population growth and loss rates such as death and predation (Bates *et al*., 2004a, b; Karthikeyan *et al*., 2012). Benthic microalgae flourish where aquatic systems are affected by anthropogenic impacts.

It has been internationally recognized that diatoms can be effectively used as water quality indicators. Research shows that assemblages of diatoms provide a valuable aid in determining water quality and more importantly, in undertaking assessments of environmental change. Approaches, particularly those that examine the ecological associations of phyto- and epiplanktonic communities provide a powerful description of the chemical and biological cause and effect pathways. Although diversity is not an adequate measure to infer water quality, diatom indices do provide a simple yet rapid measure of evaluating the integrity of a particular system.

This study will attempt to help understand the changes that were occurring in the microphytobenthos diatom community in the Mnweni river system. The source of the Mnweni River in the KwaZulu-Natal escarpment is a pristine environment that has not yet been severely impacted upon by human intervention. Consequently, this area forms an ideal control of the study in that diatom assemblages would not have been impacted upon and may be truly reflective of pristine communities.

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Materials and Methods

Study Area

The Mnweni River flows between the Royal Natal National Park (RNNP) and Cathedral Peak; in the most pristine part of the northern Drakensberg. The Mnweni area is the largest tribal land in the Drakensberg and falls under the amaNgwane Tribal Authority. Mnweni is a typical rural area that is sparsely populated. The community mainly depends on farming and natural resources for their survival. As a result, this pure untouched site has led to it being an area for ecotourism development (Fig. 1). The indigenous name for the Drakensberg mountain range is Ukhahlamba or 'The Barrier of Spears', a reference to the ridges that raises over 3000 m. This environmentally pristine area is a world heritage site and used for recreational activities such as rock climbing, hiking, camping, bird spotting and trout angling (Derwent, 2000; Bristow, 2003).

Environmental Characteristics of the Mnweni System

The unique Mnweni Valley between the Ukhahlamba Drakensberg landmarks of the Amphitheatre and Cathedral peak is one of the most vital sources of fresh water in the country that contributes to the delivery of clean water for urban, rural, domestic and industrial usage in both KwaZulu-Natal and Gauteng provinces. The scenic, cultural and archaeological resources enhance the area's potential for tourism development.

The physical biodiversity of the Mnweni area manifests itself through a rich mix of plants and animal species that are abundant in the diverse array of habitats created by the water system. The need to sample diatoms in estuaries and rivers arises from the necessity to know the water quality in terms of the South African National Water Act 36 (1998). When benthic diatoms are collected from riverine sediments and from estuaries, the selection of the precise site is either specific or random (Bate *et al*., 2004a, b). It is common knowledge that diatom species can be used as an estimate of water quality however; these assemblages may vary from habitat to habitat (Steele *et al*., 2014).

Diatom Collection and Processing

Various procedures were used in the preparation of composite algal inoculums representative of all diatom communities from the different sampling sites of the river system.

Phytoplankton

A phytoplankton net (55 µm) was swept ten times at each sampling site. This procedure was repeated until a dense phytoplankton concentrate was obtained. The concentrate was stored on ice until return to the laboratory.

Epipelon

Sampling of diatoms involved obtaining a segment of the upper 10 mm of the river bed. A simple specially devised hand held and operated corer was used. This consisted of a 20 mm diameter Perspex pipe of 2 m in length. Built into the pipe was a simple plunger, which aided in the extraction of the sediment. One end of the pipe was inserted into the sediment to a depth of a few centimetres. Cupping ones palm over the other end created a suction enabling it to be removed with the sediment in place. Upon extraction, the stopper was removed and the sediment core gently removed by depressing the plunger (Bate *et al*., 2004a, b).

Upon removal of the sediment, a knife was used to cut out the top 10 mm of the sediment. The sediment was then placed in a sterilised plastic bottle; water from the sampling site was added to cover the sample. This procedure was replicated thrice in different positions at the same site in order to get a sample that was representative of the different micro- habitats at each site. Each bottle was labelled with the site number, sample number and Global Positioning System (GPS) reading.

Laboratory Sample Processing

The sediment samples were taken to the sampling camp station where they were carefully placed in 90 mm diameter plastic petri dishes and allowed to settle overnight. The supernatant was drawn off in the morning and eight new cover slips (covering more than 90% of the surface area) were placed on the moist surface. Cover slips were carefully removed after 2 h with as little sediment as possible. This method ensured that only living cells were attached to the cover slips (Bate *et al*., 2004a, b). Glass bottles were used to transport the cover slips to the laboratory.

Epilithon

Stones and boulders were randomly selected, collected from the middle of the system and carefully shaken to remove surface organic matter and sand particles. The stones and boulders were placed in a large plastic tray together with a small amount of river water and the upper side thoroughly scrubbed with a plastic spatula. The resulting diatom suspension was placed in a labelled sampling bottle and taken to the laboratory on ice.

Slide Preparation

Aliquots of the algal inoculate were digested in saturated potassium permanganate ($KMnO_4$) and 1:1 (v/v) hydrochloric acid (HCl; 10 M). Distilled water was used to clean all samples using five repeated spins (2000 rpm for 10 min). Permanent light microscopy slides were prepared using 1 µL of diatom "digest", placed onto an acid-washed cover slip and air dried (Bate *et al.*, 2004). Slides were made in triplicate and average values taken as representative of diatoms from each site.

Cover slips treated and stored in this manner allow the drop of sample to spread more evenly (Bate *et al*., 2004). When totally dry, a small amount of Naphrax mounting medium (Saarchem-Holpro Analytical Supplies, S.A.) was placed onto the glass slide and the cover slip placed over it. The mountant was allowed to dry for two days and each slide was eventually sealed around the edge of the cover slip with Bioseal® to prevent ageing of the mountant.

Diatom Identification and Enumeration

Diatom frustules were examined under an Olympus Bx41TF light microscope with DIC optics and attached with a Sony Hyper HAD Digital video camera (SSC-DC18P). Images of the species enumerated were visualised and captured using the Analysis image analysis programme (© 2001, Soft Imaging System GmbH). From each sample 200 diatom valves were counted using 1000 × magnification). Identifications were facilitated largely by taxonomic work done by Bate *et al*. (2004) and a variety of diatom classification nomenclatures and literature (Schoeman and Archibald, 1976; Hustedt, 1976; Archibald, 1983; Krammer and Lange-Bertalot, 1986-1991; Round *et al*., 1990; Van Vuuren *et al*., 2006; Taylor *et al*., 2007).

Statistical Data Analysis

A range of formal statistical analyses were used to study patterns in diatom community structure. These included both univariate and multivariate techniques. Diversity indices that successfully collapse full sets of species counts in samples into single coefficients can be accurately treated using univariate statistical approaches (Clarke and Warwick, 1994). The Kruskal-Wallis method was used to perform analysis of variance by ranks and to compare the diversity indices amongst different reaches or seasons. When the null hypothesis of no difference was rejected at a probability $P<0.05$, differences of ranks were compared using pair-wise multiple comparisons procedure (Turkey's test). The following diversity indices were considered:

- S = total number of species recorded per sample
- N = total density of diatoms recorded per sample
- d = Margoles's species richness
  $d = (S-1)/\log N$
- H' = Shannon-Wiener diversity
  $H' = -\sum_i p_i (\log_e p_i)$ where p_i is the proportion of the total sample density arising from the ith species
- J' = Pielou's evenness index

$J' = H'observed/H'_{max}$ where H'_{max} is the maximum possible diversity which would be achieved if all species were equally abundant.

Multivariate analyses were achieved using the computer software package PRIMER v.5 (Plymouth Marine Laboratory, UK) and included methods of grouping (hierarchical group average linkage), ordination (non-metric Multi-Dimensional Scaling [MDS] and k-dominance curves (ranked species abundance plots). The adequacy of MDS in signifying similarities amongst samples in a low dimension ordination plot can be measured by the extent to which the ordination preserves the rank order of dissimilarities. A stress value is derived and gauged against points of reference (Clark and Warwick, 1994), as indicated below:

- stress <0.05 gives an excellent representation with no prospect of misinterpretation
- stress <0.1 gives a good ordination that is unlikely to lead to misinterpretation
- stress <0.2 gives a potentially useful ordination which may be interpreted with some caution
- stress >0.3 gives an ordination that should be treated with scepticism

Tests for differences in structure and composition of assemblages were achieved using analysis of similarities (2-way crossed ANOSIM). The ANOSIM test statistic (R) is defined so that:

- $-1 \leq R \leq 1$
- R =1 only if all replicates within sites are more similar to one another than any replicates from different sites.

R approaches zero if the null hypothesis is true, so that similarities between and within sites will be the same on average (Clark and Warwick, 1994).

Results

Species Abundance and Dissimilarity Analysis

In this study, a total of 14 diatom species which belonged within 9 genera were found in the Mnweni River system study sites (Fig. 1). Species belonging to the genera *Cymbella* (namely *Cymbella turgidula* Grunow) was found to be present at all sites but most abundant in sites 1 and 2. Species belonging to other genera were also found but were not dominant and occurred in 1, 2 or 3 sampling sites only (Table 1). The genus *Navicula* was more abundant than other diatom genera. Site 2 had the most number of diatom genera whilst site one was the least diverse (Table 1). The diatoms *Navicula lanceolata* Ehrenberg and *Navicula cryptonella* Lange-Bertalot were found in site 2 only.

The Multi-Dimensional Scaling (3D stress = 0.04) showed the similarity of the assemblage data and displayed a good indication of the dissimilarity of species that were in the river (Fig. 2). A SIMPER analysis of the data showed the average dissimilarity between the sites. In sites 1 and 2, the dissimilarity was 65%. At site 1, *Cymbella turgidula* was found to dominate (105 diatoms) whilst this species was greatly reduced in site 2 (44 diatoms). The average dissimilarity between sites 1 and 3 was 77%. In site 3, *Encyonopsis stafsholtii* was the most abundant diatom (102 diatoms) whilst site two was dominated by many diatoms (Table 1). The average dissimilarity of site 4 was 76%.

Table 1: Dominant diatom species in the sampling sites

Diatom species	Number of diatoms/µl of water				
	Site 1	Site 2	Site 3	Site 4	Site 5
Cymbella turgidula Grunow	105	44	29	32	41
Diploneis didyma (Ehrenberg) Cleve	0	8	12	0	27
Encyonopsis stafsholtii La Bahls	0	0	102	68	0
Fragilaria capucina Desmaziere	0	4	0	4	0
Frustulia rhomboides (Ehrenberg) De Toni	5	0	0	0	1
Geissleria decussis (Ostrup) Lange-Bertalot & Metzeltin	0	8	12	0	46
Hantzschia amphioxys (Ehrenberg) Grunow	26	5	14	0	0
Hantzschia marina (Donkin) Grunow	0	0	0	17	17
Navicula cryptocephala Kutzing	0	27	0	0	45
N. cryptonella Lange-Bertalot	0	23	0	0	0
N. gastrum (Ehrenberg) Kutzing	0	0	0	45	0
N. gregaria Donkin	33	30	0	0	30
N. lanceolata Ehrenberg	0	35	0	0	0
Placoneis dicephala W Smith	0	0	55	25	0

Table 2: Physico-chemical parameters as per each sampling site within the Mnweni River system

Parameter	1	2	3	4	5
TDS ($mg.l^{-1}$)	360	500	340	450	200
Phosphorus ($mg.l^{-1}$)	0.979	1.2975	2.475	2.438	3.145
Nitrogen ($mg.l^{-1}$)	0.438	0.983	1.721	1.238	2.375
pH	7.27	7.29	7.17	7.52	7.1

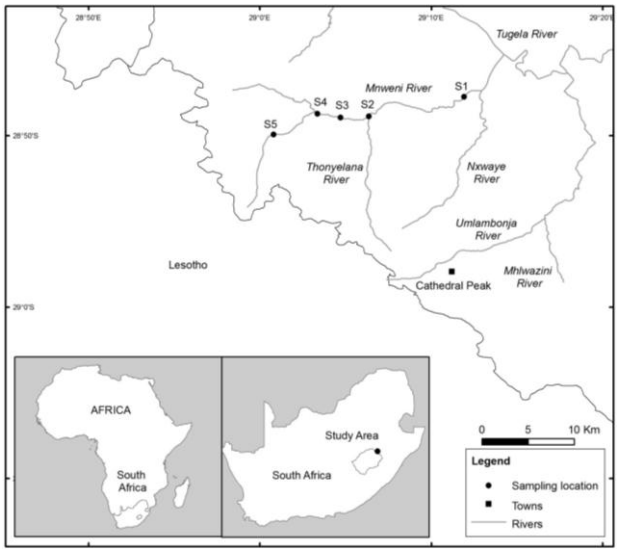

Fig. 1: Map illustrating sampling points along the Mnweni river system

At this site, the dominant specie's were *Encyonopsis stafsholtii* (68 diatoms) and *Navicula gastrum* (45 diatoms).

Site 5, had an average dissimilarity of 71%. The specie *Geissleria decussis* (Ostrup) Lange-Bertalot and Metzeltin showed the greatest abundance at this site (46 diatoms) followed by *Navicula crypotenella* with an abundance of 45 diatoms. Three diatoms, *Geisleria decussis*, *Navicula cryptocephala* and *Diploneis didyma* were more dominant in site 5 than any of the other sites. The presence of *Cymbella turgidula*, which was found in site 5, as well as all other sites, was a clear indication that the Mnweni River system was a fresh water system.

Physical and Chemical Parameters

Table 2 represents the physico-chemical parameters of the Mnweni river system in KwaZulu-Natal. The TDS concentrations were the the highest at sites 2 (500 $mg.L^{-1}$) and 4 (450 $mg.L^{-1}$). Site 5 had the lowest TDS concentration of 200 $mg.L^{-1}$ compared to all the sites that were sampled. Site 1 had a concentration of 360 $mg.L^{-1}$ and site 3 a value of 340 $mg.L^{-1}$. Relatively high phosphorus concentrations were measured in sample sites 3, 4 and 5 (Table 2). Sample sites 3 (1.721 $mg.L^{-1}$) and 5 (2.375 $mg.L^{-1}$) had the highest nitrogen levels compared to site 1 which had the lowest (0.438 $mg.L^{-1}$). Site 5 had the lowest pH (7.1). Sites with higher TDS, Phosphorous and Nitrogen concentrations had slightly suppressed pH values although all values were above 7.

Biological Parameters–diatom Richness (D), Evenness (J) and Diversity (H) Species Indices

The species diversity between the sampling sites were relatively high and varied from 1.2 – 1.5 respectively (Fig. 3). Sample site 2 showed the highest species diversity compared to the other four sites. The species richness at sites 2, 3 and 4 showed an increase in richness at these sites whereas sites 1 and 5 showed a low level of species richness (Fig. 3). Species evenness between the sites were low and varied between 0.6 – 0.8 (Fig. 3). There were noticeable differences in the richness and diversity of diatom assemblages that were indicative of variation in the water chemistry and the habitat character between the sites. In particular, richness and diversity declined at the sites with slower water flow, pebble, gravel and silt substrata.

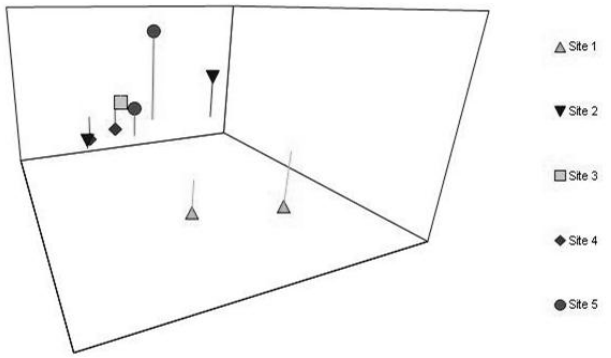

Fig. 2: MDS Plot of the Mnweni River System

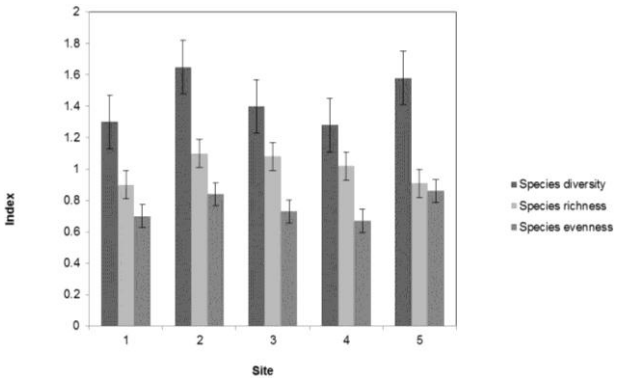

Fig. 3: Indices of species diversity, species richness and species evenness between five different sites along the Mnweni River

Discussion

Human disturbances have adversely affected the TDS levels of inland waters throughout the world (Hart *et al*., 1991). Total dissolved solid levels are a routine measurement in all freshwaters. When this parameter is measured by combining the results of separate analyses for all major ions, it is equivalent to salinity (Day, 1990). The increase in the TDS levels at sites 3, 4 and 5 may be caused by irrigation and greater turbidity caused by humans and animals. Irrigation causes salinisation of rivers in two ways. Firstly, although the irrigation water may be low in TDS, the water itself is taken up by crops or even evaporates resulting in solutes being left behind. Ion exchange processes in the soil results in the accumulation of NaCl, which is washed out of the soil into rivers. Secondly, irrigation may result in a rise in the water table and subsequent evaporation from the surface of the now wet soil (Hart *et al*., 1991). The reuse of water, whether it is recycled for immediate water consumption or returned to a stream and subsequently reused further downstream, will result in partial evaporation during the cleansing process and will consequently lead to increased TDS levels (du Plessis and van Veelen, 1991; Diekmann *et al*., 2009).

In general, it appears that many diatom species were able to survive at comparatively high salinities. Many freshwater blue greens and bacteria can adapt readily to TDS values (Hart *et al*., 1991). It was shown by Prinsloo and Pieterse (1994) that an increase in TDS concentrations in the middle of the Vaal River was accompanied by decreases in turbidity. They have also shown that production in green algae, *Monoraphidium circinale* increased in TDS values between 500 and 2500 $mg.L^{-1}$, while that of the diatom *Cyclotella meneghiniana* and the blue green *Microcystsis aeruginosa* were inhibited at those concentrations.

The high concentration of phosphorus was probably due to leaching or runoff from agricultural land. Phosphorous plays a major role in the structure of nucleic acids and in molecules, which are involved in the use, and storage of energy in cells (Addiscott *et al*., 1991). The increase in phosphorous levels at sites 3, 4 and 5 probably resulted in an increase in diatom biomass. Nitrogen occurs abundantly in nature and is essential in the constituent of proteins, which include enzymes that catalyse all biochemical processes, and are therefore a major component of all living organisms. The relatively high concentrations of nitrogen at sample sites 3 and 5 resulted in increases in electrical conductivity (Table 1). The high levels of these nutrients also add to the high TDS levels at certain sampling sites. This nutrient enrichment is detrimental to the healthy growth of all aquatic organisms in the system. The role of the processes and mechanisms that regulate the supply of bioavailable phosphate and nitrogen is essential in the management of catchments, rivers and lakes in order to avoid pollution (Webster *et al*., 2001; Trevisan *et al*., 2010).

The pH is determined largely by the concentrations of ions (H+) and alkalinity by the concentrations of hydroxyl, bicarbonate and carbonate ions in water. In very pure waters the pH can change rapidly because the rate of change is determined by the buffering capacity, which in turn is usually determined by the concentration of bicarbonate and carbonate ions in the water (Dallas and Day, 2004; Li and Zheng, 2011). The banks of the Mnweni River were denuded due to the removal of vegetation in order to increase agricultural production. The river chemistry and habitat character differed between sites, typical of agricultural land and human use of the river system. The concentrations of nitrogen at the sampling sites 3 and 5 were relatively high, probably because of the utilisation of agricultural fertilisers, as well as higher erosion rates and increased weathering and evaporation (Jenkins *et al*., 1995; Collins and Jenkins, 1996; Katiyer *et al*., 2010). It was observed during sampling that there was removal of flora along the Mnweni riverbanks due to an increase of agriculture in the area.

Ecological gradients and assemblage composition in the Mnweni River catchment suggested that aquatic chemistry, habitat structure, current type and agricultural

use were the most important environmental variables affecting diatom species composition in the Mnweni River. Volcanic rocks were present at the top of the river system and sedimentary rocks and boulders were present at the end of sampling sites. These characteristics may result in changes to the geology of the Mnweni River. Furthermore, diatom assemblages were different between agricultural catchments and this may be due to the influence of sewage or human waste from settlements. In agriculturally dominated landscapes conductivity, nutrient concentrations, catchment use and current flow are key environmental variables affecting benthic algae (Munn *et al*., 2002). Water chemistry gradients were an important component in this study but changes in the flow character, anthropogenic modification or influences on the river bank and catchment land also significantly contributed to changes in assemblage composition between sampling sites.

The results indicated that diatom diversity and assemblage composition in the Mnweni River are affected by water chemistry as well as organic pollution. In addition, diatom composition may be further influenced by habitat characteristics that are related to water flow, river bank character and catchment land use. The diatom assemblages that were found at the Mnweni river sites were characteristic of clean or slightly enriched water conditions.

The demands for water resources and changes to freshwater habitats in the Mnweni River catchment are likely to escalate in the future. The expansion of settlements will probably result in an increased use of fertilisers for crops and livestock which may cause diatom communities to reflect those changes.

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