

**Towards the successful application of diatom-based
biomonitoring in South Africa**

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**Thesis submitted in fulfilment of the requirements for the degree
Philosophiae Doctor in Environmental Sciences at the North-West University**

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Truly, the world which we are holding between our finger and thumb – this world in a globule of water – this world of rollicking, joyous, boisterous fellows, that a pin's head would take up, is even more wonderful than the shoals of whales in Baffin's Bay, or the herds of elephants that shake the earth in the forests of Ceylon. Truly the great God who made them is *maximus in minimus*.

Philip Gosse 'The Minute'

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“For by him all things were created that are in heaven and that are on earth, visible and invisible, whether thrones or dominions or principalities or powers. All things were created through Him and for Him” Colossians 1:16

To my wife, your patience has been immense and your support irreplaceable – Thank you!

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TOWARDS THE SUCCESSFUL APPLICATION OF DIATOM-BASED BIOMONITORING IN SOUTH AFRICA

SUMMARY

Water is a scarce and precious resource in South Africa. Not only is the quality and quantity of water available limited by climatic conditions but these resources are often impacted by domestic and industrial effluents, as well as run-off from agriculture. Monitoring the aquatic environment helps to determine the impact of various pollutants.

Chemical monitoring, important as it is, can only at best give a "snapshot" of water quality at a particular moment in time. Biomonitoring in particular forms a vital part of monitoring programmes as it gives a time integrated reflection of a particular pollutant or group of pollutants on the ecosystem.

Although diatoms have long been studied in South Africa, as well as being used in the past to indicate water quality, the current suite of bioindicator organisms used in this country has not until recently included diatoms, or any autotrophic organisms for that matter.

This body of work is presented with the aim of facilitating the use of diatoms as bioindicators of water quality in South Africa. Methods for the collection, preparation and analysis of diatoms have been collated and standardized. A number of European and other indices have been tested in diverse localities for use in indicating recent and historical water quality. The impact of diffuse mine effluent on diatom community structure has also been discussed. A new species common in impacted waters (i.e. a potential indicator species for these conditions) has been described and a guide to the common diatoms of South Africa is presented which, it is hoped, will be of use to those who wish to commence with a study of South Africa's diatom flora.

The results of this study show that diatom indices are successful in reflecting both current and past water quality in South Africa. This result justifies the inclusion of diatoms with the current suite of bioindicator organisms as well as in national monitoring initiatives such as the River Health Programme.

Concluding comments underline the importance of using diatoms as indicators, not only of water quality, but also as indicators of aquatic microorganism biodiversity, or loss thereof. The need for continued research in the fields of diatom ecology and taxonomy is also highlighted.

Key words: Diatoms, Biomonitoring, Water quality, Indicators, Standard methods, New species, Illustrated guide.

IN DIE AANLOOP TOT DIE SUKSESVOLLE TOEPASSING VAN DIATOOM- GEBASEERDE BIOMONITERING IN SUID-AFRIKA

OPSOMMING

Water is 'n skaars en waardevolle natuurlike hulpbron in Suid Afrika. Beide die hoeveelheid en kwaliteit van water word beperk deur klimatologiese toestande, maar word verder deur huishoudelike, industriële en landbou-afval geïmpakteer. Monitoring van die akwatiese omgewing is noodsaaklik om die impak van hierdie besoedelstowwe te bepaal.

Chemiese monitoring, alhoewel belangrik, bied slegs 'n aanduiding van die waterkwaliteit op 'n oomblik in tyd. Biomonitoring vorm 'n baie belangrike deel van monitoringsprogramme en gee 'n geïntegreerde beeld van die invloed van 'n spesifieke besoedelstof, of groep besoedelstowwe, op die ekosisteem.

Alhoewel diatome lank reeds in Suid Afrika bestudeer is en ook reeds in die verlede gebruik is as indikatore van waterkwaliteit, vorm diatome, of enige ander outotrofiese organismes, nie deel van die huidige groep bioindikator-organismes wat in die land gebruik word nie.

Die doel van die huidige studie is om die gebruik van diatome as bioindikatore van waterkwaliteit in Suid Afrika te fasiliteer. Metodologie vir die versameling, voorbereiding en analise van diatome is saamgevoeg en gestandaardiseer. Diatoomindekse van onder andere Europa is in 'n verskeidenheid lokaliteite getoets vir hul gebruik as indikatore van resente en historiese waterkwaliteit. Die impak van diffuse mynbou-afval op diatoom-gemeenskapstruktuur word ook aangespreek. 'n Nuwe diatoomspesie wat algemeen in geïmpakteerde water voorkom (en dus kan dien as 'n potensiële indikatorspesie vir hierdie toestande) is beskryf. Verder is 'n identifikasiegids tot die algemene diatome van Suid Afrika saamgestel in die hoop dat dit sal dien as inspirasie vir diegene wat wil begin met studies van Suid Afrika se diatoomflora.

Die resultate van hierdie studie toon dat diatoom-indekse betroubare aanduiders is van resente en historiese waterkwaliteit in Suid Afrika. Hiermee word die insluiting van diatome in die huidige groep bio-indikatororganismes, asook in nasionale monitoringsprogramme soos die *River Health Programme*, onomwonde geregverdig.

Ter afsluiting word die belang van diatome as indikatore, nie net van waterkwaliteit nie, maar ook van mikrobiese biodiversiteit, uitgelig. Die noodsaaklikheid van voortgesette navorsing in die velde van diatoom-ekologie en –taksonomie word ook aangespreek.

Sleutelwoorde: Diatome, Biomonitoring, Waterkwaliteit, Indikatore, Standaardmetodes, Nuwe spesies, Identifikasiegids.

PREFACE

Statement of selected format

Article format has been selected for the presentation of this thesis.

Authors contribution to jointly written Chapters and Annexures

- | | |
|---------------------|--|
| Chapter 2 - | Study planned jointly. Jonathan Taylor and Arno de la Rey wrote the paper jointly. Methods and techniques included in the paper were selected and tested by Jonathan Taylor. |
| Chapter 3 - | Paper planned jointly. William Harding wrote the main part of the paper to which Colin Archibald and Jonathan Taylor contributed. |
| Chapter 4 - | Study planned jointly. Arno de la Rey and Jonathan Taylor wrote the paper jointly. All diatom data was collected and analysed by Jonathan Taylor. |
| Chapter 5 - | Study planned jointly. Jonathan Taylor and J  an Prygiel wrote the paper jointly. Jonathan Taylor analysed all diatom data and carried out all statistical analyses. |
| Chapter 6 - | Study planned jointly. Jonathan Taylor wrote the main part of the paper to which William Harding and Colin Archibald contributed. Jonathan Taylor analysed all diatom data and carried out all statistical analyses. |
| Chapter 7 - | Study planned by Colin Archibald. Colin Archibald and Jonathan Taylor wrote the paper jointly. |
| Chapter 8 - | Study planned jointly. Jonathan Taylor and Horst Lange-Bertalot wrote the paper jointly. All analysis of diatom data, statistical analyses, light and electron microscopy was performed by Jonathan Taylor. |
| Annexure 1 - | Planned by Jonathan Taylor. Jonathan Taylor collected the majority of the images and wrote the main part of the text to which Colin Archibald and William Harding contributed. |
| Annexure 2 - | Paper was planned jointly. Jonathan Taylor wrote the text. |
| Annexure 3 - | Study planned jointly. Jonathan Taylor wrote the main part of the text and Dale Elgar performed the microscopy and photography. |
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List of publications and details

- i. TAYLOR JC, DE LA REY PA and VAN RENBURG L (2005) Recommendations for the collection, preparation and enumeration of diatoms from riverine habitats for water quality monitoring in South Africa. *African Journal of Aquatic Sciences* **30** (1): 65-75.
- ii. HARDING WR, ARCHIBALD CGM and TAYLOR JC (2005) The relevance of diatoms for water quality assessment in South Africa: a position paper. *Water SA* **31**(1): 65-75.
- iii. DE LA REY PA, TAYLOR JC, LAAS A, VAN RENBURG L and VOSLOO A (2004) Determining the possible application value of diatoms as indicators of general water quality in the Mooi River (North West Province): A comparison with SASS 5. *Water SA* **30** (3): 325-332.
- iv. TAYLOR JC, PRYGIEL J, VOSLOO A, DE LA REY PA, VAN RENSBURG L. Can diatom-based pollution indices be used for bio-monitoring in South Africa? A case study of the Crocodile West and Marico water management area. Accepted for publication in *Hydrobiologia* (04/01/2007).
- v. TAYLOR JC, HARDING WR, ARCHIBALD CGM and VAN RENSBURG L (2005) Diatoms as indicators of water quality in the Jukskei-Crocodile river system in 1956 and 1957, a re-analysis of diatom count data generated by Dr. B. J. Cholnoky. *Water SA* **31** (2): 237-246
- vi. ARCHIBALD CGM and TAYLOR JC (2004) The assessment of diffuse pollution from acid-mine drainage using an updated and revised diatom assessment procedure as an added-value bio-monitoring tool. *Presented at the Specialist Conference on Diffuse Pollution, IWA-WISA Conference 2005. Sandton Convention Centre, Johannesburg, 9-12 August 2005 and Published in Water Science & Technology* **55** (3): 151–160.
- vii. TAYLOR JC and LANGE-BERTALOT H. *Eolimna archibaldii* spec. nov. and *Navigiolum adamantiforme* comb. nov. (Bacillariophyceae): Two possibly endemic elements of the South African diatom flora tolerant to surface water pollution. *African Journal of Aquatic Science* **31** (2): 175-183.

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Dear Sir or Madam:

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- i. Recommendations for the collection, preparation and enumeration of diatoms from riverine habitats for water quality monitoring in South Africa
- ii. Determining the possible application value of diatoms as indicators of general water quality in the Mooi River (North West Province): A comparison with SASS 5
- iii. Can diatom-based pollution indices be used for bio-monitoring in South Africa? A case study of the Crocodile West and Marico water management area

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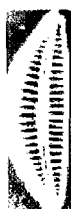
- i. The relevance of diatoms for water quality assessment in South Africa: a position paper
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Yours sincerely,



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KZN Aquatic Ecosystems



Ditot Servata Fides

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- i. *Eolimna archibaldii* spec. nov. and *Naviculolum adamantiforme* comb. nov. (Bacillariophyceae): Two possibly endemic elements of the South African diatom flora tolerant to surface water pollution

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Yours sincerely,

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Dear Jonathan

Permission is hereby given for you to present two publications from the African Journal of Aquatic Science as part of your PhD thesis.

Yours faithfully,
M. Coke

Michael Coke
Editor, African Journal of Aquatic Science

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CHAPTER 1

Introduction and overview

CHAPTER 1 – INTRODUCTION AND OVERVIEW

“The study of the algal group known as the diatoms (Bacillariophyta) provides a valuable tool for assessing the quality of natural and polluted waters. The diatoms are an integral constituent of the aquatic biota and the association of species living at any time at a given locality is the resultant of the physico-chemical conditions of the surrounding waters over a period of time. Each species in an association has its own particular ecological requirements. It is the knowledge of these specific requirements that enables the diatomist to draw conclusions concerning the composition or quality of the water.”

Dr G.G. Cillie. 1976

Director, National Institute for Water Research CSIR (South Africa)

1.1 Why include diatoms in the current suite of bio-monitoring tools in South Africa?

Biological monitoring techniques have been introduced as part of routine monitoring programmes due to certain shortcomings in standard physical and chemical methods. Because of the difficulty of chemically analysing every potential pollutant in a sample of water, and of interpreting results in terms of the severity of impact, it makes sense to turn to the monitoring of aquatic biota. Results given by biological monitoring are also more cost effective and results can be obtained more rapidly than an extensive chemical analysis. The main advantage of a biological approach is that it examines organisms whose exposure to water and any pollutants therein is continuous. Thus species present in riverine ecosystems reflect both the present and past history of the water quality at a particular point in the river, allowing detection of disturbances that might otherwise be missed (Eekhout *et al.*, 1996). The rationale for using diatoms for biomonitoring in South Africa has been laid out in some detail in Chapter 3.

No single group of organisms is always best suited for detecting the diversity of environmental perturbations associated with human activities. Current biomonitoring programmes in South Africa commonly make use of several aquatic bioindicator organisms, however until recently no methods based on autotrophic organisms. The diatoms (Bacillariophyceae) comprise a ubiquitous, highly successful and distinctive group of mostly unicellular algae, with the most obvious distinguishing characteristic the possession of siliceous cell walls (frustules). As autotrophs diatoms contribute significantly to the productivity of such ecosystems, frequently forming the base of aquatic food chains (Cox, 1996). Round (1993) lists numerous reasons why diatoms are useful tools of biomonitoring, amongst which the following bear especial relevance to the South African situation;

methods are cost effective, data is comparable, techniques are rapid and accurate, and identifications and counts can be done by non-specialists with a biological background if they are provided with illustrated guides.

Kelly *et al.* (1998) introduced the concept that diatoms are 'subcosmopolitan', *i.e.* they occur anywhere certain environmental conditions are fulfilled. This concept suggests that geographical location is not the determining factor in the distribution of diatom species and the composition of communities, but it is rather the specific environmental variables at a specific site that determine this distribution. This concept allows for the application of diatom indices developed in Europe and elsewhere and in South Africa (Taylor, 2004). However, Taylor (2004) only tested diatom indices in the Vaal River and recommended that "... future research into the efficacy of diatom-indices as bio-monitoring tools should be undertaken in different physical and geographical regions of South Africa... testing of diatom indices is needed in rivers with different physical characteristics to the Vaal..." . In addition, the study undertaken by Taylor (2004) did not include any reference to organic pollution. Thus a study is presented, which was undertaken to ascertain whether a numerical diatom index developed in Europe has a potential use for indicating general water quality in a small river in the North West Province, and how this index compares to the currently used macroinvertebrate-based indices (see Chapter 4). An additional study, on a much larger scale, was undertaken to assess the whole of the Crocodile West and Marico water management area (see Chapter 5), using diatom indices and to compare index results to those obtained by conventional chemical water quality analysis, including a measure of organic pollution to compliment the study of Taylor (2004) .

1.2 Interpretation and use of historical diatom data

South Africa is in possession of an enormous database of literature, diatom material (slides and preserved material) and most importantly diatom analysis sheets housed in the South African diatom collection at the CSIR in Durban (Harding *et al.*, 2004). To draw correct inferences about the water quality of a given river or stream using diatom analysis methods, several hours are needed behind a high power microscope to determine the relative species composition of the sampled community. In addition, to perform the diatom analysis the operator needs to have a very good knowledge of diatom taxonomy. However, the South African Diatom Collection benefits from the existence of original diatom analysis sheets. Thus the most time consuming and painstaking part of using diatom indices has been completed. It now only remains to convert these diatom analysis sheets to digital format and then generate historical ecological information based on the diatom communities using modern diatom pollution indices that have been developed and tested over several decades in Europe and elsewhere. Hürleman *et al.* (2001) came to the conclusion that

historical algae collections are of great potential value for biological assessments, especially with regard to reference or base-line conditions and biodiversity. A study was undertaken using data from the South African diatom collection as part of an assessment of the potential use of this collection (Harding *et al.*, 2004) to demonstrate the value of historical diatom analysis sheets for use in drawing conclusions about the past condition of South Africa rivers. The results of this study are presented in Chapter 6.

1.3 The use of diatoms for monitoring diffuse pollution and extreme environments

The nature of diffuse effluents and effluents in extreme environments (e.g. mine effluent canals) makes them difficult to monitor using traditional chemical-based techniques. Pollutant concentrations continuously fluctuate while the effect of these pollutants on ecosystems cannot be easily described using chemical-based techniques.

The diatoms are suitable for monitoring water quality as they, and the composition of their assemblages, are directly affected by different variables and do not have escape mechanisms. The diatoms are associated with a particular substrate and their community composition will be a direct result of water quality changes. Other organisms traditionally used for biomonitoring (e.g. macroinvertebrate insects) simply cannot survive some extremes of water quality, and in addition are restricted by habitat availability and water depth in an environment such as an effluent canal. Diatoms have no such restrictions and occur in many extremes of water quality (e.g. low pH, high salinity etc.) and can live in streams a few millimetres deep, making them ideal indicator organisms in these conditions. A study, presented in Chapter 7, was undertaken to assess the use of diatoms for monitoring the impact of diffuse mine pollution on a natural stream.

1.4 Facilitation of diatom-based monitoring studies in South Africa

If a biomonitoring technique is to be introduced and be used successfully the first and most important step is the provision of standard guidelines and best practices for the application of the method in the field and laboratory. This need has been addressed within the scope of a South African Water Research Commission Project (for details see www.dhec/diatoms). The importance of the provision of standardised methods has been recognised in Europe and has taken the form of recommendations (Kelly *et al.*, 1998) and two standard protocols (CEN, 2003; CEN, 2004). Some South African literature exists on methodology for the preparation of diatoms for examination under the microscope (e.g. Welsh, 1964) but these methods are often out-dated and make use of dangerous chemicals. It was deemed necessary to test and apply methods developed in Europe for

the collection, preparation and enumeration of diatom and to then recommend a standard set of procedures for South Africa. These standard recommendations are presented in Chapter 2.

The lack of illustration of common species together with vast changes in diatom taxonomy over the last decade has lead to misconceptions about diatom taxa encountered in South Africa (see discussion in Chapter 6). The vast majority of common diatom taxa found in South Africa are cosmopolitan both in distribution (Krammer & Lange-Bertalot, 1986-1991), and environmental tolerances. However, some poorly studied and described endemic diatom species occur as dominant species in polluted and anthropogenically impacted waters, making them valuable indicators for these conditions.

Thus there are two gaps to be filled in the knowledge of South African diatoms. 1.) The description of new diatom species, together with a description of their associated ecological requirements with the aim of eventually including these species in South African biomonitoring systems. 2.) The illustration of common diatom species found in South Africa, allowing for the easier and more rapid identification of common and ecologically critical elements of the South African diatom flora. Thus two dominant, endemic and pollution tolerant diatom species have been described and discussed (see Chapter 8), and an iconographic summary of some of the most common elements of the South African diatom flora is presented (Appendix 1).

1.5 Research aims and objectives

The aim of the study is to present a body of work to describe, standardise, test and facilitate the implementation of diatom-based water quality biomonitoring in South Africa.

2. METHODOLOGY

General methodology is laid out in Chapter 2. The methods followed when using different statistical techniques are laid out in the individual chapters.

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CHAPTER 2

**Recommendations for the collection, preparation and
enumeration of diatoms from riverine habitats for water
quality monitoring in South Africa**

African Journal of Aquatic Science

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Recommendations for the collection, preparation and enumeration of diatoms from riverine habitats for water quality monitoring in South Africa

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Diatoms have become important organisms for monitoring freshwaters and their value has been recognised for cross-border water quality monitoring in the European Union. If South Africa is to include diatoms in the current suite of bioindicators, then thorough testing of diatom-based techniques is required. This paper provides guidance on methods through all stages of diatom collection, preparation and examination for the purposes of water quality assessment.

Keywords: archiving, biomonitoring, diatom-based indices, diatoms, enumeration, preparation, sampling, water quality assessment

Introduction

Water is a scarce and valuable resource in South Africa and as such needs to be protected from excessive pollution. The only way to effectively identify current trends in pollution is by monitoring the resource, which can be done using traditional chemical methods as well as the more recent suite of biological methods that form part of the National Biomonitoring Programme for Aquatic Ecosystems (NBPAE) (Hohls 1996).

The National Water Act (1998) states that '...water is (to be) protected, conserved, managed and controlled in a sustainable and equitable manner for the benefit of all persons.' Water quality monitoring, both chemical and biological, provides information on the quality of resources, and as such is the first step in ensuring the effective implementation of the new Water Act.

Biological monitoring techniques have been introduced as part of routine monitoring programmes because of certain shortcomings in standard physical and chemical methods. It is difficult to analyse every potential pollutant in a sample of water and, when interpreting results in terms of the severity of impact, it makes sense to turn to the aquatic biota. The main advantage of a biological approach is that it examines organisms whose exposure to water and any pollutants therein is continuous, and reflects the actual impacts (both long and short-term) of pollutants on the ecosystem.

Recent studies, as well as studies in progress, have identified diatoms as useful organisms to include in the suite of biomonitoring tools currently used in South Africa (Bate *et al.* 2002, de la Rey *et al.* 2004, Taylor 2004) both for assessments of current water quality and for establishing historical conditions in rivers in South Africa (Taylor *et al.* 2005). If diatom monitoring and the use of the associated indices are to become a fully-fledged part of the

NBPAE in South Africa it is of the utmost importance that samples are collected, prepared and stored in a standard manner, which will not only provide data on current ecological conditions but can also be stored and, as such, provide reference material for future studies and investigations.

Usually, sample protocols and methodological recommendations become standardised only after years of study and validation. However, international studies on the routine use of diatoms in river monitoring studies have reached the point where such recommendations have been made for Europe and other parts of the world. Several early studies conducted in South Africa examined different preparation protocols for diatoms, especially for the counting and enumeration of diatom communities (Cholnoky 1968, Schoeman 1973).

Diatoms have been shown to be reliable indicators of specific water quality problems such as organic pollution, eutrophication, acidification and metal pollution (Rott 1991, Tilman *et al.* 1982, Dixit *et al.* 1992, Cattaneo *et al.* 2004), as well as for general water quality (AFNOR 2000). Although this paper is not intended as a motivation for the use of diatoms as bioindicators, it is perhaps important to mention the reasons why diatoms are useful tools for biomonitoring, as listed by Round (1993):

- diatoms have a universal occurrence throughout all rivers;
- field sampling is rapid and easy;
- cell cycle is rapid and they react quickly to perturbation;
- diatoms are relatively insensitive to physical features in the environment;
- cell counting by microscopic techniques is rapid and accurate;
- cell numbers per unit area of substratum are enormous, making random counts excellent assessments of diatoms;

- the ecological requirements of diatoms are in many cases better known than those of any other group of riverine organisms;
- permanent records can be made from every sample;
- unlike invertebrates, diatoms do not have specific food requirements, specialised habitat niches, and are not governed to a major extent by stream flow.

Although diatom-based water quality monitoring has many advantages, there are some problems associated with the techniques used in such monitoring. These include recent rapid changes in diatom taxonomy with the re-assignment of many taxa to new genera. Not all such changes are accepted by all diatomists. Many taxa belonging to the genera *Achnanthes* Kützinger, *Mayamaea* Lange-Bertalot and *Eolimna* Lange-Bertalot & Schiller are very small and may be difficult to identify and distinguish using light microscopy (LM). In some cases it is necessary to check the identity of these species using scanning electron microscopy (SEM); this is especially true for the fragilarioid diatoms (Morales 2001).

Despite these problems, within the last decade diatom-based indices of aquatic pollution have gained considerable popularity throughout the world. Much of the development and testing of diatom indices has been carried out in France, where that country's size and typological diversity enabled a more general application in Europe (Prygiel and Coste 1999). The design of software programmes such as OMNIDIA for the calculation of diatom indices has also facilitated the use of diatom-based biomonitoring methods (Lecointe *et al.* 1993). A variety of diatom indices have been adopted and tested in many European countries including Finland (Eloranta and Andersson 1998) and Poland (Kwandrans *et al.* 1998). In recent years, diatom-based techniques have also been used for monitoring associated with the directives of the European Union (Kelly *et al.* 1998, Prygiel *et al.* 2002). Many other countries, including Taiwan (Wu 1999), Malaysia (Maznah and Mansor 2002), Argentina (Gómez 1999), Australia (John 2000) and the US (Stevenson and Pan 1999), are now either using diatoms as part of their routine monitoring programs or are in the process of developing the necessary techniques to do so.

European diatom indices were derived, applied and tested in temperate regions, and there is little information regarding their application in the tropics and subtropics (Wu and Kow 2002). Thus the need exists for the evaluation of these indices before they can be routinely applied in warmer climates. Evaluation of numerical diatom indices has begun in earnest in South Africa, with one study already published (de la Rey *et al.* 2004) plus the existence of a large amount of unpublished data (Taylor 2004). It has become apparent during the testing of diatom-based indices that, without standard field and laboratory protocols, robust data cannot be collected in a country as large and diverse as South Africa.

De la Rey *et al.* (2004) and Taylor (2004) showed that diatom-based pollution indices may be good bioindicators of water quality in riverine ecosystems in South Africa by demonstrating a measurable relationship between water quality variables such as pH, electrical conductivity, phosphorus and nitrogen, and the structure of diatom communities as reflected by diatom index scores. The conclusion reached was that diatom indices need to be

tested further in South Africa. If further broad scale testing is to succeed, workers in the field of diatom ecology should standardise the techniques used for the collection, preparation and enumeration of diatom samples, and provide a standardised set of methods for the analysis of benthic diatom samples which will enable maximum use to be made of all samples taken by various workers. This will assist scientists to evaluate current diatom-based indices as well as the creation and refinement of new diatom-based water quality indices or other methods based on the deviations between reference and observed communities.

Kelly *et al.* (1998) made a strong case for the standardisation of methods used for the sampling of benthic diatoms for water quality studies in Europe. They argued that, if basic data could be collected in a robust and systematic manner, it could be used in a number of different ways in the future. This could facilitate the evaluation of indices in different geographical areas and enable individuals developing and refining indices to draw upon data from other regions in order to get a better idea of the environmental preferences of taxa.

It is thus the aim of this paper to present a set of standardised protocols for field collection of samples and the preparation and enumeration of these samples in a manner yielding the most reproducible data. This paper collates relevant methodological information from both European and South African studies to make these principles more readily available to those wishing to use diatoms in water quality monitoring studies in southern Africa.

Habitats of choice for diatom-based water quality monitoring

According to Round (1993) diatoms form distinct assemblages that occur closely associated with particular microhabitats, e.g. on sediments (epipelon), sand (epi-sammon), gravel, stone and bedrock (epilithon) and macrophytic plants (epiphyton). Because of these distinct associations care should be taken not to contaminate the target community with species from other microhabitats when sampling from a specific substratum type. Although diatom community structure may to some extent be governed by substrata associations (Reavie and Smol 1997), the main influences on community composition are disturbance (mainly from floods), resource supply (mainly from inorganic nutrients) and, to a lesser extent, grazing (Biggs *et al.* 1998).

Round (1993), Kelly *et al.* (1998) and Prygiel *et al.* (2002) consider cobbles and small boulders as the preferred substratum for monitoring diatoms in the riverine environment, and almost all diatom indices throughout the world can be applied to the community that develops on this substratum.

The most important reasons for this choice of substratum can be summarised as follows:

- epilithic substrata are generally widely available, throughout the length of a river from headwaters to lowland stretches, and throughout the year (Kelly *et al.* 1998);
- the type of stone sampled can usually be discounted when assessing the flora at a particular site (Kelly *et al.* 1998);

- the performance of major diatom-based indices on this substratum is well understood (e.g. Eloranta and Kwandrans 1996);
- the epilithon is ecologically better known than any other group (Round 1993).

In the absence of cobbles or small boulders, emergent macrophytes — such as *Typha* sp. or *Phragmites* sp., — or submerged macrophytes may be sampled for diatoms. If pebbles, cobbles, boulders or macrophytes are absent from the sample site, artificial substrata may be sampled if they have been submerged for at least four weeks. The advantages of using artificial substrata include the ease of sampling from smooth surfaces, an easier control over the exact area of sampling, as well as standardisation of substrata, less contamination by macrophytic algal growth, and the option of exact positioning (Round 1993).

However, there are some disadvantages to using artificial substrata (Round 1993). The flora will be somewhat unnatural and biased towards those diatoms which are fast growing and can attach to flat, smooth surfaces - depending on the period of exposure prior to sampling,

the flora may not be a 'climax' community. The smooth surfaces of some artificial substrata often lead to 'sloughing off' of the diatom film. A method and apparatus needs to be devised to hold the substratum in position. Substrata are often lost, removed or vandalised.

Another problem associated with artificial substrata is that they need to be immersed in the river for at least four weeks (although this period is dependent on the trophic status of the water). This causes a delay in the availability of data, as well as adding to the cost of the monitoring program as transport costs to and from the site in question are doubled. Further information about the use and application of artificial substrata can be found in Cattaneo and Amireault (1992) and Lane *et al.* (2003).

Field procedures for water quality monitoring Site selection

The number and location of sampling sites should be designed according to the extent and aims of the survey. Sites should be selected so as to provide representative samples, preferably where marked changes in water quality are likely to occur or where there are important river uses, for example confluences, major discharges or abstractions. If sampling is intended to monitor the effects of discharges, sampling both upstream and downstream of discharge points should be carried out. Sampling should extend for an appropriate distance to assess the effects on the river (CEN 2003).

Personal experience has shown that, in South African inland waters, diatom communities are at the peak of their development in mid-winter to early spring. In addition, when sampling during the winter in summer rainfall regions, one can assume that water levels are receding rather than rising and therefore that submerged substrata can be assumed to have well-developed diatom communities. Care should be taken to avoid sampling after events such as scouring floods, which can displace diatom communities (CEN 2003). Sampling may be impossible at the height of the wet season due to the frequency of such floods.

Sites for stream biomonitoring should be in a 'riffle', where the water is flowing over stones (Round 1993), with a current velocity $>20\text{ cm sec}^{-1}$ (CEN 2003). However, 'runs' and 'glides' are also suitable if these have suitable substrata (DARES 2004). Sampling in riffles or areas of moderate or high water velocity ensures continuous exchange of the water surrounding the algae and prevents the build-up of a local chemical environment. Furthermore, it prevents sedimentation of drifting organisms and particles, with the result that mainly organisms living on that particular spot will be collected. Where the objective is the assessment of water quality, the selection of cobble sites pools and ponded areas should be avoided for sampling purposes (Kelly *et al.* 1998). The above recommendations have, however, been made with wadeable rivers in mind and cannot at all times be applied to deep rivers.

In broad, deep, slow-flowing rivers, such as the Vaal and Orange Rivers which are not wadeable, the sampling procedure of Fore and Grafe (2002) can be followed. Cobbles or other substrata may be collected close to the riverbank from riffles with flowing water or where flow is $>20\text{ cm sec}^{-1}$. The flowing water at the edge of the main stream (littoral zone) can be assumed to be of the same physical and chemical quality as that in the main stream. When sampling in rivers or streams the safety of the operator/s should always be paramount. Cobbles and boulders (but not macrophytes) should be gently agitated in the river for a few seconds before removal (CEN 2003). This should remove any surface contamination, including small particles of organic matter and sediment (DARES 2004).

The following considerations should be taken into account before selecting the reach and specific substrata to be sampled.

- Although there is a reasonably uniform distribution of the diatom flora at any given sampling point, slight differences may occur between substrata from shallow water and those from deeper water (Round 1993). For this reason, sampling from depths greater than 1m should be avoided, especially in turbid rivers where the euphotic zone may not extend to the riverbed. Elber *et al.* (1992) state that the performance of diatom indices is not affected at depths of up to 0.5m, provided that this is still within the euphotic zone.
- Boulders without filamentous algae should be used, because filamentous algae support unique diatom communities (Round 1993). However, if the majority of the substratum is covered with filamentous algae, sampling from uncovered substrata would be non-representative. If $>75\%$ of the substrata are smothered with filamentous algae, these should be sampled in preference to substrata lacking such growths (CEN 2003). For further details of sampling methods to be used when differing proportions of the substrata are smothered with filamentous algae, see DARES (2004).
- Boulders covered with a layer of sediment should be avoided, if possible, as fine sediments may modify substrate conditions (Kelly 2003). However, in lowland rivers it may be difficult to avoid such boulders.
- Although colonisation rates of diatoms are slower in fast-flowing than in slow-flowing rivers, Elber *et al.* (1992) state

that current speeds of 0.1 to 1.6 m.s⁻¹ have no effect on the performance of diatom indices.

- Repeated sampling at the same site requires the marking of sites with bolts, paint on big stones, or other landmarks. To ensure the comparability of samples from other sites, the conditions of light, current velocity, substratum etc. should be as similar as possible (CEN 2003).

Sampling

Section Summary

Site selection

1. Select with the aim of the sampling programme in mind (e.g. the impact of point source effluent on a stream).
2. Note the location, degree of shading, dominant substrata, average stream depth, average stream width, flow rate and other features.
3. Site should preferably be in a riffle or where the river is flowing.
4. Substrata should be removed from the centre of the stream, if wadeable; if not, substrata can be removed from the flowing littoral zone.

Substratum

1. Preferably cobbles or boulders (epilithon).
2. Ideally, cobbles should be free of sediment and filamentous green algae.
3. Alternative substrata for sampling, in order of preference, are:
 - a. *In situ* artificial objects (e.g. bricks, concrete etc.),
 - b. Emergent macrophytes (e.g. *Typha* sp., *Phragmites* sp.),
 - c. Submerged macrophytes,
 - d. Introduced substrata (after allowing a minimum colonisation period of four weeks).

Procedure

1. Choose five or more cobbles (reeds, plants, objects) from a 10 m reach.
2. Scrub their upper surfaces with a toothbrush and rinse into a white tray.
3. Mix well and pour into 150 ml storage bottle.

Storage and preservation

1. Samples to be processed within 24 hours can be stored in a cool dark place.
2. Samples stored for a longer period should be preserved by adding ethanol or Lugol's iodine.

Sampling should be representative rather than random. Operators should first decide which areas in a river reach should be excluded and then search within the remaining areas for substrata with obvious diatom growths, either by appearance or by feel. Diatom growths can be identified by a golden-brown coloured mucilaginous layer on the substratum or — if this is not visible — by the feel of the rocks, which will be slimy or slippery because of the mucilage exuded by the diatoms for locomotion or attachment. Where suitable strata are very abundant, random or stratified sampling may be done in the defined reach (Kelly *et al.* 1998).

Samples should be taken from five or more cobbles (>64, ≤265 mm diameter) or small boulders (>256 mm), where possible. In the absence of boulders, due to the nature of the river at a particular site, it is also acceptable to sample vertical

faces of man-made structures such as quays and bridge supports. Other hard man-made surfaces, such as bricks, can also be sampled (CEN 2003). Alternative substrata, such as submerged or aquatic macrophytes, can also be sampled. In order to compare downstream community composition, it is important to sample from similar substrata along a river, as diatom communities vary according to substratum (Patrick 1977) and samples should be taken in such a way as to obtain the greatest possible degree of uniformity between sites. When sampling from macrophytes, it is important to sample the same species or, if this is not possible, the same morphological type of macrophyte.

Five to ten cobbles, boulders, pebbles or other substrata should be collected from a reach of at least 10 m² (DARES 2004) in the river or stream, briskly rinsed in the stream and carefully placed in a sampling tray on the river bank, together with about 50 ml of stream water. Diatoms can be removed by vigorously scrubbing the upper surface of the substratum with a small brush (e.g. toothbrush) to dislodge the diatom community. Only the upper side (the side most exposed to flowing water) of boulders should be scrubbed, so as to avoid contamination with sediment that might be present on the sides of the boulders. The resulting diatom suspension is then poured into a labelled plastic sample bottle. Care should be taken to avoid instrument contamination between sites by rinsing both the toothbrush and the plastic tray in the river, both before and after taking the diatom sample.

Sampling from macrophyte substrata should be achieved as follows. The emergent macrophyte stem is cut with a knife or similar sharp object above the water line. A plastic bottle is then inverted over the remainder of the stem and the stem is cut slightly above the point where it emerges from the sediment. The bottle is then inverted and brought to the bank. This procedure needs to be repeated until five stems have been collected (CEN 2003). The scrubbing and removal of the diatom communities can then proceed in a similar fashion to that described above for solid substrata. Submerged macrophytes can be sampled by selecting replicates from five different plants growing in the main flow of the river. Each replicate, consisting of a single stem plus associated branches of the plant from the lowest healthy leaves to the tip, should be placed in a plastic bag together with 50 ml of stream water. Diatoms should be present as a brown film associated with the macrophytes. The plants should be shaken vigorously in the plastic bag and the resulting brown suspension poured into a sample bottle (DARES 2004).

The DARES Consortium (Diatoms for Assessing River Ecological Status) has made video footage available, as well as presentations and protocols that deal with sampling diatoms both from cobbles and aquatic macrophytes. This material may be downloaded from the DARES website (<http://craticula.ncl.ac.uk/dares/methods.htm>).

Preservation of diatom material

Fresh diatom samples can be stored either in a refrigerator or — if circumstances dictate a period of storage longer than 24 hours — then the samples may be fixed with ethanol to prevent cell division. An alternative to ethanol is Lugol's

iodine, which may be used for short-term storage. Lugol's iodine is preferred if material is to be examined prior to cleaning. Ethanol should be added to reach a final concentration of 20% by volume and Lugol's iodine to a final concentration of 1% by volume. Formalin is a preservative commonly used for algal samples, but for diatom samples it should be avoided as it is carcinogenic. In addition, very weak formalin solutions might damage the fine structure of diatoms (Kolbe 1948) as formalin breaks down into alcohol and formic acid (Krammer and Lange-Bertalot 2000). Riemann (1960) demonstrated that formalin — even in extremely low concentrations — causes silicic acid to be released from diatom valves.

Laboratory procedures

Section Summary

Preparation

1. Pre-preparation examination for live cells.
2. Cleaning of cells.
 - a. In laboratory equipped with a fume cabinet: KMnO_4 + hot HCl method, hot H_2SO_4 + HNO_3 (2:1) method, hot H_2O_2 method.
 - b. In laboratory without fume cabinet: Cold H_2O_2 .
 - c. Rinsing, centrifuge available: centrifuge with distilled water until sample is circumneutral (4–5 runs for 10min. at 2 500rpm).
 - d. No centrifuge available: decant supernatant using an aspirator. Resuspend sample using distilled water and allow to settle for 8 hours (repeat 4–5 times).
3. Slide preparation
 - a. Concentrated diatom solution diluted with distilled water until only slightly cloudy,
 - b. 1.5–2ml of solution is placed on cover slip, depending on size of cover slip,
 - c. Sample allowed to air-dry,
 - d. Cover slip heated to drive off excess moisture,
 - e. Sample mounted with high-resolution mounting medium.

Archiving

1. Cleaned samples should be stored in ethanol, at a concentration high enough to prevent the growth of bacteria and fungi and to prevent the dissolution of silica.
2. Slides should be stored flat until mounting medium is dry.
3. All relevant information on the location, date of collection, substratum and collector should be stored with both the sample and the slide, and not simply with a reference number.

Pre-preparation examination

On one's return to the laboratory, a quick examination of unpreserved samples should be performed to assess whether they consist predominantly of live cells (dead cells will form part of the bio-film and are not washed away, under normal conditions). If the majority of the diatoms are dead cells (empty frustules with no chloroplasts) the sample should be discarded, as it will not be possible to obtain a true reflection of recent water quality at the particular sampling site from this sample (Bate *et al.* 2002).

Cleaning techniques

The following techniques and methods, taken mostly from Hasle (1978), Welsh (1964), Lohman (1982), McBride (1988) and Krammer and Lange Bertalot (2000), are modified according to our own personal experience.

Most structures in the diatom frustule are so fine that optimum conditions for Light Microscopy (LM) and Scanning Electron Microscopy (SEM) must be achieved. The organic components of the cell must, therefore, be removed. Many methods have been developed to do this (Hasle 1978, Krammer and Lange-Bertalot 2000), each with its own advantages and disadvantages.

Not all laboratories have the facilities to perform a particular preparation procedure. Thus, a description of various techniques, demanding different levels of technical facilities, is provided. Any method of preparation of diatoms for microscopy is acceptable, as long as the slide meets the following criteria (DARES 2004):

- the organic matter in the sample should be completely removed;
- foreign matter should either be absent or insufficient to cause problems during the enumeration or identification of the specimens;
- the distribution of valves on the cover slip should not be significantly clumped, but be evenly dense, without significant edge effects, over the whole area of the cover slip;
- ideally, there should be 5–15 valves, but not less than 1 and not more than 20 valves, per field of view when viewed at 100 x magnification;
- the mountant should be properly cured, with no air bubbles, and should spread right to the edge of the coverslip.

The small size of most diatoms makes contamination from sample to sample, due to carelessness, unclean glassware, etc., an ever-present hazard, and one that must be guarded against in all phases of preparation from the collection of the sample in the field to the final mounting of the sample on a glass slide. Only simple glassware, such as 150ml beakers, watch glasses and centrifuge tubes, are used as these are capable of being easily and thoroughly cleaned after each use. Because public water supplies often contain impurities (sometimes even diatoms) distilled water is used. As it is impossible to clean a pipette, both pipettes and pipette tips should be used only once and then discarded (Lohman 1982). A cheap alternative to a pipette is a plastic drinking straw. Care should be taken to avoid the carrying of diatoms from one beaker to another, through too violent bubbling during acid and hydrogen peroxide cleaning procedures (Welsh 1964).

'Incineration' (the burning away of organic materials) is not recommended as a means of cleaning diatom valves as the diatoms become covered by a thin film of charred protoplasmic material which obscures the exceedingly fine markings of the stria, making species identification difficult (Welsh 1964). Nevertheless, this method is useful when the structure of colonies is characteristic of a certain species, or when particularly delicate diatoms, which could be damaged by caustic preparation techniques, are present.

Acid oxidation is a common method of preparing diatoms. It effectively removes all organic parts of a cell, including the

diatopem covering membrane. It has the disadvantage that the silica structures of the cell wall are more likely to be damaged. The acids dissolve one of the solid phases of the silicic acid of the cell wall so that, when studied at high magnification under SEM, the cell wall appears more or less jagged in structure. In LM studies such damage is of little significance (Krammer and Lange-Bertalot 2000). The use of acids is dependent on the available technical facilities. In the absence of a fume cabinet, all methods employing boiling acids must be avoided. A series of techniques, including both acid and non-acid techniques (such as the hydrogen peroxide technique favored in the United Kingdom), are described below. When material is required for SEM techniques the use of acid oxidation should be avoided, and the more gentle method using hydrogen peroxide should be employed (Round *et al.* 1990), or the material should be left untreated (Taylor 2003).

With the exception of material from calcium-poor water, it is almost always necessary to dissolve traces of calcium in the sample with hydrochloric acid and then to rinse the sample (Krammer and Lange-Bertalot 2000). This is particularly important if further processing with sulphuric acid is needed, otherwise a calcium sulphate diatom precipitate will form, which will make subsequent identification of the valves difficult. Decalcification of samples emanating from South African rivers is particularly important, due to the high silt load in many of these rivers but, if method (a), (described below) — for the removal of organic remains — is followed, decalcification, as a separate step, is unnecessary.

If the sample contains a significant amount of calcium it will foam on the addition of a few drops of concentrated hydrochloric acid. In this case, dilute HCl can be added to the sample. When the sample has stopped foaming it should be rinsed by centrifugation or by a series of decantations until circumneutral.

In all the following methods the original sample should be allowed to settle for 24 hours. The sample is concentrated by pouring off the clear supernatant water, taking care not to lose any diatom material. This step is particularly important if the sample contains a low concentration of diatom material.

After cleaning, the final rinsing of the samples is essential, as any remnant of acid may react with the mounting medium when the slide is prepared (Round *et al.* 1990).

Hot HCl and KMnO_4 method

This method is based on that of Hasle (1978) and is recommended by the authors as it has yielded good results with samples taken from throughout South Africa. Round *et al.* (1990) also recommend this method of preparation.

1. Shake the sample well and pour 5 to 10ml (depending on the concentration of the material) of thick suspension into a heat-resistant beaker.
2. Mark the beaker clearly with the sample number in several places.
3. Add 10ml saturated potassium permanganate (KMnO_4) solution, mix and leave to stand for at least 24 hours.
4. In a fume cabinet, add 10ml concentrated HCl (32%), taking care not to inhale the gasses released. Cover the beaker with a watch glass and heat on a hot plate at 90°C for 1 to 3 hours until the solution becomes clear.

5. After oxidation of organic material, add 1ml of hydrogen peroxide to check if the oxidation process is complete and no organic material remains, in which case the hydrogen peroxide will not cause lasting foaming.
6. When oxidation is complete, allow the samples to cool and transfer to 10ml centrifuge tubes. Before pouring the diatom and acid samples from the beakers, the beakers must be vigorously swirled, the aim of the rotary movement being to re-suspend the diatoms, whilst causing the stone and heavier sand particles to fall to the bottom of the beaker.
7. Rinse the samples by centrifuging with distilled water at 2 500rpm for 10 minutes.
8. After centrifugation decant the supernatant and repeat the washing a further 4 times.
9. The supernatant should be poured off in a single movement, and care should be taken not to lose any diatom material. After pouring off the supernatant fluid the diatoms and small particles of sand at the bottom of the tube are loosened by means of a jet of distilled water from a wash bottle. More water is then added until reaching the required volume in the centrifuge tube.
10. After the last wash, the diatoms are again loosened by means of a jet of distilled water and then poured into small glass storage vials bearing the necessary sample information. It is important to store diatom samples in glass as opposed to plastic vials, as glass releases silica, which counters the dissolution of diatom valves.
11. Alternatively, the excess acid and soluble chlorides can be washed out by a series of timed decantations. The beaker is filled with distilled water to within 1cm of the top and allowed to settle overnight. After each decantation, the remainder is swirled to get it into suspension and the beaker is again filled with distilled water. This is repeated until the suspension is clear and it no longer turns blue litmus paper red (i.e. the sample is circumneutral). The supernatant may be decanted using an aspirator attached to a water suction pump or by siphoning. An aspirator can conveniently be made by heating and bending a glass Pasteur pipette into a u-shape.

Hot $\text{HNO}_3/\text{H}_2\text{SO}_4$ method

1. Mix the diatom suspension carefully and take a subsample (~5 to 10ml) into a beaker. The size of the sample is dependent on the sample density, which can be judged by the visible concentration of suspended material.
2. Mark the beaker clearly in several places with the sample number.
3. Add 5ml of a strong acid mixture ($\text{HNO}_3 + \text{H}_2\text{SO}_4$, 2:1) and place the beakers on a hot plate. The beakers should be covered with a watch glass to prevent contamination between flasks if boiling becomes too vigorous and splashing occurs.
4. Heat the samples at 90°C for 2–3 hours, depending on the amount of organic matter in the sample.
5. Rinse the samples and test for organic material as in points 4–11 in the previous method.

Hydrogen peroxide is much gentler than acid as it is not as corrosive. It is best used with samples that require little

cleaning, and where corrosion should be limited, as in SEM studies (Krammer and Lange-Bertalot 2000). The choice of technique (either hot or cold) depends on the availability of a fume cabinet. If one is available the peroxide can be boiled and, if not, a cold method should be used, but only in a well-ventilated room.

Hot H_2O_2

1. Mix the diatom suspension and place 5 to 10ml of the suspension in a beaker.
2. Mark the beaker clearly in several places with the sample number.
3. Add 20ml H_2O_2 and heat on a hot plate at 90°C for 1 to 3 hours.
4. Add a few drops of HCl and leave to cool.
5. Rinse the samples as in method A (4–11).

Cold H_2O_2

1. Proceed as in method C, above, with the exception of using a hotplate.
2. Cover beaker with watch glass and leave for a minimum of four days.
3. Rinse the samples as in method A (4–11).

Preparation of diatom slides

Most of the ultra-structural details of diatoms lie at the limit of resolution of light. In addition, all mounting media generally used in cytology have a refractive index similar to that of diatom valves, with the result that slides with diatoms mounted in these media are too low in contrast for satisfactory investigation. For this reason diatoms must be enclosed in a medium of higher refractive index than that of the diatom valves (Krammer and Lange-Bertalot 2000). Three types of mounting media are generally used: 'Hyrax' r.i. (refractive index) 1.71 (Hanna 1930); 'Naphrax' r.i. 1.69 (Flemming 1954) and 'Pleurax', r.i. 1.73 (Hanna 1949; refractive indices after Meller 1985). 'Naphrax' is available from Brunel Microscopes Ltd, Chippenham, SN14 6QA, England while 'Pleurax' may be obtained from the corresponding author.

Slides should be free of contamination by other diatomaceous material and should display an assemblage of diatoms that is as close as possible, in terms of composition, to that of the original sample. For this reason, strewn slides are used almost exclusively (Lohman 1982), and can be prepared following the methods of Welsh (1964), described below:

(Note: It is always necessary to keep the sample well mixed or shaken, as the larger diatom cells will tend to settle out of solution quicker than the smaller cells and thus the community counts will be skewed and unreliable).

1. Slides and cover slips should be scrupulously cleaned with detergent soap and stored in ethanol until needed.
2. Using a pipette, a portion is drawn from a well-shaken numbered vial of cleaned material. The cleaned diatom suspension is diluted until it appears only slightly cloudy to the naked eye.
3. A single drop of ammonium chloride (NH_4Cl ; 10% solution) is added for every 10ml of diluted diatom suspension to neutralise electrostatic charges on the

suspended particles and reduce aggregation (McBride 1988).

4. Using a pipette ~1.5ml of this suspension is placed on a clean, dry cover-slip (22 x 32mm).
5. After being placed on the cover slip the diatom suspension should be allowed to dry at room temperature in a dust free environment. It should not be disturbed until dry, because vibration causes clumping of the diatom valves.
6. The drying of cover slips on a hot plate is not recommended because the resultant convection currents form more or less concentric rings of diatoms, with consequent overlying.
7. After the water has evaporated, diatom-coated cover-slips are placed on a hot plate at ~350°C for 2 minutes to drive off the excess moisture and to sublimate the residual ammonium chloride.
8. After the cover slips have cooled, they can be briefly examined under 400 x magnification to determine if the concentration of diatoms in the solution was correct. At least 10, but not more than 40, valves should be visible per field. When the sample is finally viewed at 1 000 x magnification there should ideally be between 5 and 15, but not more than 20, valves visible in each field. If the concentration is too high or low, steps 1–7 need to be followed again, using a more, or less, dilute suspension, before proceeding further.
9. After the diatom-coated cover slips have been allowed to cool, one or two drops of mountant are placed onto each by means of a glass rod or pipette.
10. A previously-cleaned glass slide is then lowered onto the cover slip, inverted, and then heated at 90–120°C on a hot plate until the mounting medium 'boils' and all the solvent evaporates.
11. The solvent of the mounting medium should be evaporated quickly. If this is not done, a ring of exuded medium will harden around the edge of the cover slip, while the mounting medium under the cover slip remains more or less viscous.
12. Under no circumstances should the mounting medium be heated for too long, or at too high a temperature, because it will then turn dark in colour.
13. Depending on temperature and the quality of the mounting medium, it will be necessary to heat the slide on the hot plate for two to five minutes.
14. After the mounting medium has boiled for this length of time, but while it is still viscous, the hot slide is quickly removed from the hot plate, and laid on the work bench.
15. The cover slip is then manoeuvred into position. If this operation is not successful the first time, the slide need only be re-heated for another few moments and the positioning repeated.
16. When the slide is thoroughly cooled, all the mounting medium should be hard and brittle and capable of being easily chipped off with the point of a scalpel.
17. Surplus medium, which has been exuded and has set round the edge of the cover slip, may be carefully removed with the point of a scalpel, after which the slide is then wiped clean with a soft rag soaked in the particular mounting medium's solvent (iso-propyl alcohol

for 'Pleurax' and toluene (which is carcinogenic) for 'Hyrax').

18. The cover glass may then be ringed with shellac cement or Bio-seal® (Bate *et al.* 2002)
19. The slide should be carefully labeled. The following important details should be included on the slide label: date of collection, site location and co-ordinates, habitat, collector and type of mounting medium.
20. The slide is then ready for microscopic examination.

Archiving

It is important to retain a portion of the original sample throughout the preparation stage until the final slide has been made and examined under a microscope. After slide preparation, a portion of the cleaned suspension should be preserved and stored in a labeled vial, with ethanol added to reach a final concentration of more than 20% by volume, to prevent the growth of micro-organisms (Round *et al.* 1990). Alternatively, two or three drops of a 5% aqueous solution of phenol (caution – this chemical is carcinogenic) may be added (Welsh 1964). The archiving of diatom material is necessary in case further slides need to be made or if other workers wish to verify the results of a diatom community analysis after mounting and examining the sample, or if SEM studies on the material are to be undertaken. Finally, a slide should be stored in a herbarium or diatom collection to facilitate cross-referencing.

Enumeration

Different conventions have been evolved for the enumeration of diatoms, using either valves or frustules as the basic unit, or not distinguishing between valves and frustules. The effect that such conventions have on the final results has not been evaluated, but is likely to be small. However, it is important that the convention used be specified in advance. In the case of small diatoms, such as some *Achnanthis* and Naviculoid species, it may not be possible on all occasions to distinguish with certainty between intact frustules and isolated valves (CEN 2003). Prygiel *et al.* (2002) recommended that the required number of individuals be counted, without any distinction between valves and frustules.

The aim of counting diatom units is to produce semi-quantitative data from which ecological conclusions can be drawn. With this in mind it is important to know how many valves to count to get a reliable estimation of the relative species composition at a specific sampling site. The total number of valves to be counted for each sample varies according to the purpose of the analysis and according to the need to produce statistically good results. The statistical precision of percentage counting depends on the frequency of the taxon in the sample count in relation to the size of the sample count (Battarbee 1986). In a South African study Schoeman (1973) made a series of experimental counts in which 200, 300, 400, 500 and 800 valves per sample were counted and their relative abundance calculated. When only 200 valves were counted, compared to when 800 valves were counted, the percentage differences of the relative abundances of individual species were often as high as 6–7%. However, the results obtained from counting 400 as

opposed to 800 valves differed by only 1–2%. For this reason he concluded that counting 400 valves was satisfactory for the calculation of relative abundance of diatom species. Similarly, Battarbee (1986) demonstrated that there were marked differences in the percentages between counts of 100 and 200 valves, while there was little difference between counts of 400 and 500. For this reason he recommended that a count of 300 to 600 may be used for purposes of routine analysis. This range is supported by Prygiel *et al.* (2002) who, in an inter-comparison exercise, found that diatom index scores were not affected at counts of 300 and above. Hence, it is recommended that, for diatom community analysis in South Africa, 400 diatom valves should be counted in each sample.

Suggested rules for counting diatoms, according to CEN (2004), are summarised below:

- Counts of diatom valves on slides should be made using a microscope equipped with incident light, phase contrast optics or differential interference contrast optics (DIC) at a magnification of 1 000 x and higher (100 x oil immersion objective in combination with a 10x eyepiece).
- The eyepiece graticule or other measuring equipment must be calibrated against a stage micrometer prior to the analysis to allow for measurement of dimensions and taxonomic features.
- Either the field of view or the grid of a graticule is used as the area defining the limits of the count. All diatoms visible in the field of view (or within the grid of a graticule) are identified and counted before moving along either a horizontal or vertical traverse to the next field, or selecting a new field of view at random.
- The edge of the dried sample suspension is recommended as the position to begin counting, but if this rule is to be adopted, ensure that there are no significant 'edge effects'. If 'edge effects' consistently prove to be a problem in slide preparation and examination, the methods of McBride (1988) can be adopted, in which cover slips are immersed in a well containing the diatom suspension and allowed to dry over a period of days. Although this method needs an extra three days for drying the cover slips, it will produce random distributions of diatoms with no 'edge effects'.
- A rule is needed to cover situations where a diatom lies only partially inside a defined counting area. For example, such a rule might include taxa that are only partially visible at the upper, but not the lower, margin (in the case of vertical traverses) or the left, but not the right, margin (in the case of horizontal traverses). The precise form of the rule is less important than the consistency of its use when analysing samples.
- Whether a horizontal or vertical traverse is used, it is important that each subsequent traverse does not overlap with the previous one. No diatom valve should ever be counted twice. The distance that the stage is moved on each occasion must also account for any diatoms only partially visible in the field of view.
- If sample analysis is unlikely to be completed in a single session, then it is useful to record the position of each traverse. This ensures that subsequent traverses do not overlap with those already completed.

- Each individual specimen encountered is counted as a single unit, with no differentiation between a valve and a frustule (Prygiel *et al.* 2002).
- Girdle bands (copulae) should not be enumerated as being representative of diatom taxa.
- Occasional filaments should be recorded as the corresponding number of diatom units. If a large number of diatom units are found in filaments, a new preparation technique, using a more aggressive mix of oxidising agents, should be considered.
- In order to eliminate the risk of including separate fragments of broken valves or frustules, a consistent approach must be decided on before starting a project. Valves should be counted only if approximately three quarters are present, or alternatively broken valves may be excluded from the count altogether. Since the scale of physical damage during the sampling and preparation stages is unlikely to be significant, the presence of many small fragments of diatoms may indicate that dead diatoms are being washed in from upstream sites.
- A diatom may not be identifiable for a number of reasons, including the presentation of a girdle view, the presence of overlying material obscuring the view, or the taxon not being recognised by the analyst. If many valves are obscured, then new slides should be prepared using more diluted suspensions.
- Some taxa are identifiable from girdle (side) views, either because the girdle view is particularly characteristic (e.g. *Rhoicosphenia curvata*) or because the girdle view can be assigned with confidence to a particular taxon by 'matching' it with corresponding valve views of taxa found in the sample. However, this is not always possible and, in cases of doubt, the analyst should record the girdle views at the lowest level to which they can be assigned with confidence (e.g. 'unidentified *Gomphonema* sp.', 'unidentified pennate girdle view').
- This convention should also be applied to other individuals found on the slide but not identifiable by the analyst. A large number of such individuals may indicate a problem, either with the slide preparation, or the identification skills of the analyst. As most diatom indices presume that all taxa in a sample are identified, it is recommended that not more than five per cent of the total count should comprise unidentifiable individuals. If a diatom unit cannot be identified for any reason, photographs, digital images or detailed drawings should be made. Notes should also be taken of the shape and dimensions of the diatom unit, striae density and arrangement (at the centre and poles), shape and size of the central area, number and position of punctae and arrangement of raphe endings.

Identification

The most valuable recent flora or identification guide for Europe is that of Krammer and Lange-Bertalot (1986–1991). This flora can be used for the identification of many of the species occurring in South Africa and for the confirmation of species identifications by other authors. Other taxonomic guides that may be consulted include Schoeman (1973), Schoeman and Archibald (1976–80), Archibald (1983), Gasse

(1986), Round *et al.* (1990), Hartley (1996), Prygiel and Coste (2000), Lange-Bertalot (2001) and Krammer (2002).

Diatom taxonomy has recently undergone many changes and is currently in a state of flux. This is due mainly to the splitting of large genera such as *Navicula* and *Nitzschia*. There is now consensus amongst diatom taxonomists that the diatom genus *Navicula* is restricted to the section *Lineolatae* (Lange-Bertalot 2001). This has led to the creation of new genera by encapsulating species that used to belong to the genus *Navicula*. Examples of these new genera are *Luticola* (Mann, in Round *et al.* 1990), *Fallacia* (Sickle and Mann, in Round *et al.* 1990) and *Microcostatus* (Johansen and Sray 1998). For revised nomenclature, works such as Lange-Bertalot (2001), Krammer (2002) and Kellogg and Kellogg (2002) can also be consulted.

Possible sources of error in diatom community analysis

When implementing monitoring programs based on assessments of diatom community composition, we in South Africa have the advantage of looking to European and other studies to identify sources of error in advance. Several sources of error, at all stages of the analysis, have been highlighted by Prygiel *et al.* (2002) in an inter-laboratory comparison exercise. According to these authors sampling appears to be a very important step. When the sampling protocol is not strictly adhered to, that part of the variability due to sampling can be very high. Errors include sampling from exposed substrata — from areas subjected to water level change and from areas of low-velocity flow as compared to other parts of the river — and sampling from stones covered by abundant filamentous algae. Laboratory and counting errors may include the use of high temperatures when drying slides (leading to clumping of diatom valves) and the settling out of large taxa during the preparation of consecutive slides from a single sample. The main source of variability is, however, in the identification of individuals, as already highlighted by many other biologists (Prygiel *et al.* 2002). That is why biological quality controls focus mainly on counts and misidentification (Kelly 1999). Diatoms are suitable for such controls and proposals relating to quality control have been made by Kelly (1999).

Prygiel *et al.* (2002) made recommendations which should be seen as the way forward for South Africa in terms of quality control and the validation of diatom analysis data. Most of the variability due to sampling and slide preparation can be avoided by organising comparisons between different studies. Such comparisons are very useful because, with field and laboratory approaches, they make operators aware of the consequences of not following protocols. They are also useful because they highlight some taxonomic problems. Diatoms are good subjects for photomicrography and therefore most diatomists use the internet to check problematic identifications. This approach should be encouraged by formalising expert-practitioner exchanges, by creating iconographic databases, and by organising regular workshops to allow updating of knowledge. The archiving of permanent slides also facilitates the creation of reference collections, which are particularly useful for the identification of difficult species.

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CHAPTER 3

The relevance of diatoms for water quality assessment in South Africa: a position paper

Water SA

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The International System of Units (SI) applies. Technical and familiar abbreviations may be used, but must be defined if any doubt exists.

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Tables are numbered in Arabic numerals (Table 1) and should bear a short but adequate descriptive caption. Their appropriate position in the text should be indicated.

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Two examples of the presentation of references are the following:

GRABOW WOK, COUBROUGH P, NUPEN EM and BATEMAN BW (1984) Evaluations of coliphages as indicators of the virological quality of sewage-polluted water. *Water SA* **10** (1) 7-14.

WENZEL RG (1975) *Limnology*. WB Saunders Company, Philadelphia. 324 pp.

The relevance of diatoms for water quality assessment in South Africa: A position paper[#]

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Abstract

Water quality assessment protocols based on the use of diatoms are now well developed and their value substantiated at an international level. The use of diatoms is not designed or intended to be a "rapid" technology. The detailed level of information generated from the procedure outweighs perceived disadvantages of the additional time required for sample preparation and analysis to species level. The method is applicable across a wide range of aquatic ecosystem types, namely freshwater, brackish, and estuarine, and is inclusive of both lentic and lotic environments, wetlands and their associated damp, marginal and littoral zones. Details provided by diatom assemblages support palaeoecological investigations, historical reconstruction of water quality and the determination of prevailing water quality conditions. Deliberate determination of responses to management strategies or impacts arising from a variety of anthropogenic activities can be achieved via the simple expedient of retrieving living material from introduced artificial substrates. Previous studies in South Africa and elsewhere have shown that on a site-by-site basis the use of diatoms provides a fine level of diagnostic resolution of the causes underlying changes in water quality and environmental condition.

The South African Diatom Collection ("the Collection"), a repository of diatom specimens and records that spans the length and breadth of this country, contains an as-yet unutilised wealth of ecological and taxonomic information. More importantly, the historical data analysis records provide an insight into water quality conditions prevailing 40 to 50 years ago – in many cases prior to the "development" of many of our rivers, streams and wetlands. The real value of its existence underpins the great potential for renewed attention to the value of diatom-based approaches to water quality assessments. In addition, the Collection provides a ready-made foundation on which a locally relevant tool for water quality assessment may be established to augment the current use of invertebrate indicators.

It is now appropriate that the full potential of the use of diatoms in water quality assessments, and the information contained in the Collection, be developed and utilised for water quality assessment in South Africa.

Keywords: diatoms, water quality, Chohnoky, Archibald, biotic indices

Introduction

Assessing water quality using biotic indices

Few people involved with ecohydrology and water resource management doubt the value of water quality assessments derived from the use of biotic indices, i.e. assessments based on observations of the resident floral and faunal communities (Chutter, 1972; Patrick, 1973; Schoeman, 1976; Descy, 1979; Kelly et al., 1995; Kelly, 1998a; Bate et al., 2002). Assuming requisite levels of ecological experience and taxonomic proficiency on the part of the assessor, such evaluations provide a description of the water quality that is often not achievable from elemental analyses alone. The value of an integrative biological response provided by the analysis of diatom associations offsets the inconsistency of rapid changes in water chemistries that render the use of conventional analytical approaches inadequate. A further potential advantage is that the diatom-based approach could obviate the need for additional and

expensive toxicity testing protocols – particularly because of the attendant uncertainty of extrapolation to the real environment of the responses of selected single species testing gauged under laboratory conditions. Ecological risk assessments are more appropriately based on biological endpoints in the field than on measures of chemical constituents (Karr and Chu, 1997). Monitoring procedures based on the biota measure the health of a river and the ability of aquatic systems to support life, as opposed to simply characterising the chemical and physical components of a particular system. This is the central purpose of assessing the biological condition of aquatic communities of a river (Barbour, 1997).

Karr and Chu (1997) have stressed that chemical criteria based on laboratory-derived dose-response curves for single toxicants cannot account for cumulative, synergistic or antagonistic interactions of the suite of chemicals found in a polluted river system. Comprehensive and accurate multimetric indices explicitly embrace several attributes of the sampled assemblage including taxon richness, indicator taxa and the health of individuals. In many cases, biotic indices provide an indication of the existence or absence of life in stream water where routine chemical measurements of "indicator elements", even at the limits of analytical detection are not definitive.

Cairns (1981) highlighted the need for standard methods for the bio-monitoring profession because he recognised that the condition of individual species and of communities of indigenous biota was one of the best measures of the health of an ecosystem. In an effort to

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provide a reliable assessment tool within as short as possible a time frame, the search for rapid assessment technologies in South Africa centred largely on the use of aquatic insects, and protocols such as the South African Scoring System (SASS), now in its 5th iteration, were derived (Dickens and Graham, 2002). Subsequently the value and validity of rapid assessment biological methods have been challenged by Taylor (1997) and he contends that further refinements of these rapid techniques will be less useful than improving established methods. With proficient and professional use SASS provides a great deal of insight into the condition of invertebrates within a particular riverine environment. Nevertheless there is also an inappropriate tendency, perhaps borne of the apparent absence of any alternative, to extend the use of SASS into other environments, such as wetlands. The diatom approach provides a viable and already-developed alternative protocol for the health assessments of wetlands.

The need to augment invertebrate-based protocols

The presently preferred use and value of the aquatic invertebrate method is variably limited by hydrology, substrate(s), habitat, food availability, seasonality and distribution patchiness. The latter aspect places an obvious demand on the number of samples that need to be taken in order to produce a quantitative result within regional considerations and a host of anthropogenic factors – not least the major modifications in assemblages occurring downstream of dams and weirs (Dallas, 1997). While invertebrate indices do not provide a reliable indication of eutrophication, diffuse- and point-source impacts have previously been positively identified by direct measurements of the diatom associations found in South African river systems (Cholnoky, 1960; 1968; Archibald, 1972; Schoeman, 1976). Charles (1996) stressed that the use of algae for monitoring rivers has increased because of limitations of benthic invertebrates and fish as indicators. Coupled with significant improvements in technologies for algal assessment that increase the information/cost ratio, there is a realisation and acceptance by water authorities of the value of biological monitoring, particularly where multiple groups of organisms (i.e. from different trophic levels) are included in the evaluation.

Justification for and recognition of the value of such a scientific management approach is demonstrable in the existence of the South African Diatom Collection. The meticulous manner in which our former dedicated colleagues - Cholnoky, Giffen, Archibald and Schoeman - recorded sample localities, their results, and preserved the collected material, has provided an invaluable environmental legacy for South African aquatic systems. It has become a matter of concern therefore that since the late 1980s the real value of this material has gone unrecognised amongst water management authorities and aquatic scientists, if only because the information and its existence have not been sufficiently advertised.

While assessments such as SASS should undeniably remain in the toolbox of water quality assessment professionals, there exists a pressing demand for additional protocols that will confirm and/or augment the value of invertebrate-based evaluations. Ideal attributes for such additional tools would be:

- Wide-ranging applicability across aquatic ecosystem types and their adjacent (damp) environments
- Indicators should be retrievable under all hydrological conditions, including stagnant and dry and – most important – should lend themselves to forensic interrogation – i.e. palaeoecological assessment
- The results obtained therefrom should be incontrovertibly linked to water quality.

One group of organisms fulfils these core requirements, and more – the algal Class Bacillariophyceae. Diatoms occur in all types of aquatic ecosystems, and extend into the more saline estuarine environments as part of the river continuum. They comprise the major component of the microphytobenthos (bottom-dwellers), performing essential photosynthetically driven, microbial functions at the base of the food chain (Cox, 1996). Accordingly they respond directly to growth stimulants (nutrients) and/or stressors such as toxicants, as well as to physical factors.

The use of diatoms as an “added-value” assessment tool

Importantly, the ability to use diatoms to evaluate present and past conditions of water quality and environmental change in just about any aquatic environment has been recognised world-wide for many decades (Patrick, 1973; Van Dam, 1974; Chessman, 1986; Whitmore, 1989), but has been limited in South Africa – until now – by perceptions of seemingly onerous sample preparation and time-consuming dedication required to develop key taxonomic skills. State and regional river-monitoring programs for algae in the United States tend to rely solely on analysis of diatom assemblages (Charles, 1996).

The authors' personal experience from using the most recent and modern computer technology, supported by image analysis software, and informed by recently published literature, is that this has resulted in a marked reduction in time required and greater confidence in the results. Difficulties with the taxonomy and nomenclature of the diatoms can now be resolved through comparison of images via rapid e-mail communication with other experts – as is the common norm in many disciplines of scientific investigation. Kociolek and Stoermer (2001) hold a strong view that studies on accurate taxonomy and ecology of diatoms in the 21st Century will and must be linked. They also emphasise that this approach will be driven by integrated research programs (e.g. river health studies) and can now be facilitated by technological advances (e.g. computer toolkits and image analysis) that support both accurate taxonomy and improved ecological interpretation. They envision a research paradigm that closely integrates diatom taxonomy and ecology to develop conservation biology in which microbial communities (e.g. diatoms) can be used to define ‘natural’ habitats requiring conservation.

Diatoms have for some time, and latterly increasingly so, been used for the assessment of short- and long-term environmental change (Dixit et al., 1992). Assessment approaches based on diatom indices were developed in the lacustrine environment, and have since been extended to encompass the riverine systems (Round, 1991a; b; Stevenson and Pan, 1999 and Eloranta and Soininen, 2002). Diatom-based information can be gleaned not only from natural surfaces (sediments, stones-in-current and marginal vegetation) but also from just about any other substrate or surface type in an aquatic environment. The living component can also be deliberately gathered in a controlled fashion using the simple expedient of artificial substrates – a significant advantage in the formulation of stressor-response models (Gold et al., 2002). High frequency, multi-parameter water quality monitoring programs are simply not cost effective in the present South African situation – and an alternative for assessing change over time is urgently required. The examination of living diatoms in sediments and on stones, together with the invertebrates, provides a method that combines two independent indicator systems at different trophic levels (Smol, 1992; Hofmann, 1996).

Diatoms provide the following essential suite of diagnostic attributes (e.g. De la Rey et al., 2004):

- They collectively show a broad range of tolerance along a gradient of aquatic productivity, with individual species having specific water chemistry requirements
- They have one of the shortest generation times of all biological indicators (~2 weeks). They reproduce and respond rapidly to environmental change and provide early measures of both pollution impacts and habitat restoration
- They are sensitive to change in nutrient concentrations, supply rates and silica/phosphate ratios. Each taxon has a specific optimum and tolerance for nutrients such as phosphate and nitrogen, and this is usually quantifiable. Moreover, whereas the use of historical water chemistry data are constrained by the level of analytical sophistication prevailing at the time of sampling, the associations of diatoms with water quality remain unchanged
- Their assemblages are typically species-rich – augmenting the information gained from a diversity of ecological tolerances. Moreover, the large number of taxa provides redundancies of information and important internal checks in datasets, increasing the confidence of environmental inferences
- They respond rapidly to eutrophication. Because diatoms are primarily photoautotrophic organisms, their growth response is directly affected by changes in prevailing nutrient concentrations and light availability
- Their rapid immigration rates and the lack of physical dispersal barriers ensure that there is little lag-time between perturbation and response
- Diatom frustules have a lasting permanence in sediments, such that sediment cores provide details of changes in the quality of the overlying water for as far back as one is able to search. This attribute alone has significant and far-reaching relevance for the determination of reference conditions, not only climatic but also the condition of the system prior to intrusion from anthropogenic development
- The taxonomy of diatoms is comprehensively documented. Species identifications are largely based on frustule morphology – an attribute readily identifiable with modern light microscopy and image analysis techniques, and are not dependent on electron microscopic techniques as is commonly misconceived
- Diatoms can be found on substrata in streambeds even when dry can be sampled at most times of the year and still accurately reflect recent or prevailing conditions

Additionally the use of diatoms is supported by the attributes identified by Schoeman and Haworth (1986):

- Their ease of collection, preparation for observation, and storage (small sample volumes, no desiccation risk) for reference purposes
- The considerable amount of tried and tested ecologically-associative information already available, both nationally and world-wide
- Their suitability for diversity analysis
- The availability of interpretive software package.

Concerns that the wealth of diatom-based information developed in the north-temperate zone might not be directly applicable to the southern latitudes have been comprehensively quelled by more recent findings that diatoms are 'subcosmopolitan', i.e. they occur anywhere where particular environmental conditions are fulfilled (Kelly, 1998b). This concept suggests that geographical location is not the determining factor in the distribution of diatom species and the composition of communities but rather the specific environmen-

tal variables prevailing at a particular site (Gold et al., 2002). Comparison of South African examples of paired sets of diatom and water quality data confirms that several of the diatom indices are directly applicable locally in certain rivers (De la Rey et al., 2004). In fact Cholnoky's South African work at the CSIR 30 years ago supported the identification of water quality and environmental change parameters at relatively fine spatial scales. An analysis of the Upper Hennops River in 1979 revealed that use of the Lange-Bertalot method – or saprobial system in which diatom taxa are placed in 9 different classes according to their specific pollution tolerances was adaptable to South African conditions (Schoeman, 1979).

Importantly, the use of diatom-based approaches is now supported by a better understanding of the relationship between diatoms and environmental variables (Prygiel and Coste, 1993; Gomez, 1999; Juttner et al., 2003). Predictive models exist that demonstrate their direct applicability for use in some South African rivers (De la Rey et al., 2004). Numerous case studies that provide clear guidance on the validity and strength of the approach abound worldwide (Prygiel et al., 1999; Stevenson and Pan, 1999; John, 2000; Wu and Kow, 2002). Moreover our work indicates that a significant amount of historical water quality information (providing a 'reference condition') is contained in the SA Diatom Collection (see below).

The aforementioned attributes have led to diatoms becoming firmly established, although not yet in South Africa, as important indicators of the present and past nature and condition of the aquatic environments in which they may be found. Diatom communities typically range from opportunistic tolerant species in areas of severe pollution, giving way to less tolerant and more competitively dominant species at the most distant location from the pollution source. Given that the types of diatoms dominant in nutrient-poor waters (oligotrophic) are distinct from those in enriched (eutrophic) environments and/or in potentially toxic conditions, the pattern of cultural eutrophication can be readily discerned (Cholnoky, 1960a; Canter-Lund and Lund 1995). To quote Round, an eminent UK diatomist, "*the value of using diatoms lies in the fact that the flora reflects rather precisely the water quality at any one point and, by monitoring changes in the flora, subtle changes in water quality conditions will be detected.*"

The South African diatom collection

While many water resource managers and aquatic scientists may not realize it, South Africa possesses one of the most comprehensive collections of diatoms in the world. This substantial collection is currently housed at the offices of the CSIR (KwaZulu-Natal, Durban), and was considered to be the largest in the Southern Hemisphere. Information on this collection may be found at www.dhec.co.za/diatoms. During the early- to mid-20th centuries, two types of botanical collectors criss-crossed this country – viz. botanists and diatomists. Were it not for the untiring efforts of these early 'explorers' much of what our natural history of aquatic systems was like would still be unknown. Notable amongst these early efforts is the work of diatom specialists such as Cholnoky, Giffen, Archibald and Schoeman. Between them they conscientiously collected samples from a wide range of South and Southern African aquatic environments – many of which have been subjected to infrastructural development such as dams, weirs, water-transfer and abstraction schemes. Diatom material was also obtained from many parts of the world. The availability of computerised diatom assessment procedures now enables re-working of these data and comparison with current conditions. This information will be

invaluable in judging both the impact of man-made alterations to aquatic systems and the efficacy of remedial management strategies.

Some selected examples of the Southern African coverages are:

Gauteng

- *Diatom Indicator Groups in the Assessment of Water Quality in the Jukskei-Crocodile River System* (Schoeman, 1976)
- *Diatoms from the Vaal Dam Catchment Area, Transvaal* (Archibald, 1971).

Eastern Cape

- *Diatoms of the Swartkops Estuary* (Eastern Cape) (Cholnoky, 1960a)
- *Diatoms of the Estuaries of the Eastern Cape Province* (Giffen, 1963)
- *The Diatoms of the Sundays and Great Fish Rivers in the Eastern Cape Province of South Africa* (Archibald, 1983).

Western Cape

- *New and Rare Diatoms of the Cape Province* (Cholnoky, 1959)
- *An Account of the Littoral Diatoms from Langebaan, Saldanha Bay* (Giffen, 1975)
- *Marine Littoral Diatoms from the Gordon's Bay Region of False Bay* (Giffen, 1971)

KwaZulu-Natal

- *Contributions to Our Knowledge of the Diatom Flora of Natal (Tugela, Mooi, Mkomas, Umgeni, Pongola, and Umfolozi Rivers)* (Cholnoky, 1960b)
- *New and Rare Diatoms from Africa – Diatoms from the Tugela System (I, II and III)* (Cholnoky, 1956; 1957)
- *Diatom Associations of St Lucia Lake* (Cholnoky, 1968b)

Other Southern African areas

- *Diatoms of the Okavango* (Cholnoky, 1966a)
- *Diatoms of Bechuanaland* (Cholnoky, 1966b)
- *Diatoms from Sewage Works in the Republic of South Africa and South West Africa* (Schoeman, 1972a)
- *Diatoms of Swaziland* (Cholnoky, 1962).

Evaluation of the SA diatom collection

The findings of the aforementioned studies were documented and stored in slide collections, but have remained in almost permanent disuse since the early 1990s. More recently, new research efforts have contributed to the revival of interest in diatom associations as indicators of water quality in South African rivers (Watt, 1998 and Bate et al., 2002). The main aim of the WRC-funded work of the latter has been to survey benthic diatom flora and to relate the dominant taxa to the chemical water quality of selected river systems of the Eastern Cape, Western Cape and Mpumalanga. This study concluded that “*benthic diatoms have the potential to be used as biological indicators as they are ubiquitous members of riverine systems, react rapidly and predictably to water quality and their taxonomy has been well described*”. Unfortunately, the historical value of the resources contained in the South African Diatom collection were not fully realised, perhaps leading to the comment that “*so far the use of benthic diatoms as indicators of river water quality in South Africa has been limited*”. They did conclude, however that “*diatoms appear to be very suitable biomonitoring organisms. They give an accurate indication of water chemistry within water quality classes*”.

Diatom assemblages: Indices and interpretive tools

Within the last decade diatom-based indices have gained considerable popularity throughout the world as a tool to provide an integrated reflection of water quality, and in support of management decisions for rivers and streams (Kelly, 1998b; 2002; Prygiel and Coste, 1993). Work on the applied use of diatoms as bio-indicators has proceeded such that diatom indices have replaced those of invertebrates as the biomonitoring method of choice in certain situations e.g. canalised waterways (Prygiel and Coste, 1993)

The bulk of the developmental diatom work has been carried out in French drainage basins – with testing on the scale of a territory as large, and as typologically diversified, as France; enabling the general application of these indices on the European continent (Prygiel and Coste, 1993). In other examples diatoms have been integrated into a suite of testing methods required to support nutrient reduction directives (Kelly, 2002). The design of software programs such as OMNIDIA for the calculation of diatom indices has greatly enhanced the use of diatom-based methods (Le Cointe et al., 1993). A variety of diatom indices have been adopted and tested by many European countries including Finland (Eloranta, 1999) and Poland (Kwandrans et al., 1998).

The majority of the diatom indices are based on the weighted average equation of Zelinka and Marvan (1961) and have the basic form:

$$\text{index} = \frac{\sum_{j=1}^n a_j s_j v_j}{\sum_{j=1}^n a_j v_j}$$

where:

a_j = abundance (proportion) of species j in sample

v_j = indicator value

s_j = pollution sensitivity of species j

The performance of the indices depends on the values given to the constants s and v for each taxon and the values of the index ranges from 1 to an upper limit equal to the highest value of s . Diatom indices differ in the number of species used and in the values of s and v which have been attributed after compiling the data from literature and from ordinations (Prygiel and Coste, 1993).

Concluding remarks

The use of diatoms as a diagnostic tool, and the value of the historical information contained in the SA Diatom Collection, can no longer be ignored. International precedents and local experience clearly indicate the versatility and durability of this protocol as a scientifically robust tool. It should be used in conjunction with the SASS invertebrate method as two independent indicator systems comprising a more comprehensive ecosystem health screening protocol in South Africa. If the ability to inform strategic water resource assessments is to be appropriately developed at a higher level of confidence, then any delays in attention to the diatom-based assessment methods would be both administratively and functionally negligent. Not least, a failure to do so would flout the considerable amount of effort that has been historically invested in the development of the resource base that is the SA Diatom Collection.

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CHAPTER 4

Determining the possible application value of diatoms as indicators of general water quality in the Mooi River (North West Province): A comparison with SASS 5

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GRABOW WOK, COUBROUGH P, NUPEN EM and BATEMAN BW (1984) Evaluations of coliphages as indicators of the virological quality of sewage-polluted water. *Water SA* **10** (1) 7-14.

WENZEL RG (1975) *Limnology*. WB Saunders Company, Philadelphia. 324 pp.

Determining the possible application value of diatoms as indicators of general water quality: A comparison with SASS 5

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Abstract

The applicability of a European numerical diatom index, the Specific Pollution sensitivity Index (SPI), was tested in a river system where the SPI scores were compared both to chemical water quality and to scores yielded using a macro-invertebrate index of riverine health namely the South African Scoring System (SASS 5). This investigation showed that the SPI reflects certain elements of water quality with a high degree of accuracy. Due to the broad species base of SPI, few problems were encountered when using this system in the Southern Hemisphere. The conclusion is that SPI or a similar diatom index will provide a valuable addition to the suite of biomonitoring tools currently in use in South Africa.

Keywords: biomonitoring, diatoms, SASS 5, SPI diatom index, general water quality

Introduction

We live on a subcontinent recognised for its unpredictable rainfall. South Africa is a semi-arid country, and the decline in the quality of available water is one of the major problems currently facing the country (Davies and Day, 1998). There are several factors that contribute to the decline in water quality, the most important being industry, intensive and careless agricultural practices and the population explosion, which increases the demand for domestic water supply. The National Water Act 36 of 1998, repealed and replaced over 100 previous acts. The preliminary section of the Act, states, "...water is (to be) protected, conserved, managed and controlled in a sustainable and equitable manner for the benefit of all persons ...".

Under Act 36 of 1998, activities that pollute or degrade water resources require a licence issued by the Department of Water Affairs and Forestry (DWAF). The Act stipulates that "...an applicant may be required to provide an assessment of the likely effect of the proposed activity on the resource quality...". Licences will not be issued for periods longer than 40 years. Provision is made for the periodic review of the licence at intervals that must not exceed 5 years. The important component of this periodic review is that quality monitoring forms an essential part of the conditions of many such water licences.

Biological monitoring techniques have been introduced as part of routine monitoring programmes due to certain shortcomings in standard physical and chemical methods. Because of the difficulty and cost of chemically analysing every potential pollutant in a sample of water, and of interpreting results in terms of impact severity, it makes sense to monitor aquatic biota. Results from biological monitoring are cost effective and the results can be obtained rapidly. The main advantage of a biological approach is that it examines organisms whose exposure to pollutants is continuous. Thus species present in riverine ecosystems reflect both the present and past history of the water quality in the river, allowing

detection of disturbances that might otherwise be missed (Eekhout et al., 1996).

Biological communities reflect the overall ecological integrity by integrating various stressors, thus providing a broad measure of their synergistic impacts. Aquatic communities, both plant and animal, integrate and reflect the effects of chemical and physical disturbances that occur over extended periods of time. These communities can provide a holistic and an integrated measure of the integrity or health of the river as a whole (Chutter, 1998).

Numerous methods have been developed for the bioassessment of the integrity of aquatic systems. Some of these are based on one or other aspect of a single species, but most are based on the attributes of whole assemblages of organisms such as fish, algae or invertebrates. Although methods have been available for many years, biomonitoring has only as recently as 1996 become a routine tool in the management of South Africa's inland waters (Hohls, 1996).

Benthic macro-invertebrates are recognised as valuable organisms for bioassessments, due largely to their visibility to the naked eye, ease of identification, rapid life cycle often based on seasons and their largely sedentary habits (Dickens and Graham, 2002). Currently, the backbone of the National River Health Programme is SASS (South African Scoring System), a macro-invertebrate index developed by Chutter (1998). The SASS system has undergone several refinements to suit all conditions; the most recent of these modifications is SASS 5 (Dickens and Graham, 2002). However, Round (1991) lists several reasons why animal components of an ecosystem may not provide a satisfactory index system:

- Animals have complex reproductive cycles which are often linked to the seasons,
- Animals are largely motile and this may cause difficulty during sampling,
- Animals may have many different life stages and may undergo metamorphosis,
- Animals have specific habitats and niches;
- They are actively grazed; and closely linked to flow conditions and thus will not usually be evenly distributed from headwaters to estuaries, and

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- Watercourses, which are too deep to wade across, may prove difficult if not impossible to evaluate using a macro-invertebrate index along the length of the stream.

Finally, the composition of the aquatic invertebrate community is always modified immediately downstream of dams and weirs. This is also often true downstream of bridges (Chutter, 1998) and therefore decreases the potential uses of SASS.

No single group of organisms is always best suited for detecting the diversity of environmental perturbations associated with human activities. If the maintenance of ecosystem integrity is the aim of river management, there is a need to monitor the status of different taxonomic groups. Diatoms provide interpretable indications of specific changes in water quality (Kwandrans et al., 1998), whereas invertebrate and fish assemblages may better reflect the impact of changes in the physical habitat in addition to certain chemical changes (McCormick and Cairns, 1994).

The diatoms (Bacillariophyceae) comprise a ubiquitous, highly successful and distinctive group of unicellular algae, with the most obvious distinguishing characteristic the possession of siliceous cell walls (frustules). As autotrophs, diatoms contribute significantly to the productivity of such ecosystems, frequently forming the base of aquatic food chains (Cox, 1996).

Diatoms are abundant, diverse and important components of algal assemblages in freshwater bodies. They comprise a large portion of total algal biomass over a broad spectrum of trophic states (Kreis et al., 1985). Diatoms are used as biological indicators for a number of reasons:

- They occur in all types of aquatic ecosystems.
- They collectively show a broad range of tolerance along a gradient of aquatic productivity, individual species have specific water chemistry requirements (Werner, 1977; Round, 1991).
- They have one of the shortest generation times of all biological indicators (Rott, 1991). They reproduce and respond rapidly to environmental change and provide early warnings of both pollution increases and habitat restoration success. It takes two to three weeks before changes are reflected to a measurable extent in the assemblage composition (Round, 1991; Kelly et al., 1998).
- They are sensitive to change in nutrient concentrations, (Pan et al., 1996). Each taxon has a specific optimum and tolerance for nutrients such as phosphate (Hall and Smol, 1992; Reavie et al., 1995; Fritz et al., 1993; Bennion, 1994; Bennion et al., 1996) and nitrogen (Christie and Smol, 1993), which can usually be quantified to a high degree of certainty.
- Assemblages are usually diverse and therefore contain considerable ecological information. For this reason, and because it is easy to obtain large numbers of individuals, robust statistical and multivariate procedures can be used to analyze assemblage data. (Dixit et al., 1992).
- They respond rapidly to eutrophication and recovery (e.g. Zeeb et al., 1994). Because diatoms are primarily photoautotrophic organisms, they are directly affected by changes in nutrient and light availability (Tilman et al., 1982).
- Rapid immigration rates and the lack of physical dispersal barriers ensure there is little lag-time between perturbation and response (Vinebrooke, 1996).
- The taxonomy of diatoms is generally well-documented (Krammer and Lange-Bertalot, 1986-91). Species identifications are largely based on frustule morphology.
- Diatoms can be found on substrata in streambeds even when

dry, so they can be sampled at most times of the year (Stevenson and Pan, 1999).

Round (1993) lists numerous reasons why diatoms are useful tools of biomonitoring, amongst which the following bear especial relevance to the South African situation; methods are cost-effective, data are comparable, techniques are rapid and accurate, and non-specialists with a biological background can do identifications and counts if they are provided with illustrated guides.

Concern has been expressed about the transfer and comparison of data between the Northern and Southern Hemisphere (Round, 1991). It is well known that some species have the same morphology, but questions still remain concerning the range of ecological tolerances of these various species. This is a valid concern when distance, climatic condition, and other environmental pressures are taken into account. However, Kelly et al., (1998) discuss the concept, that diatoms are 'subcosmopolitan', i.e. they occur anywhere when certain environmental conditions are fulfilled. This concept suggests that geographical location is not the determining factor in the distribution of diatom species and the composition of communities, but it is rather the specific environmental variables at a specific site that determine this distribution.

Diatom indices may be able to provide answers to the problems involved in monitoring rivers for the inorganic nutrients that cause eutrophication, organic loading, ionic composition and dissolved oxygen (Kwandrans et al., 1998).

The aim of the study was to ascertain whether the numerical diatom index developed in Europe has a potential use for indicating general water quality in the North West Province. Bate et al. (2002), in a study on South African rivers, came to the conclusion that benthic diatoms could be a useful addition to the national biomonitoring programme as they give a time-integrated indication of specific water quality components. However, Bate et al. (2002) went on to state that the particular data set tested in their study that of Van Dam et al. (1994), could not be transposed directly to South African conditions. For this reason the current study investigates the potential use of another autecological diatom index developed in Europe (France).

A further aim of the study was to establish whether diatom species are indeed sub-cosmopolitan as stated by Kelly et al., (1998), by determining the number of species actually used in the calculation of the chosen index.

SASS 5 was chosen for comparison as it is widely used in biomonitoring river systems in South Africa and is currently considered as the industry standard for biomonitoring.

Materials and methods

Sampling sites

Twelve sampling sites in the Mooi River in the North West province of South Africa were chosen for this study. The study was conducted during May 2003. Study sites were chosen to represent a range of water quality and the impact of some of the tributaries entering the Mooi River. The study sites (Fig. 1) extended from below Klerkskraal Dam (M1; 26°30,86' S, 27°07,40' E), downstream to the Prozesky Bird Sanctuary in Potchefstroom (M5; 26°34,13' S, 27°06,03'E). The four tributaries that formed part of the study were the Wonderfontein Spruit (WFS), an unnamed tributary near Boskop Dam (T3), Wasgoed Spruit (WS) in Potchefstroom as well as Loop Spruit (LS) entering the Mooi River at the Prozesky Bird sanctuary.

Land use in the upper reaches of the Mooi River catchment is



Figure 1
The Mooi River system (North West Province, South Africa) showing the location of the sampling sites used in the study

mainly agricultural with activities such as peat and informal diamond mining occurring further downstream. Gold mining and sewage effluent enters the Mooi River through the Wonderfontein Spruit. The unnamed tributary (T3) introduces water from a canal into the Mooi River just above Boskop Dam, from an unknown source. Effluents from heavy industry (e.g. a fertilizer manufacturer) as well as storm water drain into the Mooi River from Potchefstroom via the Wasgoed Spruit. Loop Spruit is mainly influenced by agricultural activities.

The study also included samples above and below two major dams in the system namely the Boskop Dam and Potchefstroom Dam.

SASS 5 and ASPT scores

Macro-invertebrates were collected as prescribed by the SASS 5 protocol and the SASS 5 and ASPT (average score per taxon, calculated as the total SASS score divided by the number of taxa contributing to the SASS score) indices calculated according to standard methods (Dickens and Graham (2002); Chutter (1998)).

Diatoms

Sample collection

Three to five different boulders at any particular site (Round, 1993) were sampled from different positions within a defined 10m reach, in a riffle if possible. As far as possible, boulders (>256 mm) free of filamentous algae and obvious siltation were selected. The diatoms were removed to provide a composite sample. The diatoms

were sampled from the upper surface of the boulder with a stiff toothbrush and the epilithon collected in a 250 ml sample bottle, suspended in distilled water (Kelly et al., 1995).

Preparation and identification

Samples were allowed to settle for 24 h and the supernatant decanted. Samples were first examined live to establish if a considerable number of dead cells were present. This was done, as only living cells will be able to provide a reflection of recent water quality. The samples were then oxidised in a saturated solution of potassium permanganate. Carbonates were removed using concentrated (32%) hydrochloric acid (Pienaar, 1988). Samples were then rinsed with distilled water and collected by centrifugation, using five successive runs at 2 500 r·min⁻¹. Clean valves were then mounted in Pleurax (Hanna, 1949).

Diatoms were identified under phase contrast using an oil-immersion lens at 1 000 x magnification. The nomenclature follows Krammer and Lange-Bertalot (1986-91). At least 400 valves (400-500) were identified for each sample (Prygiel, 2002).

Description of the SPI diatom index

The index used is based on the weighted average equation of Zelinka and Marvan (1961) and has the basic form:

$$\text{index} = \frac{\sum_{j=1}^n a_j s_j v_j}{\sum_{j=1}^n a_j v_j}$$

where:

- a_j = abundance (proportion) of species j in sample
- v_j = indicator value
- s_j = pollution sensitivity of species j .

The performance of the index depends on the values given to the constants s and v for each taxon and the values of the index ranges from 1 to an upper limit equal to the highest value of s . For SPI (Specific Pollution sensitivity Index; CEMAGREF, 1982), the maximum value of 5 (converted to 20 by the software package OMNIDIA; Lecointe et al., 1993) indicates clean water. SPI is a comprehensive index, with values of s and v available for over 1 300 species (Coste et al., 1991).

Chemical analysis

Chemical analyses were performed according to *Standard Methods* (1995) by accredited laboratories namely Mogale City local municipality water laboratory and the Agricultural Research Council: Institute for Soil, Climate and Water, Pretoria.

The following water quality variables were analysed in the water quality laboratories: total nitrogen (total N), ammonium (NH₄), total phosphate (total P), chemical oxygen demand (COD), five day biological oxygen demand (BOD₅), sulphate (SO₄) and chloride (Cl).

Several variables were determined in-stream with a calibrated temperature/pH/conductivity/oxygen meter (YSI 556 MPS Multimeter, USA) at the time of sampling. These included: temperature (temp.), pH, electrical conductivity (EC), dissolved oxygen (DO₂) and turbidity.

The variables were chosen to represent general water quality according to the monitoring requirements for domestic and industrial wastewater release (DWAF, 1999). The BOD₅ was added to this suite to provide an indication of organic load according to the analysis list of Kwadrans et al. (1998).

Data analysis

Correlation and stepwise forward multiple regressions were carried out using STATISTICA version 6. Prior to statistical analysis, the distribution of the water quality data was analyzed for normality (STATISTICA Version 6). Where the data showed a skewed distribution the data were $\log_{10}$ transformed. The SPI diatom index was calculated in the database OMNIDIA (Lecointe et al., 1993).

Multiple regressions were performed on the data to establish if there were any physical or chemical variables that influenced the indices other than the ones that showed clear significant correlations in Table 4. Forward stepwise regression was used for this purpose. This regression method takes the independent variable with the greatest contribution and adds it to the model first. Independent variables are then selected for inclusion based on their incremental contribution over the variable(s) already in the equation. Independent variables that are closely correlated in the correlation matrix may not all be included but rather other variables that also contribute to the variation in the index scores. For this reason this method can give important additional information about the factors that influence the various index scores over and above pure correlations. Adjusted R^2 values are used as indicators of the level of success with which the independent variables are able to explain the variation in the index values. This value was chosen as the Adjusted R^2 takes into account the sample size as well as the number of variables used (Hair et al., 1998). Since 12 sites may be deemed a relatively small sample size the value will give more reliable confidence values than the R or R^2 values.

In this study the ASPT value (7) for Site M2C was deemed to be an outlier due to its exaggerated residual value by comparison with the other sites. According to Hair et al. (1998) the definition of an outlier, in strict terms, is an observation that has a substantial difference between its actual and predicted values of the dependent variable (a large residual) or between its independent variable values and those of other observations. The objective of denoting outliers is to identify observations that are inappropriate representations of the population from which the sample is drawn, so that they may be discounted or even eliminated from the analysis as unrepresentative. For this reason M2C was not used in the calculation of the correlation matrix nor in the multiple regression for ASPT.

Results and discussion

The results of the water quality analysis are given in Table 1. When assessing the water quality data qualitatively, according to the variables tested, it appears that the lowest water quality was observed in the Wasgoed Spruit. The stream contained elevated levels of chloride, sulphate, ammonia and other minerals and displayed the highest electrical conductivity in the system.

The highest levels of biological oxygen demand and sulphate, as well as elevated levels of chemical oxygen demand, chloride and total nitrogen were observed in the Wonderfontein Spruit. The influence of the Wonderfontein Spruit on the Mooi River can be seen when comparing the chemical data from sites M1 and M2. Sulphate levels increased considerably from M1 to M2 due to the confluence of the Mooi River with the Wonderfontein Spruit. Increases in chemical oxygen demand, chloride and total nitrogen were also observed in the Mooi River after the confluence of these two streams.

Table 2 shows the values produced for the various indices for the different sites in the Mooi River catchment. For the interpretation of the various indices limit classes are given in Table 3 and 4. The lowest SASS 5 and ASPT scores were recorded in the Wonderfontein Spruit (WFS) that shows major deterioration in water quality, while the diatom index showed the water to be of moderate quality. The lowest SPI score was recorded in the Wasgoed Spruit (WS), which displays a value that can be interpreted as bad water quality, while SASS 5 and ASPT for the same site show values that indicate only some deterioration in water quality.

From Table 2 it would seem as though the diatom index (SPI) is more sensitive to the elevated physical and chemical parameters (that were measured for this study) in the Wasgoed Spruit than the two other indices tested. This would concur with Willemsen et al. (1990), who in a study of the impact of stormwater in the Netherlands, concluded that diatoms were more sensitive to these discharges than were benthic invertebrates; they attribute this to the inability of diatoms to migrate away from unfavourable conditions and to recolonise when conditions have improved.

SASS 5 showed a very low index value for the Wonderfontein Spruit that can be explained by the influence of organic pollution.

TABLE 1
General water quality variables as measured in the Mooi River (May 2003)

Site code	Temp.	O ₂	EC	pH	Turbidity	Total P	COD	BOD ₅	NH ₄	Total N	Cl	SO ₄
M1	16.12	13.16	408	7.67	3.20	0.18	6	0.00	0.05	0.25	13.77	7.05
WFS	12.11	11.72	617	7.19	2.00	0.14	46	1.70	0.04	2.20	64.61	270.99
M2	13.10	9.59	538	7.42	1.40	0.23	43	0.00	0.04	1.45	40.50	158.68
T3	20.94	10.46	668	7.30	0.60	0.22	6	0.00	0.03	4.37	51.02	205.77
M2C	13.41	10.22	537	7.33	1.50	0.23	25	0.00	0.03	1.69	42.40	168.10
MBB	12.49	11.12	506	7.34	2.20	0.17	104	0.00	0.04	1.50	26.67	87.52
M3	15.25	10.74	518	7.37	4.87	0.22	27	0.00	0.04	0.27	46.98	164.02
WS	12.65	8.08	1678	6.78	8.08	0.35	37	0.14	0.84	3.84	543.43	207.24
M3C	15.53	8.84	540	7.74	4.80	0.26	6	0.00	0.05	0.82	60.23	180.24
M4	15.43	10.50	541	7.00	12.40	0.15	6	0.00	0.04	0.29	52.01	165.05
LS	14.16	11.76	568	7.55	20.20	0.28	31	0.50	0.08	2.63	55.50	199.59
M5	16.20	12.97	554	7.23	20.20	0.17	6	0.00	0.04	0.41	53.45	151.03

Variables were measured in $\text{mg}\cdot\text{L}^{-1}$ except for temperature ($^{\circ}\text{C}$), electrical conductivity ($\mu\text{S}\cdot\text{cm}^{-1}$) and turbidity (NTU).

TABLE 2 Recorded scores for SASS 5, ASPT and SPI in the Mooi River (May 2003)			
Site code	SASS 5	ASPT	SPI
M1	116	5.50	15.2
WFS	48	4.00	12.6
M2	62	5.20	13.5
T3	96	4.80	12.4
M2C	70	7.00	13.5
MBB	82	4.80	14.0
M3	89	5.20	12.6
WS	80	4.44	4.5
M3C	78	4.58	12.6
M4	64	4.27	9.1
LS	105	4.56	12.4
M5	80	4.71	9.9

TABLE 4 Class limit values for SPI (Eloranta & Soininen, 2002)	
Class	SPI score
high quality	>17
good quality	15 to 17
moderate quality	12 to 15
poor quality	9 to 12 ⁺
bad quality	<9

According to Dallas and Day (1993) the enrichment of a water body with organic waste almost certainly results in a decrease in invertebrate species richness, diversity and an alteration in the composition of those communities. Chutter (1998) also observed that SASS scores were very low in organically polluted water. SPI scores did not accurately reflect the degree of organic loading in the Wonderfontein Spruit. This can also be seen in the correlation matrix (Table 5), which shows that SPI has no significant correlation to biological oxygen demand.

It is clear from these two sites (WFS and WS) that the various indices do not give the same indication of water quality. This might be due to a difference in response to environmental changes between the different groups of organisms used for calculation of SASS5 and SPI indices. This is a reason for the use of a suite of bio-indicators to assess the status of an ecosystem properly. Table 5 shows the correlation matrix of the various indices together with physical and chemical parameters. A significant correlation ($p < 0.05$) was observed between SASS 5 and ASPT scores. A similar correlation was not observed between the macro-invertebrate indices and the diatom index used. However, a decline in all the indices (Table 2) can be observed from M1 to M5 as would be expected from studying the water quality data (Table 1).

SPI was significantly correlated ($p < 0.05$) with several of the measured water quality variables (Table 5); these included negative correlations with electrical conductivity, chloride and ammonium. A positive correlation was observed between the pH and the SPI score.

TABLE 3 Interpretation of SASS 5 scores (Chutter, 1998)		
SASS 5 score	ASPT score	Class
>100	>6	water quality natural; habitat diversity high
<100	>6	water quality natural; habitat diversity reduced
<100	<6	border line good/bad water quality. Interpretation based on extent that SASS <100, ASPT <6
50-100	<6	some deterioration in water quality
<50	variable	major deterioration in water quality

TABLE 5 Correlations of physical and chemical water quality variables and water quality indices Marked correlations are significant at $p < 0.050$											
	O ₂	EC	pH	Turbidity	Total P	COD	Cl	SO ₄	NH ₄	Total N	BOD ₅
SPI	0.4576 $p=0.135$	-0.855 $p=0.000$	0.8133 $p=0.001$	-0.494 $p=0.103$	-0.3569 $p=0.255$	0.0559 $p=0.863$	-0.8945 $p=0.000$	-0.4157 $p=0.179$	-0.7714 $p=0.003$	-0.3301 $p=0.295$	0.0323 $p=0.921$
SASS 5	0.3369 $p=0.311$	-0.1731 $p=0.611$	0.4567 $p=0.158$	0.1069 $p=0.754$	0.3432 $p=0.302$	-0.2917 $p=0.384$	-0.3015 $p=0.368$	-0.5653 $p=0.070$	0.0731 $p=0.831$	0.0507 $p=0.882$	-0.4569 $p=0.158$
ASPT	0.2248 $p=0.506$	-0.4191 $p=0.200$	0.5196 $p=0.101$	-0.2648 $p=0.431$	0.1302 $p=0.703$	-0.1039 $p=0.761$	-0.543 $p=0.084$	-0.7463 $p=0.008$	-0.2201 $p=0.515$	-0.3219 $p=0.334$	-0.6066 $p=0.048$

TABLE 6 Regression summary for dependent variable: SASS 5						
R= 0.933 R ² = 0.851 Adjusted R ² = 0.741 F(5,5)=6.718 p<0.028 Std.Error of estimate: 9.968						
	Beta	Std.Err.	B	Std.Err.	t(5)	p-level
Intercept			106.920	30.920	3.458	0.018
SO ₄	-0.552	0.206	-0.157	0.059	-2.681	0.044
Total P	0.745	0.232	119.363	37.139	3.214	0.024
O ₂	0.655	0.228	8.095	2.816	2.875	0.035
COD	-0.230	0.168	-9.575	6.975	-1.373	0.228
Total N	0.296	0.219	3.950	2.927	1.349	0.235

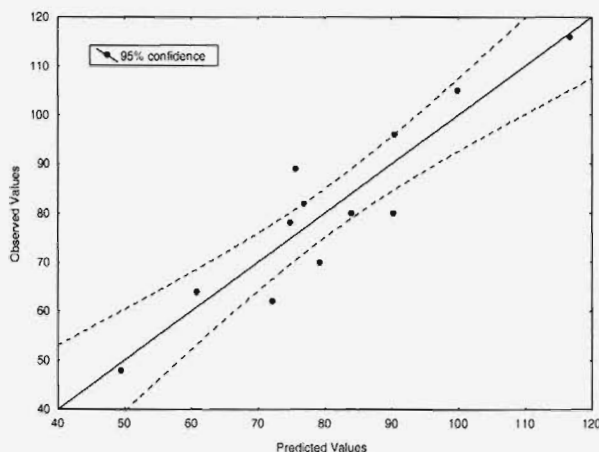


Figure 2

Predicted SASS 5 values vs. observed SASS 5 values

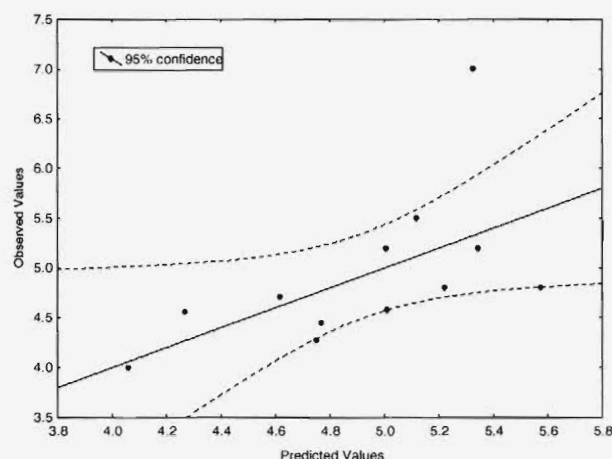


Figure 3

Predicted ASPT values vs. observed ASPT values

ASPT scores correlated significantly with the biological oxygen demand of the water as well as sulphate levels. Biological oxygen demand indicates the degree of organic loading of a stream (Viessman and Hammer, 1998). SASS 5 index scores did not show any significant correlation with any specific water quality variables. This lack of significant correlation was not unexpected as attempts, which have been made to find direct correlation between SASS4 results and water quality variables have, so far, been unsuccessful (Vos et al., 2002).

Table 6 shows the regression results of the multiple regression performed on the physical and chemical variables and the SASS 5 index scores. From the results it can be seen that five of the independent variables were used to account for the variation in the SASS 5 index values. Sulphate, total phosphate and dissolved oxygen all contributed significantly to the variation in the data while COD and total nitrogen also contributed, but not significantly. From the adjusted R² (Table 6) it is clear that the proposed linear model can successfully account for approximately 74% of the variation in the index values. This would mean that about 26% of the variation in the data could not be accounted for by the proposed model and might be accounted for by factors such as habitat.

Figure 2 shows the predicted vs. observed SASS 5 index values. The closer the observations are to the straight line the better the observations could be explained by the proposed multiple regression model. As can be seen from the graph the model was fairly successful in predicting the actual SASS 5 scores.

Table 7 shows the multiple regression results for the ASPT scores and environmental variables. Four variables were taken into account by the multiple regression for the ASPT scores. Sulphate (p<0.05) and possibly total phosphate (p<0.05) contributed to the variation in the ASPT scores while ammonia and turbidity also contributed, but not significantly. The model predicted approximately 68% of the variation in the ASPT scores (Adjusted R² of 0.678).

The graph of predicted vs. observed variables shows that the model was also fairly effective (when compared to the SASS 5 model) in predicting the actual index scores.

The Adjusted R² (Author to check throughout the doc. whether it should be Adjusted R² OR adjusted R²) for the SPI multiple regression (Table 8) is very high with approximately 99% of the variation in the data explained by various water quality variables. The variables included in the regression model were chloride, pH, turbidity, chemical oxygen demand, sulphate and oxygen. All of the variables except oxygen contributed significantly (p<0.05) to the model.

The graph of predicted vs. observed SPI values (Fig. 4) shows that the model was highly effective in predicting the SPI index values.

A total of 112 diatom species, representing 18 genera, were found in the 12 samples. Of the 112 species encountered only 3 (*Psamodictyon constricta* (Gregory) DG Mann, *Nitzschia flexoides* Geitler and *Nitzschia agnita* Hustedt) were not relevant to the

calculation of the SPI index scores. Round (1991) suggested (without experimental evidence) that caution should be observed when transferring index data from the Northern Hemisphere to the Southern Hemisphere as some species may exhibit different ecological tolerances. However, the fact that the SPI values can almost fully be accounted for by the physical and chemical variables in the Mooi River and tributaries (Tables 1 & 8) should satisfy this concern. In addition, 97% of the diatom species encountered in this investigation were useful for SPI and hence cosmopolitan in nature.

Conclusions

According to our results, the diatom index is sensitive to changes in electrical conductivity, ammonia, chemical oxygen demand, chloride, sulphate and turbidity. From this we can conclude that SPI gives a good reflection of general water quality. It would seem as though SPI is able to give a more accurate reflection of the ionic composition of water than the macro-invertebrate index. This is indicated by the strong correlation between electrical conductivity and SPI. Chutter (1998) states that SASS is less sensitive to increases in total dissolved solids (total dissolved solids = electrical conductivity x 6.5 on average) than to other types of chemical change.

From the correlation matrix (Table 5) and the multiple regressions (Tables 6 to 8) it can be deduced that the diatom index is more closely influenced by water quality than the ASPT or the SASS 5 indices. It seems that the macro-invertebrate indices cannot be fully explained by the water quality variables used in this study and may also be affected by other factors such as habitat diversity or other water quality variables not included.

There is therefore, still a need for a biological indicator (such as the diatom index used in this study) that can be indicative of specific water quality variables.

The fact that the diatom sampling also has fewer restrictions in terms of habitat requirements than macro-invertebrates could facilitate its use in monitoring water quality in small tributaries, for instance mining and industrial effluent. This conclusion is strengthened by Round's (2001) statement that "...river diatoms can colonize massive rivers but also "rivers" millimetres deep and centimetres wide..."

From the results of this study it would seem fair to say that there is definite potential for the use of numerical diatom indices as indicators of general water quality and the usefulness of these indices should be verified by further studies that cover a broader geographical area and a broader range of variables.

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TABLE 7
Regression summary for dependent variable: ASPT

R= 0.898 R ² = 0.807 Adjusted R ² = 0.678 F(4,6)=6.272 p<0.025 Std.Error of estimate: 0.249						
	Beta	Std.Err.	B	Std.Err.	t(5)	p-level
Intercept			6.549	0.551	11.896	0.000
SO ₄	-0.781	0.183	-0.005	0.001	-4.260	0.005
Total P	0.599	0.252	2.151	0.903	2.381	0.055
NH ₄	-0.427	0.264	-0.466	0.289	-1.614	0.158
Turbidity	-0.198	0.192	-0.178	0.173	-1.028	0.344

TABLE 8
Regression Summary for Dependent Variable: SPI

R= 0.998 R ² = 0.995 Adjusted R ² = 0.990 F(6,5)=174.740 p<0.00001 Std.Error of estimate: 0.291						
	Beta	Std.Err.	B	Std.Err.	t(5)	p-level
Intercept			-16.604	4.877	-3.405	0.019
Cl	-0.609	0.078	-4.705	0.605	-7.777	0.001
pH	0.428	0.047	4.563	0.497	9.183	0.000
Turbidity	-0.238	0.041	-1.400	0.240	-5.827	0.002
COD	0.195	0.033	1.230	0.205	5.990	0.002
SO ₄	0.141	0.042	0.006	0.002	3.372	0.020
O ₂	0.071	0.052	0.133	0.097	1.380	0.226

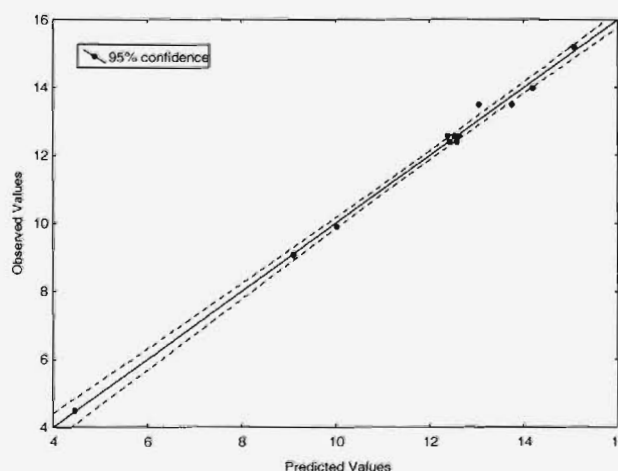


Figure 4
Predicted SPI values vs. observed SPI values

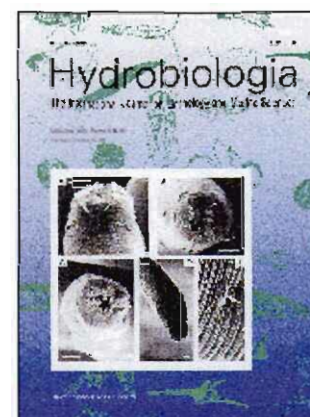
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CHAPTER 5

Can diatom-based pollution indices be used for bio-monitoring in South Africa? A case study of the Crocodile West and Marico water management area



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Can diatom-based pollution indices be used for bio-monitoring in South Africa? A case study of the Crocodile West and Marico water management area

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Key words: diatoms, indices, water quality, biomonitoring

Abstract

The inclusion of diatoms into the current suite of biomonitoring tools in use in South Africa, as well as the use of European and other diatom indices in South Africa, and in particular the Crocodile and West Marico water management area, is discussed. The indices, when compared to chemical analyses, proved useful in providing an indication of the quality of the investigated waters. Several widely distributed diatom species were shown to have similar ecological tolerances in South Africa when compared to Europe. Although most of the diatoms encountered in the study were cosmopolitan, several possibly endemic species were recorded. The occurrence of endemic species, not included in existing diatom indices may lead to miscalculations of diatom indices. It is concluded that although diatom indices developed in Europe and elsewhere are useful, at the present, to indicate water quality, a diatom index unique to South Africa including endemic species will have to be formulated.

Introduction

Water resources in South Africa are scarce, often naturally ephemeral and are difficult to manage. Large volumes of water are transferred from both inside and outside of South Africa to supply the steadily growing demands of industrial and urban centres. Together with the increased demand on use of South Africa's water resources, comes the eventual return of effluent water (usually after some form of ameliorative treatment) to rivers, streams and impoundments. Effluent returns and diffuse discharges (e.g. runoff from agricultural land) may significantly alter the natural state of the receiving water body by the addition of chemical compounds, colloidal material and suspended solids, often resulting in changes in salinity, nutrient status and turbidity of water resources. These changes will in turn alter the structure of aquatic communities to a larger or smaller extent.

Chemical and physical changes in water bodies around South Africa are monitored by the Department of Water Affairs and Forestry (DWAF) and part of the National Chemical Monitoring Programme. However, as with many chemical monitoring programmes, it is simply too expensive to monitor resources at the spatial and temporal frequency required to develop an accurate reflection of the true state of the water body in question. A chemical sample of water quality in essence provides a single "snapshot" bound to a certain brief time when the sample was collected. In addition, no information is gained on the impact of the chemical *milieu*, or changes thereof, on aquatic fauna and flora.

For the above and other reasons, a biomonitoring programme was developed for South Africa (Hohls, 1996) which uses riverine and riparian biota as well as riverine habitat status to assess the water quality and habitat integrity of rivers and streams. The biomonitoring programme is carried out under the umbrella of a national initiative known as the River Health Programme (www.csir.co.za/rhp). The programme makes use of a number of indices to describe the state of the rivers within certain selected catchments around the country. The ultimate goal of the programme is to provide meaningful and accurate data, on both the water quality and overall condition of a water resource, which can then be used as the basis of sound management decisions regarding that particular resource. The previously used indices include aquatic invertebrate fauna, fish, riparian vegetation and river habitats. Up till now

this programme has included no aquatic autotrophic organisms in the monitoring regime, the lowest trophic level being aquatic invertebrate fauna.

Algae are widely used for river quality assessments (Prygiel et al., 1999; Whitton & Kelly 1995; Rott et al., 2003) and are regaining importance in Europe due to the reinforcement of the legislation with the Urban Wastewater Directives (EEC, 1991), more recently the Water Framework Directive (EC, 2000) indicates that the phytobenthos may be used as a relevant indicator of water quality. The diatoms form a large component of the attached flora found on various substrata in rivers and streams. The community composition of these attached or locally motile organisms is directly impacted by the chemical and physical characteristics of the surrounding water body. Undoubtedly diatoms are the most largely used phytobenthic algae (Stevenson & Pan, 2003). The advantages of using these organisms have been largely described (McCormick & Cairns, 1994; Reid et al., 1995). With the exception of Patrick et al. (1954) in the USA who developed a methodology based on structure of diatom assemblages, early methodology using diatoms as indicators of water quality largely originated from Europe in the framework of the saprobic system. At first, methods were devoted to monitoring organic pollution and afterwards for salinity, eutrophication, acidification, and general water quality (Ector & Rimet, 2005; Kelly, 1998; Prygiel et al., 1999 in Prygiel et al., 1999; Rott et al., 2003). Numerous studies describing relationships between European diatoms indices and water chemistry have been carried out by specific programs (Lecointe et al., *in* Ector et al., 1999) and largely confirmed the validity of diatom indices for monitoring rivers in Europe (Dokulil et al., 1997; Eloranta & Soininen, 2002; Kwandrans et al., 1998; Montesanto et al., 1999) but also on other continents (Fawzi et al., 2002; Rott et al., 1998; Sgro & Johansen, 1998). It should be clear that if European indices can give a good indication of the river water quality, they have to be optimized. A preliminary examination of the regional situation is needed before applying diatom indication methods (Rott et al., 2003).

Recently the potential of diatom indices for monitoring water quality in rivers and streams has been explored by authors such as de la Rey et al. (2004) and Taylor et al. (2007) who found that index

scores accurately reflected present conditions as well as being useful to back cast water quality (Taylor et al., 2005). Diatoms have also been used as indicators of water quality in the recent assessment of the state of the rivers in the Crocodile West and Marico water management area (Crocodile West and Marico WMA) as part of the national River Health Programme (River Health Programme, 2005). Diatom indices developed in France and other parts of Europe were used in the South African context to provide a numerical reflection of water quality as well as to classify the rivers and streams in a particular water quality class.

Concerns have been raised as to the transfer and comparison of bio-monitoring data between the Northern and Southern Hemispheres. It is well known that some species have the same morphology, but questions still remain regarding the range of ecological tolerances of the various species. Concerns about ecological tolerances are valid when distance, climatic condition, and other environmental pressures are taken into account (Round, 1991). However, the present study has provided evidence for the concept discussed by Kelly et al. (1998), namely that diatoms are “sub-cosmopolitan” meaning that they may potentially occur anywhere in the world where a certain set of environmental conditions exist which favour the proliferation of a particular species (Padisák, 1998). The sub-cosmopolitan concept suggests that geographical location is not the determining factor in the distribution of diatom species and the composition of communities, but it is rather the specific environmental variables at a site that determine this distribution.

Up to the present only diatom indices developed elsewhere have been used and tested in South Africa. The application of the indices was possible because of the cosmopolitan distribution of many common diatoms as discussed above. Taylor et al. (2007) found that most species of benthic diatoms present in the heavily impacted Vaal River (98%) in central South Africa were cosmopolitan. However, even under these heavily polluted conditions (high nutrient load, high pH and high salinity), a few possibly endemic species may be found; for a discussion on this see Taylor & Lange-Bertalot (2006). The level of endemism may increase dramatically in smaller streams having a better water quality. For example, a distinctive species of *Achananthes* (*Achnantheidium*), *A. standeri* was described by Cholnoky and is

found in abundance in certain mountain streams (Cholnoky, 1957). This will necessitate the eventual development of unique diatom indices for accurate water quality assessment in South Africa.

This paper presents the relationship between measured water quality in the Crocodile West and Marico WMA and diatom index scores. This paper also shows that many widely distributed diatom taxa have similar environmental tolerances as compared to data recorded from Europe and elsewhere. Several problems encountered when using European diatom indices in South Africa are discussed.

Materials and methods

Study region

The Crocodile West and Marico water management area (Fig. 1) borders on Botswana to the north-west. The catchment is spread across three provinces (Gauteng, North West Province and Limpopo). Its main rivers, the Crocodile and Marico, give rise to the Limpopo River at their confluence. The climate is generally semi-arid. Extensive irrigation as well as grain, livestock and game farming occur in the catchment. Economic activity in the water management area is dominated by the urban and industrial complexes of northern Johannesburg and Pretoria together with platinum mining, north-east of Rustenburg. The Crocodile West and Marico water management area is the second most populous water management area in the country. Usage of surface water naturally occurring in the water management area has reached its potential and is being fully utilised. Large dolomitic groundwater aquifers occur along the southern part of the water management area. These are extensively utilised for urban and irrigation purposes. Some aquifers also underlie the border with Botswana and are shared with that country. A substantial portion of the water used in the water management area, is transferred into the area from the Vaal River and further afield. Transfers out of the water management area are to Gabarone in Botswana as well as to Nylstroom in the Limpopo water management area. Increasing quantities of effluent return flow from the urban/industrial areas are a major source of nutrients, salts and changes in pH in some rivers (DWAF, 2004).

Chemical and biological sample collection

Fifty diatom samples were collected during the period 29/06/2004 to 25/07/2004 in the Crocodile-Marico Catchment. Diatom samples were collected and prepared using standard methods as summarised by Taylor et al. (2005). The diatom communities were then analysed by counting between 400 and 450 valves. The flora of Krammer & Lange-Bertalot (1986-91) was used for identification. Other taxonomic guides consulted include Schoeman and Archibald (1976-80), Round et al. (1990), Hartley (1996) and Prygiel & Coste (2000). For revised nomenclature the works of Lange-Bertalot (2001), Krammer (2002) and Kellogg & Kellogg (2002) were consulted. The community counts were entered into the diatom database and index calculation tool OMNIDIA version 3.1 (Lecointe et al., 1993) and several diatom indices were calculated.

The indices tested in the present study are known as the Generic Diatom Index or GDI (Coste & Ayphassorho, 1991), the Specific Pollution sensitivity Index or SPI (Coste *in* Cemagref, 1982), the Biological Diatom Index or BDI (Lenoir & Coste, 1996), the Artois-Picardie Diatom Index or APDI (Prygiel et al., 1996), Sládeček's index or SLA (Sládeček, 1986), the Eutrophication/Pollution Index or EPI (Dell'Uomo, 1996), Rott's Index or ROT (Rott, 1991), Leclercq and Maquet's Index or LMI (Leclercq & Maquet, 1987), the Commission of Economical Community Index or CEC (Descy & Coste, 1991) Schiefele and Schreiner's index or SHE (Schiefele & Schreiner, 1991), the Trophic Diatom Index or TDI (Kelly & Whitton, 1995), and the Watanabe index or WAT (Watanabe et al., 1986). In all cases except in the CEC, SHE, TDI and WAT index, the diatom indices were calculated using the formula of Zelinka and Marvan (1961). For all of the above indices, except TDI (maximum value of 100), the maximum value of 5 (converted to 20 by the software package OMNIDIA; Lecointe et al., 1993) indicates pristine water.

The Department of Water Affairs and Forestry (DWAF) collected the water quality data used in this study as part of their National Chemical Monitoring Programme. The samples collected for this programme are analysed in the laboratories of Resource Quality Services (RQS), Pretoria, and the data

is stored on DWAF's database and information management system, namely the Water Management System (WMS) from which the environmental data were drawn.

All chemical data was normalized using $\log_{10}$ transformation with the exception of the data recorded for pH. Pearson correlation was used to determine the relationship between the calculated index scores and the measured water quality variables and was carried out in STATISTICA version. 6.1.

Canonical Correspondence Analysis (CCA), using CANOCO version 4.5 (Ter Braak & Šmilauer, 1998), was used to determine the relationship between diatom assemblages and measured environmental variables. Abbreviations used in the CCA diagram may be found in Table 4.

Results

The results of the correlation analysis between measured environmental variables and diatom index scores generated for sites in the Crocodile West and Marico WMA are presented in Table 2.

The results of the Canonical Correspondence Analysis (CCA) are presented in Figure 2. The first four axes of the species-environment plot accounted for 71% of the total variance in the community due to measured environmental variables (Table 4). Measured values (mean, median, minimum and maximum) for the physical and chemical variables ($n = 113$) in the Crocodile West and Marico WMA are presented in Table 1. These values include the calculated mean for the chemical variables; this value can be equated with the epicentre of the CCA plot. A summary of the acronyms used in Figure 2 may be found in Table 4.

Discussion

In general, the diatom indices show significant correlations to water quality ($p < 0.05$). The correlations obtained in the present study are comparable to those demonstrated by Taylor et al. (2007) in South Africa and by Kwandrans et al. (1998), Prygiel & Coste (1993) and Prygiel et al. (1999) in Europe. Significant correlations indicate the success with which diatom indices may be used to reflect

changes in general water quality (Table 2) and organic pollution (as reflected by COD; Table 2). Temperature did not correlate significantly with any of the indices and this may be due to differing temperature regimes between Europe and South Africa, or due to different temperature tolerance of South African diatoms. Changes in temperature are more likely to be associated in South Africa with stream depth than with temperature, as even in winter, shallow streams and canals heat up very quickly (unpubl. data) while deeper streams and rivers tend to be more thermally stable e.g. the Vaal River (Taylor, 2004).

CCA (Figure 2) was used to demonstrate that certain widely distributed taxa have similar ecological characteristics in widely separated geographic areas. It is clear from Figure 2 that those species commonly associated with poor water quality e.g. *Eolimna subminuscula* Lange-Bertalot, *Nitzschia frustulum* (Kützinger) Grunow ordinate on the right hand side of the diagram together with elevated levels of water quality variables. Another typical example is *Diatoma vulgare* Bory, which commonly occurs in freshwaters with elevated levels of phosphate-phosphorus and is closely associated with the vector for this variable in Figure 2. Taxa typical of cleaner, less impacted waters ordinate out on the left hand side of the diagram e.g. *Achnanthes minutissimum*, *Achnanthes affine* (Grunow) Czarnecki, *Encyonopsis microcephala* (Grunow) Krammer and *Fragilaria capucina* var. *rumpens* (Kützinger) Lange-Bertalot. This diagram helps to demonstrate that the widely distributed species encountered in the Crocodile West and Marico WMA are not simply just morphologically similar to those encountered in Europe, but have similar environmental tolerances.

Achnanthes standeri Cholnoky and *Gomphonema venusta* Passy, Kociolek & Lowe are shown in Figure 2 on the far left hand of the ordination diagram. Thus far, these two species have only been recorded from South Africa; therefore, there is a strong likelihood that these two species are endemic. Both of these species were recorded as dominant (< 5% of species relative abundance) in several samples, but did not dominate at all the sites. These two species are not included in any of the index calculations and their omission could result in an under or overestimation of the index scores.

The above evidence would suggest that although European diatom indices may be used in South Africa there are several problems to be considered:

- i) The list of taxa included in the indices needs to be adapted to the studied region. Most European diatom indices may be used in many regions and also in South Africa as they are based on the ecology of widely distributed or cosmopolitan taxa. This is particularly true for organic pollution as indicative taxa are ubiquitous and in addition, many European indices are based on Cholnoky's (1968) data for heterotrophy. Special attention should be paid to taxa occurring in pristine water (e.g. *Achnanthes standeri*) as well as endemic taxa which are absent in the indices reference lists (e.g. *Gomphonema venusta*). When these taxa are abundant, water quality may be misinterpreted.
- ii) Diatom taxonomy is undergoing rapid changes, especially at the genus level. Local floras, guides and methods to be used must be consistent. Some European indices have been proposed in the seventies or in the eighties and have never been revised. Thus, several common and abundant taxa, some of which being newly or recently described, may not be taken into account and lead to erroneous results. There are also several different approaches to taxonomy when calculating index scores. For example, *Achnanthidium pyrenaicum* (Hustedt) Kobayasi is part of the BDI taxa list, even if lumped with *Achnanthidium minutissimum* (Kützinger) Czarnecki, but is not considered in other European indices such as TDI for example. Such an exclusion will possibly change index scores as these two taxa have a different ecology, *A. biasolettianum* is characteristic of pristine calcareous rivers and *A. minutissimum* is considered as a cosmopolitan pioneer taxon. In the case of BDI, many taxa have been lumped because of the difficulty to separate them in routine surveillance, even if their ecology is different. These associations of similar taxa lead to miscalculations of water quality in polluted rivers.
- iii) It has been highlighted in other studies that classification systems based on species tolerances should be carefully considered as built, to a greater or lesser extent, from local data. For example, Rott et al. (2003) noted that when using BDI, resulting index scores classified Austrian rivers as relatively good, even though large nutrient loads should have led them to be

classified as eutrophic, poor quality rivers. It should be noted that BDI was developed from data collected from the French national monitoring network which was aimed almost solely at monitoring impacts on water quality.

Conclusions

It is concluded that the index approach is still deemed useful in South Africa to provide information on water quality impacts on rivers and streams. It has also been demonstrated that many widely distributed diatom species have similar environmental tolerances to those recorded for these species in Europe and elsewhere. However, the occurrence of possible endemic species will necessitate the eventual compilation of a diatom index unique to South Africa. In the mean time, diatom indices can be used in a) gaining support and recognition for diatom-based approaches to water quality monitoring, b) providing an indication of water quality for programmes such as the RHP and allowing for the dissemination of simplified useful information to resource managers, conservationists and the general public, and c) allowing for sample and data collection which can then be used later in the formulation of a South African diatom index.

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Tables

Table 1. Summary table of measured chemical and physical environmental variables in the Crocodile West and Marico WMA during the study period. n = 50.

	Unit	Mean	Std. Error	Median	Std. Deviation	Range	Min.	Max.
Temperature	°C	13.49	0.63	12.54	4.42	21.10	5.90	27.00
Conductivity	mS/m	44.62	5.94	26.45	41.99	153.60	1.70	155.30
pH		7.56	0.10	7.52	0.74	3.43	5.87	9.30
NO ₃ +NO ₂ -N	mg/l	4.57	3.23	0.06	22.84	159.00	0.03	159.03
NH ₄ -N	mg/l	0.06	0.04	0.02	0.28	2.00	0.02	2.01
TAL	mg/l	124.33	11.59	136.29	81.97	300.00	4.00	304.00
Na ⁺	mg/l	31.94	5.88	10.31	41.56	209.11	2.54	211.66
Mg ²⁺	mg/l	20.46	2.48	17.40	17.54	72.30	1.37	73.67
SiO ₂ -S	mg/l	6.21	0.43	5.86	3.06	17.07	0.01	17.08
PO ₄ -P	mg/l	3.83	3.66	0.01	25.89	183.15	0.01	183.17
SO ₄ ²⁻	mg/l	43.85	7.95	9.72	56.20	212.99	3.00	215.99
Cl ⁻	mg/l	43.31	8.71	8.21	61.59	253.63	2.50	256.13
K ⁺	mg/l	6.47	3.09	1.40	21.83	154.69	0.31	155.00
Ca ⁺	mg/l	53.55	20.79	28.25	147.04	1051.77	2.05	1053.81

Table 2. Pearson correlation coefficients between measured environmental variables and diatom index scores generated for sites in the Crocodile West and Marico WMA. Numerical values indicate significant correlations at $p < 0.05$, $n = 50$ (casewise deletion of missing data), while (..) indicates no significant correlation. *Index acronyms explained in last row of table. # $n = 40$.

	SPI	SLA	LMI	SHE	WAT	TDI	EPI	ROT	GDI	CEC	BDI	APDI
Temperature	..	..	..	..	..	..	..	..	..	..	..	..
EC mS/m	0.48	0.54	0.53	0.52	-0.40	0.66	0.56	0.55	0.55	0.52	0.60	-0.53
pH	-0.4	0.25	0.36	0.31	..	0.31	0.30	0.29	0.55	0.42	0.49	-0.34
PO ₄ -P	0.44	0.41	0.48	0.45	-0.45	0.28	0.55	0.49	0.28	0.45	0.30	-0.49
NO ₃ + NO ₂ -N	0.50	0.59	0.51	0.59	-0.49	0.55	0.55	0.63	0.23	0.45	0.40	-0.50
NH ₄ -N	0.51	0.39	0.40	0.39	..	0.18	0.27	0.28	0.25	0.39	0.28	-0.29
Total Alkalinity	0.34	0.42	0.37	0.40	-0.18	0.59	0.39	0.40	0.42	0.38	0.42	-0.33
Na ⁺	0.67	0.69	0.69	0.68	-0.52	0.75	0.76	0.66	0.60	0.70	0.71	-0.68
Mg ²⁺	0.50	0.57	0.52	0.57	-0.36	0.72	0.54	0.55	0.50	0.52	0.55	-0.50
SiO ₂ -S	..	..	..	..	..	..	..	..	..	..	..	..
SO ₄ ²⁻	0.69	0.73	0.68	0.70	-0.49	0.83	0.75	0.66	0.64	0.71	0.74	-0.67
Cl ⁻	0.70	0.70	0.71	0.68	-0.54	0.77	0.79	0.67	0.64	0.73	0.73	-0.70
K ⁺	0.53	0.54	0.57	0.52	-0.46	0.59	0.60	0.55	0.49	0.57	0.60	-0.57
Ca ⁺	0.42	0.48	0.44	0.45	-0.29	0.59	0.48	0.45	0.42	0.45	0.45	-0.43
COD #	0.45	0.43	0.59	0.50	0.50	..	0.45	0.61	..	0.39	..	0.57
SPI; Specific Pollution Sensitivity Index, SLA; Sládeček's Index, LMI; Leclercq & Maquet's Index, SHE; Schiefele and Schreiner's Index, WAT; Watanabe's Index, TDI; Trophic Diatom Index, EPI; Eutrophication/Pollution Index, ROT; Rott's Index, GDI; Generic Diatom Index, CEC; Council for European Communities Index, BDI; Biological Diatom Index, APDI; Artois-Picardie Diatom Index.												

Table 3. Summary of the canonical correspondence analysis (CCA) for the Crocodile West and Marico WMA

	Axis order			
	1	2	3	4
Eigenvalue	0.71	0.38	0.33	0.27
Species-environment correlation	0.95	0.84	0.86	0.75
Cumulative percentage variance of:				
species data	9.1	13.9	18.2	21.7
species-environment relation	30.3	46.3	60.5	72

Table 4. Acronyms used in the CCA biplot (Fig. 3)

Taxon	Acronym
<i>Achnantheidium minutissimum</i> (Kützing) Czarnecki	ADMI
<i>Achnantheidium affine</i> (Grunow) Czarnecki	AMAF
<i>Achnantheidium saprophila</i> (Kobayasi & Mayama) Round & Bukhtiyarova	ADSA
<i>Amphora pediculus</i> (Kützing) Grunow	APED
<i>Cocconeis placentula</i> var. <i>euglypta</i> (Ehrenberg) Grunow	CPLE
<i>Cymbella kappii</i> (Cholnoky) Cholnoky	CKAP
<i>Diatoma vulgaris</i> Bory	DVUL
<i>Encyonopsis microcephala</i> (Grunow) Krammer	ENCM
<i>Eolimna subminuscule</i> (Manguin) Lange-Bertalot & Metzeltin	ESBM
<i>Fragilaria capucina</i> var. <i>rumpens</i> (Kützing) Lange-Bertalot	FCRU
<i>Gomphonema minutum</i> (Agardh) Agardh	GMIN
<i>Gomphonema parvulum</i> Kützing	GPAR
<i>Gomphonema parvulum</i> var. <i>lagenula</i> (Kützing) Frenguelli	GPLA
<i>Gomphonema pumilum</i> (Grunow) Reichardt & Lange-Bertalot	GPUM
<i>Navicula cryptocephala</i> Kützing	NCRY
<i>Navicula tripunctata</i> (O.Müller) Bory	NTPT
<i>Nitzschia archibaldii</i> Lange-Bertalot	NIAR
<i>Nitzschia dissipata</i> (Kützing) Grunow	NDIS
<i>Nitzschia frustulum</i> (Kützing) Grunow	NIFR
<i>Nitzschia paleacea</i> (Grunow) Grunow in Van Heurk	NPAE
<i>Navicula tenelloides</i> Hustedt	NTEN
Total alkalinity	TAL
Electrical conductivity	Cond
Temperature	Temp

Figures

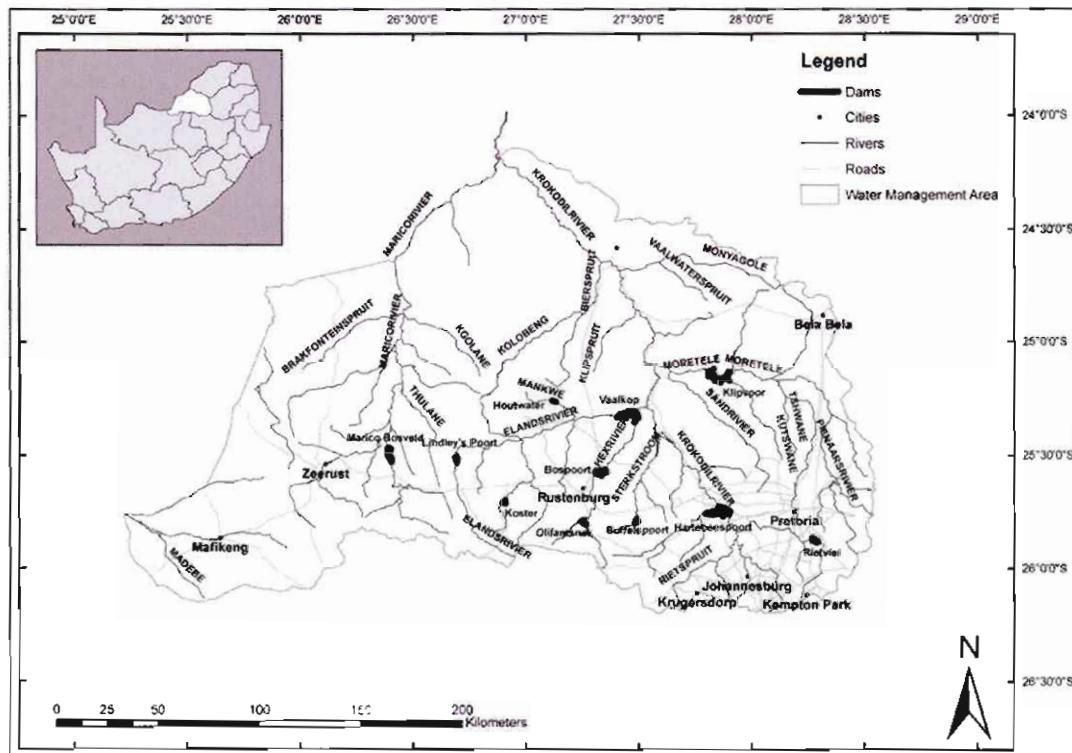


Figure 1. Map showing location of some of the larger rivers in the Crocodile West and Marico water management area.

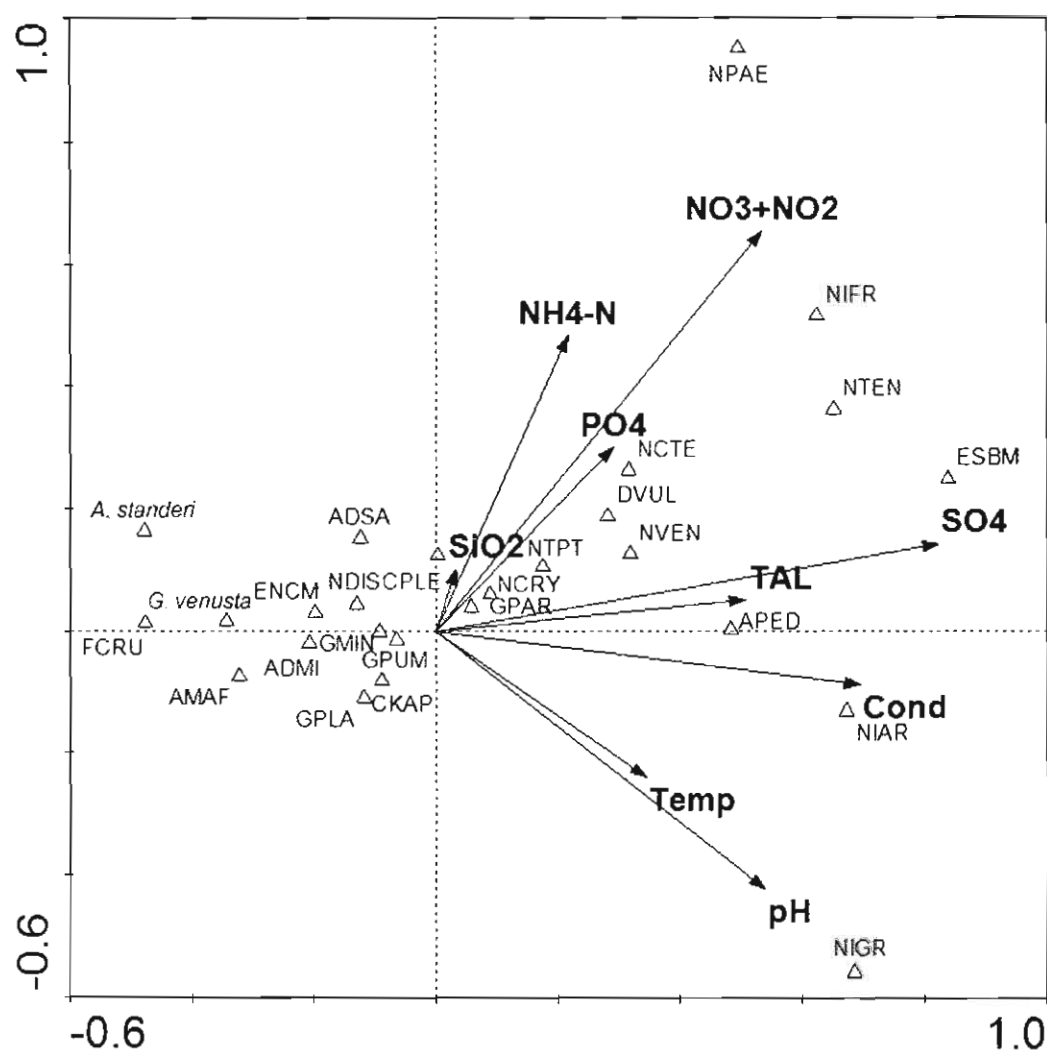


Figure 2. CCA biplot showing the relationship between measured environmental variables and some diatom species in the Crocodile West and Marico water management area. Species with a weight range of 1-100% are shown. Acronyms are presented in Table 4

CHAPTER 6

Diatoms as indicators of water quality in the Jukskei-Crocodile river system in 1956 and 1957, a re-analysis of diatom count data generated by Dr. B. J. Cholnoky

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Diatoms as indicators of water quality in the Jukskei-Crocodile river system in 1956 and 1957, a re-analysis of diatom count data generated by BJ Chohnoky

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Abstract

South Africa has a long legacy of diatom research. The eminent diatomist Dr BJ Chohnoky spent much of his working life examining and enumerating diatom communities found in Southern Africa. Most if not all of Chohnoky's collected diatom material in the form of mounted material on glass slides accompanied by diatom analysis sheets is stored in the South African Diatom Collection currently housed at the CSIR in Durban. As Chohnoky only employed enumeration methods yielding a margin of error of 2% or less, Chohnoky's results should provide an accurate reflection of the structure of the diatom communities that he examined. It is the aim of the present study to demonstrate the value of these historical diatom analyses for inferring past water quality conditions using the diatom-based index method. Data for the Jukskei-Crocodile River system were obtained from the South African Diatom Collection for the period 1956/1957. The nomenclature of the diatoms listed on Chohnoky's data sheets was modernised and the data then entered into OMNIDIA v3.1. Diatom index scores generated from OMNIDIA v3.1 were in general in agreement with Chohnoky's own assessment of water quality (especially with reference to organic pollution). It is concluded that the diatom analysis records housed in the South African Diatom Collection constitute a valuable resource for the assessment of past conditions of rivers and streams.

Keywords: BJ Chohnoky, historical diatom analyses, diatom indices, historical water quality

Introduction

Over many years the work of Dr BJ Chohnoky provided an invaluable contribution to the knowledge of the taxonomy and ecology of diatom species he encountered in a variety of southern African habitats. Chohnoky's ecological work attempted to provide a reflection of water quality based on the specific pollution tolerances of diatom species, and especially to nitrogenous compounds. In addition Chohnoky was one of the first people to predict pH of a water-body based on its diatom community (Chohnoky, 1958). Chohnoky was only able to relate several key species from a particular diatom community to different pollutants; later workers have had the luxury of using statistical techniques such as correspondence analysis (Ter Braak and Prentice, 1988) to determine the relationships between the abundances of all diatom species encountered in a certain community and the chemical composition of their aquatic environment. Consequently inferred tolerances can be assigned to diatom species for a whole range of water quality variables rather than just for nitrogen or pH.

When Chohnoky's (1968) definitive work on the diatoms *Die Ökologie Der Diatomeen in Binnengewässern* is examined it is noted that Chohnoky painstakingly dealt with all practical aspects relevant to diatom ecological studies. He first stressed that any person studying ecology should have a sound taxonomical

background; secondly he carefully determined margins of error for diatom analysis. Most importantly he tested various counting procedures and determined whether different slides from the same site need to be counted to generate an accurate result, how many individual cells should be counted and the manner in which diatom cells should be counted. Chohnoky only employed methods yielding a margin of error of 2% or less. Thus, Chohnoky's diatom analysis sheets should provide an accurate reflection of the structure of the diatom communities that he encountered. If Chohnoky's diatom community analysis is considered to be accurate then the ecological conclusions drawn from his data should be equally sound.

An explanatory note follows about working with diatom species encountered in South Africa: When diatom publications were written by various authors (Chohnoky, Giffen, Schoeman and Archibald) it was with the intention either to describe all diatom species encountered in a given sample (i.e. community structure), or to describe novel species from a particular locality. The method of illustrating these publications was with line drawings, which are both time-consuming and difficult to generate. Thus common species were usually not illustrated and the reader is most often referred to the works of Hustedt or other authors for illustrations of the species in question. Thus we have a large amount of South African literature that has few, or no, illustrations of commonly encountered diatom species, only novel and rare species. In the late 1980s workers such as Schoeman and Archibald used photographic images to illustrate articles, such as the work done in Namibia at the Gross Barmen Thermal Springs (Schoeman and Archibald, 1988). In this work common species with their variations are illustrated using photomicrograph images.

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Another obstacle encountered in relating older publications to current data and literature lies in the taxonomy and nomenclature of the diatoms. Internationally, diatom nomenclature has undergone several major upheavals and changes in the past 15 years. Since the publication of Round et al. (1990) *The Diatoms: Morphology and Taxonomy of the Genera*, the taxonomical trend has been to split large genera into smaller groups, establish synonyms between con-specific taxa, and to generally rearrange the diatom species into more natural groupings. In addition, many of the species described by Cholnoky have been established as synonyms for taxa described from Europe, while on the other hand many of his species have been validated and found to occur in Europe. Cholnoky also described many "African" forms of extant species, adding to taxonomical confusion. Schoeman (1973), writing after Cholnoky's death, comments: "*transitional forms (of diatoms), linking certain species with their forms and varieties... clearly indicate that the demarcation into varieties or forms is often entirely superfluous and can serve no purpose at all.*" This comment creates doubt about the validity of Cholnoky's "African" forms.

The lack of illustration of common species together with vast changes in diatom taxonomy over the last decade has led to misconceptions about diatom taxa encountered in South Africa. The vast majority of common diatom taxa found in South Africa are cosmopolitan both in distribution (see Krammer and Lange-Bertalot, 1986 to 1991), and environmental tolerances. There are a number of diatom species endemic to South or Southern Africa (see Schoeman and Archibald, 1976 to 1980), but the dominant diatom species in a given community are well-known, well-documented cosmopolite species. This is illustrated in the present analysis where the majority of species occurring on Cholnoky's analysis sheets were described from Europe. Although the nomenclatural changes mentioned above can be problematic for the practical diatomist, the taxonomy and nomenclature of diatom species encountered in South Africa can be quickly updated using the wealth of modern literature and electronic databases such as OMNIDIA (Lecointe et al., 1993) - as this study demonstrates.

South Africa is in possession of an enormous database of literature, diatom material (slides and preserved material) and most importantly diatom analysis sheets currently housed in the South African Diatom Collection at the CSIR in Durban. To draw correct inferences about the water quality of a given river or stream using diatom analysis methods, several hours are needed behind a high-power microscope to determine the relative species composition of the sampled community. In addition, to evaluate the structure of a diatom community the person performing this analysis needs to have a very good knowledge of diatom taxonomy. However, the South African Diatom Collection has many original diatom analysis sheets composed from samples taken over a number of decades, commencing in the mid 1950s. These analysis sheets are available for many of South Africa's rivers. Thus when historical data is needed for a particular river system (e.g. to determine the extent of degradation over a period of time), the most time-consuming and painstaking part of using diatom indices has already been completed. It now only remains to convert these previously composed diatom analysis sheets to digital format and then generate historical ecological information based on the diatom communities using modern diatom pollution indices that have been developed and tested over several decades in Europe and elsewhere.

Diatom indices function in the following manner: In a sample from a body of water with a particular level of determinand (e.g. salinity), diatom taxa with their optimum close to that level

will be most abundant. Therefore an estimate of the level of that determinand in the sample can be made from the average of the optima of all the taxa in that sample, each weighted by its abundance. This means that a taxon that is found frequently in a sample has more influence on the result than one that is rare. A further refinement is the provision of an "indicator value" which is included to give greater weight to those taxa which are good indicators of particular environmental conditions. In practice, the first step to be completed when using diatom indices is the compilation of a list of taxa in a sample, together with their absolute abundance. It is this step which has been completed for many samples by Cholnoky and his co-workers. The final index value is expressed as the mean of the optima of the taxa in the sample, weighted by the abundance of each taxon. The indicator value acts to further increase the influence of certain species (Kelly, 1998).

The diatom indices used in this analysis are known as Descy's Index or DES (Descy, 1979); the Generic Diatom Index or GDI (Coste and Ayphassorho, 1991); the Specific Pollution Sensitivity Index or SPI (Coste in CEMAGREF, 1982); the Biological Diatom Index or BDI (Lenoir and Coste, 1996); the Eutrophication/Pollution Index or EPI (Dell'Uomo, 1996); the Artois-Picardie Diatom Index or APDI (Prygiel et al., 1996); Sládeček's Index or SLA (Sládeček, 1986); Leclercq and Maquet's Index or LMI (Leclercq and Maquet, 1987); the Commission of Economical Community Index or CEC (Descy and Coste, 1991); Schiefele and Schreiner's Index or SHE (Schiefele and Schreiner, 1991); Rott's Index or ROT (Rott, 1991); the Trophic Diatom Index or TDI (Kelly and Whitton, 1995); and the Watanabe Index or WAT (Watanabe, 1986; 1990). In all cases except in the CEC, SHE, TDI and WAT Index, the diatom indices are calculated using the formula of Zelinka and Marvan (1961). For all of the above indices, except TDI (maximum value of 100), the maximum value of 5 (converted to 20 by the software package OMNIDIA; Lecointe et al., 1993) indicates a high quality or pristine water resource.

Most of the diatom indices listed above were designed to give an indication of general water quality. The indices differ in respect to the diatom species included in the calculation and in the number of taxa included in the calculation. The first index to be developed was that of Descy. This index was followed by the SPI, which has the broadest species base of all of the indices. Several refinements followed on the SPI index that eventually culminated in the BDI, which incorporates 14 parameters of water quality. 70% of the variation in the scores of the BDI index can be explained using 14 water quality variables. The remaining 30% of the variation is ascribed to physical factors such as light penetration, current speed and general habitat integrity (Lenoir and Coste, 1996). Several indices were designed to reflect eutrophication including the EPI and the TDI. The calculation of correct scores for the TDI index is dependent on the percentage of pollution-tolerant diatom taxa in the sample (%PT), more than 20% PT valves indicate organic pollution rather than eutrophication. Sládeček (SLA index) and Watanabe (WAT index) developed diatom indices which were designed to reflect degrees of organic pollution.

At this point it may be useful to define the terms organic pollution and eutrophication in the context of the present paper. Organic pollution refers to unnatural addition of dissolved and particulate organic matter to an aquatic ecosystem. Organic discharges are those produced or derived from living organisms. Organic pollution of an aquatic ecosystem may result in various chemical (dissolved oxygen, nutrient levels) and physical (turbidity and suspended solids) changes that in turn drive biological changes in the receiving water body (Dallas and Day,

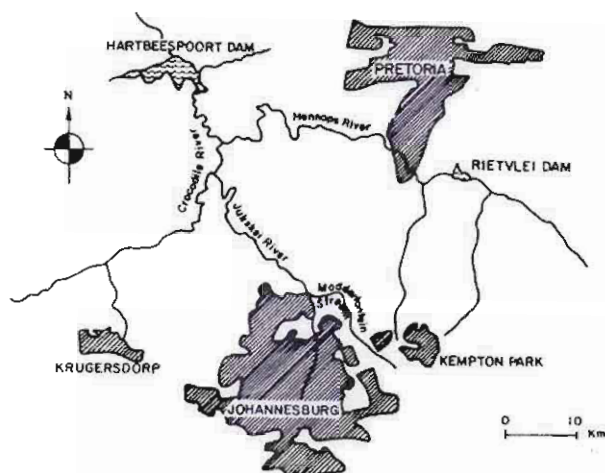


Figure 1
Location of the Jukskei-Crocodile River catchment area
(Schoeman, 1982)

2004). A widely accepted definition of eutrophication is that of the Organisation for Economic cooperation and Development (OECD, 1982) which describes the process as "... the nutrient enrichment of waters which results in the stimulation of an array of symptomatic changes, amongst which is the increased production of algae and aquatic macrophytes, deterioration of water quality and other symptomatic changes that are found to be undesirable and interfere with water uses". In the classification of Nauman (1919) and Rast and Thornton (1996) the term oligotrophic means the presence of low levels of nutrients and water quality problems; mesotrophic means intermediate levels of nutrients, with emerging signs of water quality problems; and eutrophic means high levels of nutrients and an increased frequency of water quality problems.

The aim of this study is to demonstrate the value of historical diatom analysis sheets by showing that they can be used as the basis for calculating a diatom index score for a particular site. This diatom index score can in turn be useful for drawing conclusions about the past condition (up to 50 years ago) of South African rivers.

Methods

The Jukskei-Crocodile River system drains an area of 2 046 km² between Johannesburg and the Hartbeespoort Dam at an altitude of between 1 200 and 1 800 m (see Fig. 1). Climatically this region is cold and dry in winter and warm to hot in summer. About 80 to 90 % of the rainfall occurs in the six summer months, i.e. between November and April (Allanson, 1961). The southern catchment area (northern Johannesburg) is densely populated and heavily industrialised, whereas the northern part consists mainly of agricultural areas. At the time of Cholnoky's work the Jukskei-Crocodile River system received effluent from many different sources including power station blow-down (mineralising effect), industrial and sewage effluent (Allanson, 1961; Schoeman, 1976). The Crocodile River drained what was then a predominantly agricultural area and accordingly contained water of a higher quality (Schoeman, 1982).

This study aims to demonstrate the usefulness of historical diatom community analysis sheets. However, several problems were encountered in using these analysis sheets. Firstly the data sheets needed to be converted to a digital format. In the present

study this was achieved by entering the data first into spreadsheets and then into the OMNIDIA database. The first entry into MICROSOFT EXCEL was necessary as the data had to be electronically captured. However, if the person entering the data is proficient in the use of the OMNIDIA Database the data can be directly entered without a first, time-consuming, entry into spreadsheets. OMNIDIA (Lecointe et al., 1993) was developed for the management of diatom samples and calculation of diatom indices from abundance data generated from diatom community analysis. Data entry into OMNIDIA only requires a species acronym together with absolute abundance of the relevant diatom species. From these data the program generates the full species name and relative abundance of the species in the community, and hence is far less time-consuming than entering species data into spreadsheets. Results obtained from OMNIDIA are in the form of individual diatom analysis sheets together with site information, relative abundance of the species, population, diversity, evenness and a number of diatom index scores generated from the diatom community data (see discussion above). Alternatively diatom analysis sheets can be grouped together up to 20 at a time saving repetitive mention of species. These files can then in turn be exported to EXCEL or some other similar program.

The entry of diatom data of a historical nature using the acronym method poses several problems for the inexperienced user. The first complication that arises is whether the species name used by the original author of the analysis sheets is currently valid and recognized by the software? The validity of species names can be checked in OMNIDIA or, failing that, in a number of literature resources. If the specific or generic epithet is no longer valid then the accurate synonym can be obtained in the OMNIDIA program, or alternatively from literature resources such as Krammer and Lange-Bertalot (1986 to 1991) or Kellogg and Kellogg (2002). Secondly, the relevant acronym for data entry needs to be identified. There is a printable list of acronyms in OMNIDIA for about 9 000 species, or alternatively an electronic search may be conducted by typing the full species name into OMNIDIA. The acronym construction follows certain rules and once the operator is familiar with these rules most of the acronyms can many times be determined without resorting to either a manual or electronic search.

Once the data had been entered, the database (OMNIDIA) calculated the indices listed above in the introduction. In the following section the diatom index results for this analysis will be presented and discussed.

Results and discussion

A complete species list (including synonymy) composed from the analysed data sheets is presented in **Appendix 1**. Species names highlighted in bold are those used in Cholnoky's original analysis sheets.

The diatom index scores generated from the diatom community analysis sheets are presented in Table 2, and should be interpreted using Table 1.

The results as presented in Table 2 can be seen to give an accurate indication of a highly impacted river system (as per Schoeman, 1976). However, caution should be exercised in interpreting the data yielded by those samples with a population lower than the 350 minimum recommended by Cholnoky in his book (Cholnoky, 1968), and later by European authors (e.g. 300 by Prygiel et al., 2002). All of the species listed on Cholnoky's diatom analysis sheets could be entered into the OMNIDIA database with the exception of *Cymbella bengalensis*. There is no

TABLE 1 Class limit values for diatom indices (Eloranta & Soininen, 2002)		
Class	Trophy	Index score
high quality	oligotrophy	>17
good quality	oligo-mesotrophy	15 to 17
moderate quality	mesotrophy	12 to 15
poor quality	meso-eutrophy	9 to 12
bad quality	eutrophy	<9

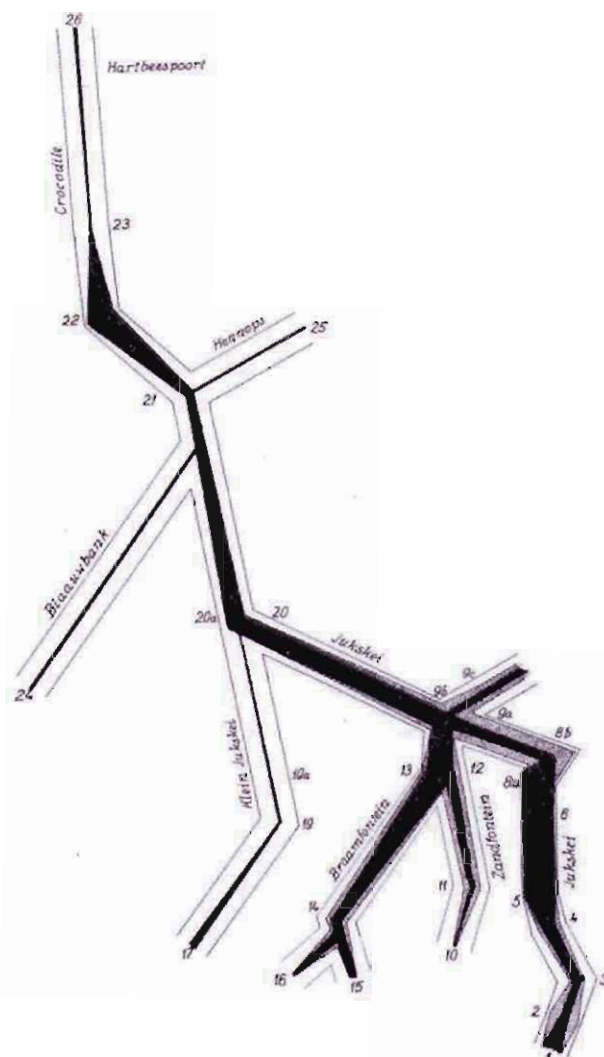


Figure 2
Abundance of the pollution tolerant diatom species *Nitzschia palea* (black) and *Sellaphora (Navicula) seminulum* (grey) in the Jukskei-Crocodile River system (Cholnoky, 1968)

acronym for this species or any of its synonyms in OMNIDIA v3.1. However, *C. bengalensis* was present in only two samples (5 and 7 individuals respectively), and its absence from the index calculation is not considered to exert an influence on the final score in any way. Prygiel (2003) cautions that when dominant species are not included in the index calculation then one runs the risk of incorrect assessments; however, this does not hold true for sub-dominant species.

It is interesting to compare the diatom-index data with the diagram that Cholnoky drew of the Jukskei-Crocodile River system, based on his diatom analysis at the same sampling stations he used in 1956/7 (see Fig. 2). Cholnoky constructed the diagram based on the relative abundance of two diatom species, *Nitzschia palea* and *Sellaphora (Navicula) seminulum*, both species known for their tolerance to organic pollution. It is evident from a comparison of Fig. 2 with Table 2 that the sampling stations on Cholnoky's diagram having the lowest percentage of *N. palea* and *S. seminulum* in the communities (hence higher quality water) have the highest scores generated from modern diatom indices. Cholnoky's diagram also agrees with the calculated percentage of pollution tolerant diatoms (%PT) using the TDI index of Kelly and Whitton (1995; Table 2).

It can also be deduced from Table 2 that some of the sampling stations have a diatom-index score that is representative of pristine water quality. The authors of this report consider this to be an erroneous assessment. If the samples classified as pristine (diatom index score >17; Table 2) are related to the abundance sheets it can be noted that all these sites have high abundance of *Achnanthes minutissimum* (*Achnanthes minutissima*). At several of the sites with index scores indicating pristine conditions, there is a high abundance of *Gomphonema parvulum*. *G. parvulum* is known to be tolerant to several forms of pollution and indicates disturbed conditions; Cholnoky was later to add *G. parvulum* to his list of pollution tolerant species (Cholnoky, 1970). The occurrence of *G. parvulum* in a community dominated by *A. minutissimum* alerts one to the fact that there is at least moderate pollution at the site. From a re-examination of the original material it can be seen that although *A. minutissimum* composed some portion of the diatom community, the additional portion of the diatoms recorded as *A. minutissimum* is in fact *A. saprophilum* (*Achnanthes minutissima* var. *saprophila*). This species or variety cannot have been noted as separate to *A. minutissimum* in 1956 or 1957 as it was only described in 1982 (Kobayasi and Mayama, 1982) from severely polluted rivers in the vicinity of Tokyo. The valve morphology of this taxon closely resembles *A. minutissimum*. *A. saprophilum* has a very high tolerance to organic pollution and often occurs as the dominant taxon even in poly-saprobic water. If the TEM illustrations of *A. minutissimum* from Pretoria salt pan found in the work of Schoeman and Ashton (1982) are compared with those presented by Mayama and Kobayasi (1989), it can be seen that several of the photographs depicting *A. minutissimum* are undoubtedly *A. saprophilum* (see Fig. 3). The similarity in outline, pore structure and arrangement of striae should be noted in this figure.

The error of identification between the two species is very easy to remedy, in the samples where it is suspected that a portion of the count of *A. minutissimum* could be *A. saprophilum*, the original slide needs to be examined and the ratio between these two species calculated. Once the diatom analysis has been corrected in this way, the data can once more be used in accurate historical ecological assessments. It is important to do this when diatom indices are being used for assessment as the two species have different tolerance values in the diatom index equation. On re-counting the abundance of *A. minutissimum*, it was found that a percentage of the diatom valves was in fact *A. saprophilum*, the resultant relative abundance was re-calculated and when used in the diatom index calculation, lowered the index score by several points and in some cases transferred the sample to a lower water quality class (Table 1) as demonstrated in Table 3.

Of the specific indices, the EPI shows that most of the sites are eutrophic, with several falling into the meso-eutrophic class and others which could be classified as mesotrophic; no sites

TABLE 2 Diatom index scores generated from diatom analysis sheets for the Jukskei-Crocodile River system in 1956/57 (Authored by Dr BJ Choinoky)															
Sample number	Population	SPI	DES	LMI	SHE	ROT	CEC	APDI	BDI	GDI	SLA	WAT	EPI	TDI	%PT
JK 1 STA 8A	565	1.2	1.1	5.8	1.3	6.7	1.6	6.6	6.7	1.3	7.7	1.7	7.5	75.6	96.5
JK 2 STA 8A	604	1.3	1.2	5.9	1.6	5.5	1.8	6.8	6.5	1.5	7.9	2.3	7.0	75.7	95.2
JK3 STA 8A	323	2.0	1.5	6.0	3.2	5.5	1.6	7.8	6.5	2.5	7.9	4.5	7.5	75.6	84.2
JK 5 STA 6	288	5.0	3.6	7.4	4.5	9.7	4.8	8.2	9.9	9.0	9.2	10.5	7.1	93.5	83.0
JK 6 STA 6	549	1.7	1.2	6.2	1.6	7.0	1.8	7.9	6.9	2.0	8.2	2.9	6.7	75.0	89.6
JK 7 STA 6	387	11.1	8.5	10.5	8.9	11.2	9.6	12.0	14.0	11.8	11.5	13.2	10.6	64.3	31.5
JK 8 STA 5	538	1.5	1.2	5.8	2.3	4.1	1.6	4.9	6.5	1.8	8.0	2.6	7.7	75.4	91.1
JK 9 STA 4	562	1.5	1.4	5.8	2.0	6.0	2.1	6.3	6.2	2.0	7.8	2.8	7.2	75.2	92.7
JK10	471	2.7	2.2	6.5	4.2	6.1	2.7	7.1	7.3	3.8	8.8	5.9	7.7	78.7	79.8
JK 11	471	2.1	1.7	6.0	3.9	5.1	2.0	7.5	6.5	2.7	8.1	4.9	7.7	77.4	83.9
JK 12	532	1.8	1.4	5.8	3.2	4.3	1.8	7.6	6.2	2.3	7.7	4.1	7.6	76.1	89.8
JK 13 STA 20	507	12.3	6.7	6.8	13.0	13.9	7.7	5.7	11.0	16.5	12.8	5.8	9.6	92.1	25.2
JK 14 STA 20 A	509	4.3	3.7	7.6	6.1	9.6	6.3	5.4	11.3	3.8	10.9	7.9	8.1	70.2	56.2
JK 15	532	11.2	14.8	10.5	17.8	18.6	18.3	9.6	8.8	14.3	11.7	19.8	8.8	10.4	0.4
JK 16	528	12.9	14.5	10.5	18.1	18.4	17.2	9.5	13.9	14.3	12.1	19.8	9.8	9.4	0.2
JK 17	562	16.9	15.4	14.0	18.1	18.0	18.3	10.4	14.6	18.4	15.3	18.8	12.6	5.0	2.0
JK 18	523	11.4	13.5	10.6	16.5	16.8	16.8	9.6	9.3	14.2	11.8	19.6	9.1	26.4	0.8
JK 19	533	11.8	13.7	10.5	18.4	18.9	17.2	9.5	10.3	14.4	11.9	19.6	9.1	12.1	1.3
JK 101 STA 1	265	4.9	5.7	8.3	8.0	11.9	6.1	9.4	11.6	3.1	10.9	10.5	12.0	77.2	54.7
JK 102 STA 2	282	2.9	4.9	6.4	4.8	6.6	5.2	2.2	10.1	7.9	12.5	4.6	11.7	94.4	50.0
JK 103 STA 3	247	6.8	9.9	7.2	5.4	7.8	5.9	5.4	10.9	9.2	9.3	8.9	10.6	93.3	62.3
JK 103B STA 3	268	5.1	7.1	8.1	6.4	8.8	4.2	5.2	9.7	7.8	12.1	9.2	8.8	87.5	48.1
JK 104 STA 4	325	2.9	4.0	6.5	4.2	7.4	3.9	4.8	7.9	6.9	10.4	8.1	8.8	90.9	79.7
JK 105 STA 5	349	5.7	6.2	6.2	5.4	5.7	4.4	5.4	9.7	11.5	8.2	10.7	9.2	95.1	88.0
JK 106 STA 8A	347	6.4	9.1	8.9	10.8	8.9	4.2	8.6	8.7	10.4	12.9	11.5	8.0	83.2	46.4
JK 106B STA 8B	258	4.5	6.7	6.7	7.7	9.5	4.2	7.5	9.3	6.5	12.0	10.8	9.9	85.1	65.1
JK 107A STA 9A	318	4.1	5.5	6.0	2.9	8.4	4.8	5.0	9.1	9.3	10.9	14.0	9.5	97.5	78.6
JK 107B STA 9B	265	4.0	7.0	7.6	7.3	9.3	5.2	6.6	9.3	9.5	11.4	9.8	8.9	93.4	70.2
JK 107C STA 9C	287	5.0	8.2	8.0	7.0	9.2	5.4	5.5	10.4	8.0	12.1	8.8	9.2	90.0	36.6
JK 108 STA 10	301	18.5	14.3	14.3	15.3	15.2	18.7	5.8	17.0	13.4	14.7	20.0	12.5	100.0	10.3
JK 109A STA 11	349	4.7	4.5	7.6	6.4	7.1	3.3	2.3	7.3	8.9	10.2	11.0	8.3	95.5	25.5
JK 109B STA 11	338	3.9	4.7	6.7	5.1	6.8	4.6	4.8	8.0	8.5	9.8	10.5	9.2	94.7	64.2
JK 109C STA 11	313	4.2	5.8	7.3	6.4	6.7	3.7	2.6	7.5	9.1	10.2	10.3	9.1	95.3	26.2
JK 110A STA 12	157	6.1	6.8	8.5	8.6	9.9	5.8	7.7	9.6	9.8	11.1	9.6	9.5	87.7	55.4
JK 110B STA 12	310	12.9	14.7	14.6	13.4	14.0	12.0	14.2	12.3	12.2	14.5	11.0	8.8	55.2	6.8
JK 111 STA 13	299	2.6	3.2	6.3	3.2	11.3	4.4	8.1	8.5	3.3	9.6	6.2	10.7	82.4	81.6
JK 112 STA 14	272	4.0	6.2	6.6	5.8	7.7	7.1	5.6	10.1	8.4	10.1	9.7	10.3	93.8	72.8
JK 113 STA 15	351	12.0	10.6	12.1	13.7	15.2	16.2	8.9	16.1	1.8	13.7	16.4	12.8	78.7	20.2
JK 114 STA 16	263	10.4	11.8	11.1	11.5	11.7	11.1	7.8	12.8	9.8	12.3	16.4	10.3	87.5	31.2
JK 115 STA 20	262	3.8	4.8	7.3	5.4	8.6	3.5	4.6	7.9	8.8	12.3	8.1	9.1	91.7	87.4
JK 116 STA 20A	259	1.5	2.2	5.9	2.3	5.5	3.9	4.1	8.0	9.6	10.3	2.7	9.2	95.1	94.6
JK 117 STA 21	234	7.0	7.3	8.2	9.6	12.5	7.1	7.9	10.6	9.5	11.8	10.8	11.2	75.3	43.6
JK 119 STA 8B	347	3.3	2.9	6.7	4.5	5.5	2.9	1.8	10.7	7.5	12.9	2.4	8.0	93.6	24.2
JK 120 STA 10	345	18.4	14.3	14.4	15.6	15.2	13.5	5.8	17.3	10.8	14.8	19.8	12.6	98.3	8.1
JK 122 STA 9A	267	2.8	2.1	6.0	2.9	5.2	4.6	1.7	9.7	7.2	12.5	3.9	9.3	92.7	43.4
JK 123 STA 17	297	14.0	13.5	12.3	14.9	15.3	16.2	12.4	13.3	8.6	13.8	15.7	11.6	46.6	6.1
JK 124 STA 19A	367	17.5	15.9	14.0	15.9	16.2	17.3	7.6	16.8	13.7	14.6	18.8	12.2	45.4	6.3
JK 125 STA 20	309	10.3	14.0	10.1	14.3	11.8	11.5	7.2	13.0	11.4	10.7	12.3	8.1	64.2	13.6
JK 126 STA 2	280	6.1	7.0	6.5	3.2	6.8	5.9	4.6	9.5	12.3	11.3	16.1	9.5	98.7	70.0
JK 127 STA 3	313	4.2	2.6	7.2	3.9	9.6	4.4	9.4	9.5	6.9	8.4	7.9	7.1	89.6	87.5
JK 128 STA 4	164	6.7	9.4	8.4	9.6	8.1	6.3	5.9	9.7	7.0	10.3	12.9	8.0	95.1	48.2
JK 129 STA 5	281	1.6	1.5	6.1	2.3	7.9	3.5	8.8	7.3	2.6	8.0	3.3	7.3	79.4	93.6
JK 130 STA 6	347	4.4	2.9	6.7	9.2	9.0	1.8	5.9	6.9	2.0	8.5	7.9	7.0	80.1	36.6
JK 131 STA 8B	347	3.2	2.9	6.5	4.5	7.5	3.9	7.4	7.8	5.5	8.5	7.8	8.0	87.1	82.1
JK 132 STA 12	301	6.0	13.1	8.9	12.7	10.5	4.4	5.9	7.2	2.1	9.3	10.6	6.8	85.7	17.3

TABLE 2 (continued)															
Sample number	Population	SPI	DES	LMI	SHE	ROT	CEC	APDI	BDI	GDI	SLA	WAT	EPI	TDI	%PT
JK 133 STA 16	308	10.8	13.5	10.9	11.8	12.8	11.1	8.9	10.7	9.3	12.3	13.7	8.9	63.7	18.5
JK 134 STA 17	313	15.1	14.1	10.9	16.2	14.2	14.9	11.3	13.1	14.3	12.0	16.8	10.8	59.5	3.5
JK 135 STA 19	375	15.3	14.0	12.3	15.3	17.0	16.4	11.5	15.0	15.2	14.7	17.8	12.6	45.0	5.3
JK 136 STA 20A	300	2.0	2.0	6.2	1.6	7.0	3.5	6.3	6.2	4.1	8.3	6.8	7.7	85.6	92.3
JK 137 STA 21	423	17.6	15.0	14.7	17.5	16.9	17.7	8.0	16.1	16.8	15.3	18.9	12.7	9.9	0.5
JK 138 STA 22	344	3.3	2.0	6.7	4.5	7.0	4.6	6.1	5.7	3.7	8.0	6.2	7.6	75.4	89.0
JK 139 STA 23	351	12.3	14.4	12.3	11.8	10.2	12.4	7.1	14.2	11.0	11.4	15.6	10.7	82.4	2.8
JK 140 STA 24	338	14.5	14.4	13.0	15.3	15.4	15.6	12.9	14.3	12.4	13.6	16.5	11.9	55.8	7.1
JK 141 STA 24	328	16.3	13.7	12.5	15.9	15.7	17.0	13.9	15.5	15.1	13.8	16.6	11.5	43.2	3.4
JK 142 STA 26(1)	431	13.3	13.3	12.0	14.0	13.4	14.3	12.2	16.5	11.4	12.6	16.1	11.6	62.2	9.5
JK 143 STA 26(2)	363	16.0	17.5	13.5	18.4	13.2	17.0	14.4	15.4	12.3	14.1	7.9	12.5	89.5	0.3
JK 144 STA 26 (3)	407	14.6	15.1	12.9	14.3	13.1	14.3	11.5	16.3	12.7	13.1	17.8	12.1	54.6	4.4
JK 145 STA 1	341	15.3	12.7	13.3	15.3	15.0	18.1	5.8	16.9	15.8	14.2	17.9	12.9	77.7	10.9
JK 146 STA 2	404	3.2	2.7	5.9	3.2	5.0	5.0	1.7	10.4	8.7	12.6	4.2	9.4	96.7	35.4
JK 147 STA 3	300	3.3	3.1	6.4	4.5	6.3	3.7	4.3	8.1	5.8	9.3	6.6	7.0	89.3	67.0
JK 148 STA 4	163	4.4	4.6	7.1	6.1	8.6	5.0	5.6	8.3	6.3	10.2	9.3	9.6	90.1	61.3
JK 149 STA 5	204	3.4	3.2	6.5	5.4	6.2	3.5	7.5	7.4	5.0	8.5	7.5	8.0	82.7	71.6
JK 150 STA 6	361	7.0	3.5	8.1	6.7	9.2	4.2	9.5	10.8	11.3	11.2	10.5	6.6	89.6	59.6
JK 151 STA 8B	259	3.4	2.8	6.6	5.1	6.3	2.9	6.4	7.3	4.5	9.1	6.5	7.3	80.2	71.0
JK 152 STA 9A	623	1.2	1.0	5.8	1.3	4.8	1.6	1.8	7.0	1.7	8.6	1.3	8.3	77.7	91.3
JK 153 STA 9B	424	2.3	1.7	6.0	2.9	5.3	3.3	2.3	8.2	5.1	10.8	4.1	8.9	87.7	67.9

SPI; Specific Pollution Sensitivity Index, DES; Descy's Index, LMI; Leclercq & Maquet's Index, SHE; Schiefele and Schreiner's Index, ROT; Rott's Index, CEC; Council for European Communities Index, APDI; Artois-Picardie Diatom Index, BDI; Biological Diatom Index, GDI; Generic Diatom Index, SLA; Sládeček's Index, WAT; Watanabe's Index, EPI; Eutrophication/Pollution Index, TDI; Trophic Diatom Index, %PT; Percentage Pollution Tolerant Species

TABLE 3 Diatom index scores before and after reclassification of <i>Achnan-</i> <i>thidium minutissimum</i> and <i>A. saprophilum</i>					
Site	Index score with all species as <i>A. minutissimum</i>				
	SPI	SHE	BDI	WAT	ROT
JK 141 STA 24	16.3	15.9	15.5	16.6	15.7
JK 143 STA 26 (2)	16	18.4	15.4	7.9	13.2
JK 144 STA 26 (3)	14.6	14.3	16.3	17.8	13.1
Site	Index score after splitting of <i>A. minutissimum</i> and <i>A. saprophilum</i>				
	SPI	SHE	BDI	WAT	ROT
JK 141 STA 24	15.5	13.7	13.4	14.2	13.4
JK 143 STA 26 (2)	14.9	16.8	12	5.8	9.3
JK 144 STA 26 (3)	13.6	12.4	14.9	15.6	11.3

SPI; Specific Pollution Sensitivity Index, SHE; Schiefele and Schreiner's Index, BDI; Biological Diatom Index, WAT; Watanabe's Index, ROT; Rott's Index

warrant the classification of oligotrophic. The TDI is included for the %PT values as the index itself was developed for monitoring sewage outfall (orthophosphate-phosphorus concentrations) and not organic pollution or general stream quality (Kelly and Whitton, 1995). The index cannot be used accurately if the %PT values are above 20. The %PT values do however, demonstrate that for the most part the Jukskei-Crocodile system was

subject (as it still is) to high loading with organic pollutants. The GDI has a lower resolution being based only on the genus of the taxa composing the diatom communities. Although far simpler to use than indices that rely on species level identification it seems to yield comparable results in most cases.

The diatom index scores were correlated to the average water quality variables at 10 of the sites for which average annual data were available and the results are presented in Table 4.

It is interesting to note that the strongest correlations are between nitrogen and the diatom index scores, with no significant correlation to either orthophosphate-phosphorus or to total phosphate. This would suggest that the major impact in the system is from waste containing nitrogenous compounds. This finding is in agreement with Chloňok's assessment at the time, showing that at some sites almost all of the diatom species encountered were tolerant to organic pollution, which in turn may result in nutrient enrichment

(Dallas and Day, 2004). Other strong correlations exist between the diatom index scores and electrical conductivity and the major ions. This correlation between ionic compounds matches a descriptive assessment of the Jukskei-Crocodile system as being heavily impacted by industrial and agricultural runoff and effluents.

TABLE 4 Correlation between water quality variables at selected sites in the Jukskei-Crocodile system and diatom index scores generated from re-analysis of historical data sheets Marked correlations are significant at $p < 0.05$ $n = 10$ (Casewise deletion of missing data)												
	SPI	DES	LMI	SHE	ROT	CEC	APDI	BDI	GDI	SLA	WAT	EPI
pH	0.67	0.64	0.77	..	0.64	0.74	..	0.81	..	0.64	0.77	0.77
EC	..	-0.68	..	..	-0.67	..	-0.86	..	-0.81	..	..	..
Temp.	0.68	0.64	0.69	..	0.73	0.76	..	0.72	0.35	0.86	0.69	0.81
COD	..	..	..	..	..	..	-0.89	..	-0.65	..	..	..
NH ₄ -N	-0.85	-0.86	-0.86	-0.79	-0.84	-0.82	-0.78	-0.85	..	-0.69	-0.86	-0.76
NO ₂ -N	-0.78	-0.82	-0.76	-0.74	-0.79	-0.74	-0.85	-0.74	..	..	-0.77	-0.64
NO ₃ -N	..	..	..	..	..	..	-0.76	..	-0.73	..	..	..
KJ-N	-0.82	-0.85	-0.82	-0.79	-0.83	-0.78	-0.87	-0.78	..	-0.65	-0.82	-0.68
PO ₄ -P	..	..	..	..	..	..	..	..	..	..	..	..
Total P	..	..	..	..	..	..	..	..	..	..	..	..
Na ⁺	..	-0.65	..	..	-0.64	..	-0.87	..	-0.76	..	..	..
K ⁺	..	..	..	..	..	..	..	..	-0.75	..	..	..
Ca ²⁺	..	..	..	..	..	..	-0.80	..	-0.86	..	..	..
Mg ²⁺	-0.74	-0.75	-0.66	-0.74	-0.79	-0.70	..	-0.67	..	-0.69	-0.68	..
SO ₄ ⁻	-0.77	-0.80	-0.73	-0.74	-0.80	-0.73	-0.90	-0.68	-0.71	-0.66	-0.74	..
Cl ⁻	-0.69	-0.73	-0.66	-0.67	-0.73	-0.66	-0.90	..	-0.74	..	-0.67	..
Variables were measured in mg.l ⁻¹ except for temperature (°C), electrical conductivity (μS.cm ⁻¹)												
SPI; Specific Pollution Sensitivity Index, DES; Descy's Index, LMI; Leclercq & Maquet's Index, SHE; Schiefele and Schreiner's Index, ROT; Rott's Index, APDI; Artois-Picardie Diatom Index, CEC; Council for European Communities Index, BDI; Biological Diatom Index, GDI; Generic Diatom Index, SLA; Sládeček's Index, WAT; Watanabe's Index, EPI; Eutrophication/Pollution Index												

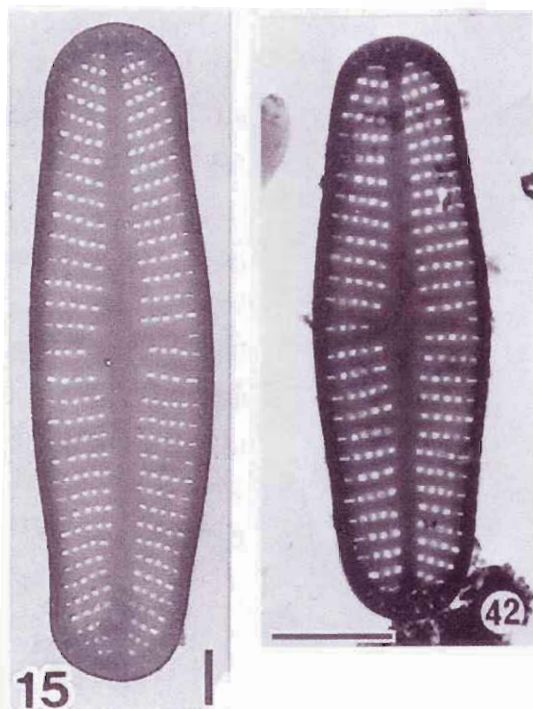


Figure 3

15; *Achnanthes saprophilum* (Mayama and Kobayasi, 1989), scale bar = 1 μm. 42; *Achnanthes minutissimum* (Schoeman and Ashton, 1982), scale bar = 2 μm

Conclusions

In general it can be concluded from the preceding sections that the diatom analysis sheets authored by Dr Chloňoký constitute a valuable resource from which accurate inferences may be drawn concerning the past ecological status of the rivers and streams for which data exist in the SA Diatom Collection dating from the mid 1950s. The classification of the various sampling stations carried out by Chloňoký yields similar results to those gained by using modern diatom indices. The use of diatom indices relies on information stored in a database rather than the operator's own knowledge, and thus provides a relatively rapid and more efficient assessment technique when compared to those employed by Chloňoký half a century ago.

The diatom analysis sheets contained in the SA Diatom Collection are likely to prove to be a valuable resource for obtaining historical data against which present-day and/or future environmental assessments may be compared, and provide a measure of either degradation or restoration since the time of original sampling. OMNIDIA proves to be both useful as a database and as a tool for calculating diatom index scores.

It has been demonstrated that the species listed on the diatom analysis sheets can be related to current nomenclature (synonyms; see Appendix 1) when necessary, and entered into the electronic database OMNIDIA. The diatom analysis sheets provide enough data for the calculation of accurate diatom index scores. Results generated from diatom analysis sheets with a population count of less than 300 should be regarded with caution.

Besides the difficulties encountered caused by the identification of *Achnanthes minutissimum*, the historical species data sheets contained in the SA Diatom Collection are of a quality sufficient to allow for the generation of accurate, high-confidence results that support the formulation of ecologically-based inferences on past ecosystem condition.

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APPENDIX 1	
Species list and updated nomenclature	
Taxon	
<i>Achnanthes amoena</i> Hustedt	
<i>Achnantheidium exiguum</i> (Grun.) Czarn.	
<i>Achnanthes exigua</i> Grun. in Cleve & Grun.	
<i>Achnantheidium exiguum</i> var. <i>heterovalva</i> (Krasske) Czarn.	
<i>Achnanthes exigua</i> var. <i>heterovalva</i> Krasske	
<i>Achnantheidium minutissimum</i> (Kütz.) Czarn.	
<i>Achnanthes minutissima</i> Kütz.	
<i>Achnantheidium microcephalum</i> (Kütz.) vide Rabenh.	
<i>Achnanthes microcephala</i> (Kütz.) Grun.	
<i>Amphipleura pellucida</i> Kütz.	
<i>Amphora coffeaeformis</i> (Agardh) Kütz.	
<i>Amphora montana</i> Krasske	
<i>Amphora submontana</i> Hustedt	
<i>Amphora ovalis</i> (Kütz.) Kütz.	
<i>Amphora pediculus</i> (Kütz.) Grun.	
<i>Amphora ovalis</i> var. <i>pediculus</i> (Kütz.) Van Heurck	
<i>Amphora veneta</i> Kütz.	
<i>Anomoeoneis sphaerophora</i> (Ehr.) Pfitzer	
<i>Aulacoseira granulata</i> (Ehr.) Simonsen	
<i>Melosira granulata</i> (Ehr.) Ralfs	
<i>Aulacoseira granulata</i> var. <i>angustissima</i> (O. Müll.) Simonsen	
<i>Melosira granulata</i> var. <i>angustissima</i> O. Müll.	
<i>Aulacoseira italica</i> (Ehr.) Simonsen	
<i>Melosira italica</i> (Ehr.) Kütz.	
<i>Brachysira vitrea</i> (Grunow) Ross in Hartley	
<i>Anomoeoneis exilis</i> (Kütz.) Cleve	
<i>Caloneis aequatorialis</i> Hustedt	
<i>Caloneis bacillum</i> (Grun.) Cleve	
<i>Caloneis schumanniana</i> var. <i>biconstricta</i> (Grun.) Reichert	
<i>Caloneis silicula</i> (Ehr.) Cleve	
<i>Caloneis ventricosa</i> (Ehr. Donkin) Meister	
<i>Cocconeis pediculus</i> Ehr.	
<i>Cocconeis placentula</i> Ehr.	
<i>Craticula ambigua</i> (Ehr.) Mann in Round, Crawford & Mann	
<i>Navicula cuspidata</i> var. <i>ambigua</i> (Ehr.) Cleve	
<i>Craticula cuspidata</i> (Kütz.) Mann in Round, Crawford & Mann	
<i>Navicula cuspidata</i> Kütz.	
<i>Cyclotella meneghiniana</i> Kütz.	
<i>Cyclotella operculata</i> (Agardh) Kütz.	
<i>Cyclotella stelligera</i> Cleve et Grun. in Van Heurck	
<i>Cymatopleura solea</i> (Bréb.) W. Smith	
<i>Cymatopleura librile</i> (Ehrenberg) Pantocsek	
<i>Cymbella amphicephala</i> Naegeli	
<i>Cymbella amphicephala</i> var. <i>hercynica</i> (A. Schmidt) Cleve	
<i>Cymbella aspera</i> (Ehr.) Cleve	
<i>Cymbella begalensis</i> Cleve	
<i>Cymbella cistula</i> (Ehr.) Kirchner	
<i>Cymbella kappii</i> Cholnoky	
<i>Cymbella kolbei</i> Hustedt	
<i>Cymbella turgida</i> Gregory	
<i>Diademesmis confervacea</i> Kütz.	
<i>Navicula confervaceae</i> (Kütz.) Grun.	
<i>Diademesmis contenta</i> var. <i>biceps</i> (Grun. ex V. Heurck) Mann in Round, Crawford & Mann	
<i>Navicula contenta</i> Grun.	
<i>Diploneis ovalis</i> (Hilse) Cleve	
<i>Diploneis smithii</i> var. <i>pumila</i> (Grun.) Hustedt	

<i>Diploneis subovalis</i> Cleve
<i>Encyonema minutum</i> (Hilse in Rabenhorst) Mann in Round, Crawford & Mann
<i>Cymbella minuta</i> Hilse ex Rabenhorst
<i>Cymbella ventricosa</i> Kütz.
<i>Encyonema muelleri</i> (Hustedt) Mann in Round, Crawford & Mann
<i>Cymbella muelleri</i> Hustedt
<i>Encyonopsis aequalis</i> (W. Smith) Krammer
<i>Cymbella aequalis</i> W. Smith
<i>Encyonopsis microcephala</i> (Grun.) Krammer
<i>Cymbella microcephala</i> Grun.
<i>Eolimna minima</i> (Grun.) Lange-Bertalot
<i>Navicula minima</i> Grun.
<i>Eolimna subminuscule</i> (Manguin) Lange-Bertalot & Metzeltin
<i>Navicula subminuscule</i> Manguin
<i>Navicula frugalis</i> Hustedt
<i>Navicula perparva</i> Hustedt
<i>Fallacia pygmaea</i> (Kütz.) Stickle & Mann in Round, Crawford & Mann
<i>Navicula pygmaea</i> Kütz.
<i>Fragilaria capucina</i> Desmazieres
<i>Synedra rumpens</i> Kütz.
<i>Fragilaria capucina</i> var. <i>acuta</i> (Ehr.) Rabenhorst
<i>Fragilaria capucina</i> var. <i>vaucheriae</i> (Kütz.) Lange-Bertalot
<i>Synedra vaucheriae</i> Kütz.
<i>Fragilaria construens</i> (Ehr.) Grun.
<i>Fragilaria delicatissima</i> (W. Smith) Lange-Bertalot
<i>Synedra acus</i> var. <i>radians</i> (Kütz.) Hustedt
<i>Frustulia rhomboides</i> (Ehr.) De Toni
<i>Frustulia vulgaris</i> (Thwaites) De Toni
<i>Geissleria decussis</i> (Østrup) Lange-Bertalot & Metzeltin
<i>Navicula decussis</i> Østrup
<i>Navicula canoris</i> Hohn & Hellerman
<i>Navicula exiguiiformis</i> Hustedt
<i>Gomphonema clavatum</i> Ehr.
<i>Gomphonema clevei</i> Fricke
<i>Gomphonema gracile</i> var. <i>subcapitata</i> Gandhi
<i>Gomphonema gracile</i> Ehr.
<i>Gomphonema gracile</i> var. <i>lanceolata</i> (Kütz.) Cleve
<i>Gomphonema parvulum</i> Kütz.
<i>Gomphonema pumilum</i> (Grun.) Reichardt & Lange-Bertalot
<i>Gomphonema intricatum</i> var. <i>pumila</i> Grun. in V. Heurck
<i>Gomphonema schweickerdtii</i> Cholnoky
<i>Gomphonema truncatum</i> Ehr.
<i>Gomphonema truncatum</i> var. <i>capitatum</i> (Ehr.) Patrick
<i>Gyrosigma nodiferum</i> (Grun.) Reimer
<i>Gyrosigma spencerii</i> var. <i>nodifera</i> (Grun.) Cleve
<i>Gyrosigma scalproides</i> (Rabenhorst) Cleve
<i>Gyrosigma spencerii</i> (Quekett) Griffith
<i>Hantzschia amphioxys</i> (Ehr.) Grun. in Cleve & Grun.
<i>Hantzschia amphioxys</i> var. <i>africana</i> Hustedt
<i>Hippodonta capitata</i> (Ehr.) Lange-Bertalot & Metzeltin in Witkowski
<i>Navicula capitata</i> Ehr.
<i>Hippodonta hungarica</i> (Grun.) Lange-Bertalot & Metzeltin in Witkowski
<i>Navicula hungarica</i> Grun.
<i>Kobayasiella subtilissima</i> (Cleve) Lange-Bertalot
<i>Navicula subtilissima</i> Cleve
<i>Lemnicola hungarica</i> (Grun.) Round & Basson
<i>Achnanthes hungarica</i> Grun. in Cleve & Grun.
<i>Luticola mutica</i> (Kütz.) Mann in Round, Crawford & Mann
<i>Navicula mutica</i> Kütz.

<i>Luticola nivalis</i> (Ehr.) Mann in Round, Crawford & Mann
<i>Navicula mutica</i> var. <i>nivalis</i> (Ehr.) Hustedt
<i>Mayamaea atomus</i> var. <i>permitis</i> (Hustedt) Lange-Bertalot
<i>Navicula atomus</i> var. <i>permitis</i> (Hustedt) Lange-Bertalot
<i>Navicula muralis</i> Grun.
<i>Melosira varians</i> Agardh
<i>Navicula bryophila</i> Boye Petersen
<i>Navicula capitatoradiata</i> Germain
<i>Navicula cryptocephala</i> var. <i>intermedia</i> Grun.
<i>Navicula cincta</i> (Ehr.) Ralfs in Pritchard
<i>Navicula cryptocephala</i> Kütz.
<i>Navicula cryptotenella</i> Lange-Bertalot
<i>Navicula radiosa</i> var. <i>tenella</i> (Bréb.) Cleve & Möll.
<i>Navicula gregaria</i> Donkin
<i>Navicula kotschyi</i> Grun.
<i>Navicula grimmei</i> Krasske
<i>Navicula lanceolata</i> (Agardh) Ehr.
<i>Navicula viridula</i> var. <i>avenacea</i> (Brébison in Grun.) V.Heurk
<i>Navicula menisculus</i> Schumann
<i>Navicula menisculus</i> var. <i>upsaliensis</i> Grun.
<i>Navicula minusculoides</i> Hustedt
<i>Navicula muticoides</i> Hustedt
<i>Navicula radiosa</i> Kütz.
<i>Navicula rhynchocephala</i> Kütz.
<i>Navicula rostellata</i> Kütz.
<i>Navicula schroeteri</i> Meister
<i>Navicula tenelloides</i> Hustedt
<i>Navicula zannoni</i> Hustedt
<i>Neidium affine</i> (Ehrenberg) Pfitzer
<i>Nitzschia acicularis</i> (Kütz.) W.M.Smith
<i>Nitzschia acidoclinata</i> Lange-Bertalot
<i>Nitzschia perminuta</i> (Grun.) M. Peragallo
<i>Nitzschia amphibia</i> Grun.
<i>Nitzschia capitellata</i> Hustedt in A.Schmidt et al.
<i>Nitzschia allanssoni</i> Cholnoky
<i>Nitzschia clausii</i> Hantzsch
<i>Nitzschia communis</i> Rabenhorst
<i>Nitzschia debilis</i> (Arnott) Grun.
<i>Nitzschia denticula</i> Grun.
<i>Nitzschia desertorum</i> Hustedt
<i>Nitzschia dissipata</i> (Kütz.) Grun.
<i>Nitzschia elliptica</i> Hustedt
<i>Nitzschia epiphytica</i> O.Müll. sensu Hustedt 1949
<i>Nitzschia fonticola</i> Grun. in Cleve & Möll.
<i>Nitzschia frustulum</i> (Kütz.) Grun.
<i>Nitzschia frustulum</i> var. <i>perpusilla</i>
<i>Nitzschia intermedia</i> Hantzsch in Cleve
<i>Nitzschia linearis</i> (Agardh) W.M.Smith
<i>Nitzschia microcephala</i> Grun. in Cleve

<i>Nitzschia nana</i> Grun. in V.Heurk
<i>Nitzschia ignorata</i> Krasske
<i>Nitzschia palea</i> (Kütz.) W.Smith
<i>Nitzschia paleacea</i> (Grun.) Grun. in V.Heurk
<i>Nitzschia bacata</i> Hustedt
<i>Nitzschia parvuloides</i> Cholnoky
<i>Nitzschia pusilla</i> (Kütz.) Grun.
<i>Nitzschia kuetzingiana</i> Hilse
<i>Nitzschia sigma</i> (Kütz.) W.M.Smith
<i>Nitzschia sinuata</i> var. <i>tabellaria</i> (Grun.) Grun.
<i>Nitzschia solgensis</i> Cleve-Euler
<i>Nitzschia interupta</i> (Reichelt) Hustedt
<i>Nitzschia tropica</i> Hustedt
<i>Nitzschia umbonata</i> (Ehr.) Lange-Bertalot
<i>Nitzschia thermalis</i> (Kütz.) Auerswald
<i>Pinnularia eburnea</i> (Carlson) Zanon
<i>Pinnularia gibba</i> Ehr.
<i>Pinnularia gibba</i> var. <i>sancta</i> (Grun.) Meister
<i>Pinnularia interrupta</i> W.M.Smith
<i>Pinnularia viridis</i> (Nitzsch) Ehr.
<i>Placoneis dicephala</i> (W.Smith) Mereschkowsky
<i>Navicula dicephala</i> (Ehr.) W.Smith
<i>Navicula dicephala</i> var. <i>neglecta</i> (Krasske) Hustedt
<i>Planothidium lanceolatum</i> (Bréb.) Round & Bukhtiyarova
<i>Achnanthes lanceolata</i> (Bréb.) Grun.
<i>Rhopalodia gibba</i> (Ehr.) O.Müll.
<i>Rhopalodia gibberula</i> (Ehr.) O.Müll.
<i>Sellaphora pupula</i> (Kütz.) Mereschkowsky
<i>Navicula pupula</i> Kütz.
<i>Navicula nyassensis</i> O.Müll.
<i>Sellaphora seminulum</i> (Grun.) Mann
<i>Navicula seminulum</i> Grun.
<i>Stauroneis anceps</i> Ehr.
<i>Stauroneis construens</i> var. <i>venter</i> (Ehr.) Hamilton
<i>Fragilaria constuens</i> f. <i>venter</i> (Ehr.) Hustedt
<i>Stauroneis pinnata</i> (Ehr.) Williams & Round
<i>Fragilaria pinnata</i> Ehr.
<i>Stephanodiscus hantzschii</i> Grun. in Cleve
<i>Surirella angusta</i> Kütz.
<i>Surirella ovalis</i> Bréb.
<i>Surirella tenera</i> Gregory
<i>Synedra ulna</i> (Nitzsch) Ehr.
<i>Tabellaria fenestrata</i> (Lyngbye) Kütz.
<i>Tryblionella apiculata</i> Gregory
<i>Nitzschia apiculata</i> (Gregory) Grun.
<i>Tryblionella hungarica</i> (Grun.) Mann in Round, Crawford & Mann
<i>Nitzschia hungarica</i> Grun.
<i>Tryblionella levidensis</i> W. Smith
<i>Nitzschia levidensis</i> (W.Smith) Grun. in V. Heurk
<i>Tryblionella victorinae</i> Grun.
<i>Nitzschia levidensis</i> var. <i>victorinae</i> Grun.

CHAPTER 7

**The assessment of diffuse pollution from acid-mine drainage
using an updated and revised diatom assessment procedure
as an added-value bio-monitoring tool**

INSTRUCTIONS FOR AUTHORS

Preparation of typescripts for selected proceedings to be published in *Water Science and Technology*, *Water Science and Technology: Water Supply*, and *Water Environment Management Series*

These notes are to help authors prepare papers which may be selected for publication in *Water Science and Technology*, *Water Science and Technology: Water Supply*, and *Water Environment Management Series (WEMS)*. To achieve rapid publication papers are reproduced from a disk version of the article (see §2). However, it is essential that a typescript is submitted in case there are any problems in reading the disk. The typescript and the version on disk must be identical. It is your responsibility as author to follow the instructions below.

In the case of WEMS publication, the conference organizer is responsible for any publication issues, but the template provided by IWA-Publishing must be applied. The instructions below may be used as guidelines.

In any other case, IWA Publishing must receive the electronic version and the original typescript together with the original figures (the paper cannot be reproduced from a photocopy). Please note that your figures may be reduced to approximately **75%** of their original size during reproduction, and it is important to ensure that lettering on diagrams will remain legible after this reduction.

1. Authors' responsibilities

- 1.1 Corrections cannot be undertaken by either the editor or the publishers, and **no proofs are provided**. Thus if your paper is not prepared in accordance with these instructions it may be omitted from the proceedings.
- 1.2 Papers should be in good, grammatically correct English. If English is not your native language you should seek the help of a colleague or professional translator.
- 1.3 Have your typescript read by at least two other persons to avoid typing errors.
- 1.4 If your paper is selected for publication in *Water Science and Technology* you will be expected to sign our copyright transfer form. Where necessary you must obtain permission to publish material and for using any other material protected by copyright.
- 1.5 **Papers accepted for publication by IWA Publishing must not have been published, submitted for publication elsewhere or offered for presentation at conferences without the consent of IWA Publishing.**

2. Preparation of electronic versions

Articles should be provided in electronic form (on disk or by e-mail). This will help us to achieve more rapid publication and fewer errors, and enable your article's inclusion in electronic archives. Although we can cope with most formats, the following basic instructions should be kept in mind.

- 2.1. **Text files.** We can accept most of the popular word-processing packages in PC or Macintosh formats; however, MS Word is recommended. Authors should not add their own macros. Please supply only the final version of your file (with no hidden text) so as to avoid any risk of old versions of the text being used in error.

The main body text should be typed flush left with no indents, single line space using the wrap-around feature. Insert one return between paragraphs, and a double return between paper title, authors' names, and addresses on the first page.

- 2.2 **Graphic files.** Illustrations should be produced using the following software packages: Adobe Illustrator, Aldus Freehand, Cricket Graph, Macdraw, Chemdraw, Corel Draw for PC. However, we can accept any of the popular drawing programs for the Macintosh or PC. Please supply TIFF or EPS files separately from the Word file (i.e. *not* embedded).

Artwork should be drawn and lettered for finished size and subsequent reduction to 75% using a Times or Helvetica typeface giving a final size after reduction of 8 point type with appropriate line weights.

Scanned artwork should be saved to TIFF format for both line and halftone, and scanned at a suggested setting of 300 dpi for halftones and 800 dpi for line work. If it is necessary to compress the scans please indicate the software used. It is essential that a hard copy print of the scans be included. Illustrations should be logically named and saved as individual files to 3.5" disk, ZIP disk, or CD.

2.3. Please label all disks with:

- the operating system;
- the word-processing package (including version number);
- the filename of the article;
- the short title of the article and the authors' names.

When naming files it is strongly recommended that you incorporate the name of the first author or the paper's reference number into the filename. Please do **not** use names such as "IWApaper", "confpaper2001" or similar - we get so many variants of these names that it is difficult to keep track of them!

2.4. Please ensure that the typescript and disk versions of your paper are identical in content. In the event of differences between disk and hard copy, the hard copy will be considered as the definitive version.

3. Preparation of the typescript

- 3.1 Papers should be typed or laser printed on standard A4 metric or 11" × 8.5" white paper sheets. In either case, text should be typed to occupy an area of **250mm depth × 170mm width**.
- 3.2 Text should be typed **single-spaced** on one side of the paper only. Do not exceed the dimensions given above. Please use a Times 12 pt justified typeface.
- 3.3 The title of the paper, author's name(s), affiliation(s), author's full postal address(es) and e-mail, abstract and keywords should be set out following the example on p4 of these notes.
- 3.4 If any figures or tables are not already fixed in their correct position in the text insert a brief note specifying which figure should be placed there.
- 3.5 Write name(s) of author(s) and page number **lightly in blue pencil** at the top right-hand corner of each page.
- 3.6 **The conference organisers will inform you of the maximum acceptable length of article (normally 6 or 8 pages): please do not exceed this limit or your paper may be rejected.**

4. Content

Papers should be well structured: i.e. they must comprise:

- (1) **Title**, author name(s), full postal and e-mail addresses for each author.
- (2) **Abstract**: no more than 200 words briefly specifying the aims of the work, the main results obtained, and the conclusions drawn.
- (3) **Keywords**: 3–6 keywords (in alphabetical order) which will enable a subsequent information retrieval system to locate the paper.
- (4) **Main text**: for clarity this should be subdivided into:
 - (i) *Introduction* - describing the background of the work and its aims.
 - (ii) *Methods* - a brief description of the methods/techniques used (the principles of these methods should not be described if readers can be directed to easily accessible references or standard texts).
 - (iii) *Results and Discussion* - a clear presentation of experimental results obtained, highlighting any trends or points of interest.

Do not number or letter section headings.

- (5) **Conclusions**: a brief explanation of the significance and implications of the work reported.
- (6) **References**: these should be to accessible sources. Please ensure that all work cited in the text is included in the reference list, and that the dates and authors given in the text match those in the reference list. References must always be given in sufficient detail for the reader to locate the work cited (see below for formats).

5. Nomenclature and Units

- 5.1 Please take care that all terminology and notation used will be widely understood. Abbreviations and acronyms should be spelled out in full at their first occurrence in the text. In describing wastewater treatment processes authors should consult "Notation for Use in the Description of Wastewater Treatment Processes". *Water Research* **21**, 135-139 (1987).

- 5.2 SI units are strongly recommended. If non-SI units must be used, SI equivalents (or conversion factors) must also be given.
- 5.3 Write equations in dimensionless form or in metric units.

6. Figures and Tables

- 6.1 Figures and tables should appear in numerical order, be described in the body of the text and be positioned close to where they are first cited.
- 6.2 If possible supply figure file(s) separate from the text file.
- 6.3 Make sure all figures and tables will fit inside the text area.
- 6.4 Original line drawings are acceptable but glossy prints are preferred (for quality of reproduction). Avoid using shading in figures. Photographs should be in black and white, and should be sharp and well contrasted. **Please supply originals, as photocopies will not reproduce and graphic files may not provide adequate resolution for printing.**
- 6.5 Because figures may be resized in the course of production, scale bars, not magnification values, should be used where applicable.

7. References: citations in text

- 7.1 Use surname of author and year of publication: Jones (1982) or (Jones, 1982).
- 7.2 Insert initials only if there are two different authors with the same surname and same year of publication.
- 7.3 Two or more years in parentheses following an author's name are cited in ascending order of year, and two or more references published in the same year by the same author are differentiated by letters a, b, c, etc. For example: Brown (1969, 1972, 1973a, b).
- 7.4 Different references cited together should be in date order, for example: (Smith, 1959; Thomson and Jones, 1982; Green, 1990).
- 7.5 If a paper has been accepted for publication but has not been published the term "(in press)" should be used instead of a date.
- 7.6 If a paper has been submitted but not definitely accepted the term "(submitted)" should be used. If the paper is still being prepared the term "(in preparation)" should be used.
- 7.7 The abbreviation "*et al.*" should be used in the text when there are more than two co-authors of a cited paper.

8. List of references

- 8.1 References should be listed alphabetically at the end of the paper. Although "*et al.*" is preferable in the text, in the list of references all authors should be given.
- 8.2 Journal reference style:
Asano T., Maeda M. and Takaki M. (1996). Wastewater reclamation and reuse in Japan: overview and implementation examples. *Wat. Sci. Tech.*, **34**(11), 219-226.
Note that to unambiguously identify articles in *Water Science and Technology* the issue number as well as the volume number is needed.
- 8.3 Book reference styles - (i) article in compilation; (ii) multi-author work; (iii) standard reference; (iv) report; (v) thesis:
- (i) Abell B. C., Tagg R. C. and Push M. (1974). Enzyme catalyzed cellular transaminations. In: *Advances in Enzymology*, A. F. Round (ed.), vol 2, 3rd edn, Academic Press, New York, pp. 125-247.
- (ii) Henze M., Harremoës P., LaCour Jansen J. and Arvin E. (1995). *Wastewater Treatment: Biological and Chemical Processes*. Springer, Heidelberg.
- (iii) *Standard Methods for the Examination of Water and Wastewater* (1995). 19th edn, American Public Health Association/American Water Works Association/Water Environment Federation, Washington DC, USA.
- (iv) Water Research Centre (1990). *Proposed Water Quality Criteria for the Protection of Aquatic Life from Intermittent Pollution*, Report PRS 2498-NM, Water Research Centre, Medmenham, UK.
- (v) Tamminen T. (1990). *Eutrophication and the Baltic Sea: Studies on Phytoplankton, Bacterioplankton and Pelagic Nutrient Cycles*. PhD thesis, Department of Environmental Conservation, University of Helsinki.

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Updated March 2002.

Sample layout: title of article should concisely and accurately specify subject of paper

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Abstract

This example is to demonstrate the layout of the first page of a "camera-ready" article for *Water Science and Technology*. The title, authors' names and addresses should be indented 1.5 cm from the left-hand margin of the text area; the abstract is indented 1.5 cm from both margins. The abstract itself, set in 10 pt type like the authors' addresses, should start about 9 cm down from the top of the text area. Please do not make reference citations in the abstract and please keep within the limit of 200 words.

Keywords

Abstract; author details; example; headings; layout; title page

MAJOR HEADINGS

Major headings are typed bold in 12pt upper case (capital letters), with two lines space above and none below. Text should be justified (extend to the right-hand margin) and be typed to fill the full depth of the text area. Please use a 12pt Times typeface. Do not indent the text paragraphs.

Minor headings

Minor headings are in 12pt bold type, not indented, with one line of space above and none below. Do not number headings.

Sub-headings. Sub-headings are typed in 12pt italic (or underlined if italics are not available), not indented, with the paragraph running on after a full stop; there is one line of space above.

New paragraphs are not indented, but are preceded by a line of space. Also please avoid using footnotes or splitting tables over two (or more) pages.

Table 1. This is an example of table layout. It shows the dimensions of the text area to be used for *Water Science and Technology* articles and of international and US paper sizes (and the consequent recommended margin settings). Note that a minimum number of horizontal rules and (usually) no vertical rules are used.

	Text area (mm)	A4 paper		US (Imperial) paper	
		(mm)	(in)	(mm)	(in)
Depth	250	297	11.69	279.32	11.0
Top/bottom margin	-	25	1.0	15	0.6
Width	170	210	8.27	215.84	8.5
Left/right margin	-	20	0.8	23	0.9

The assessment of diffuse pollution from acid-mine drainage using an updated and revised diatom assessment procedure as an added-value bio-monitoring tool.

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Abstract

Three data sources (physico-chemistry, bio-monitoring and eco-toxicology) are used in South Africa presently to establish Environmental Water Quality conditions. Environmental water quality in turn is key information required for the “Ecological Reserve Determination” of river reaches. Bio-monitoring in South Africa has been limited in recent times mostly to the use of the SASS procedure which relies on invertebrates only. This paper describes the re-introduction of a diatom-based water quality assessment as an added-value bio-monitoring tool. A specific example is discussed citing the response of diatom assemblages to diffuse pollution from acid mine drainage and how effective diatoms are as indicators of ecological integrity and river recovery measured downstream of the area of impact. The advantages of applying this bio-monitoring technique over other biological measures are presented in the context of technological advances in rapid image processing, species identification and software applications of diatom-based water quality indices. The valuable records of the diatom assemblages of the past, held in the South African Diatom Collection at the CSIR (KwaZulu-Natal), can now be accessed and interpreted as historical environmental water quality reference points for several rivers in South Africa.

Keywords

Acid mine drainage; bio-monitoring; diatom-based water quality assessment; environmental water quality

INTRODUCTION

Some of the earliest studies benchmarking the impact of drainage from coal mines on biota and river chemistry in KwaZulu-Natal were undertaken by Kemp (1962, 1967), and Oliff (1963) in the upper reaches of the Thukela River. These investigations were confined to the study of invertebrates and/or water chemistry of river systems draining the active coal mining operations around the towns of Newcastle, Glencoe and Dundee in the north-west of the province. Chohnoky (1960) produced the first ad hoc diatom records from various northern KwaZulu-Natal rivers affected by drainage from coal mines.

Acidic drainage water generated by coal mining activities is derived from underground oxidation of pyrites (iron sulphide contained in the coal and associated strata) to ferrous and ferric sulphate (Kemp, 1967). The formation of such acidic conditions can be a natural process but mining activities also accelerate the process by disturbance of mineral matter. Kemp (1962) estimated that if the rate of coal output was maintained in these catchments, the pollution of rivers in Northern Natal would double by the year 2000. Perpetuation of these conditions was expected to seriously threaten the ecological quality and biological integrity of small streams rendering them unfit for various uses.

Over thirty years have elapsed since the completion of a province-wide investigation of rivers in Northern Natal and Zululand when many coal mines were still in full operation (Archibald *et al.*, 1970). However these investigations essentially only covered the bacteriology and water chemistry of the principal rivers of the north-western parts of the province.

Deterioration of the water quality was already evident in some localities and this was ascribed to organic pollution and mineralisation from drainage out of coal mines in the headwaters of the Mkuze and Mfolozi rivers. Both these rivers drain into Lake St Lucia – a world heritage site.

Several coal mining operations in north-western KwaZulu-Natal have ceased to operate because of unfavourable economic conditions but the flow of acidic drainage from these mines did not necessarily stop when mining activities were discontinued (Kemp, 1962).

Several small unprofitable operations were simply abandoned without recourse to rehabilitation because there was no protocol for measuring environmental damage to the aquatic life. However environmental management plans are now required by the Department of Minerals and Energy as part of a proper decommissioning process.

Monitoring aquatic resources forms the basis of constructing and implementing successful management plans for river systems. Using aquatic organisms to indicate water quality and habitat integrity has certain advantages over simply monitoring the chemical and physical components of an ecosystem. Aquatic organisms provide a reflection of integrated water chemistry over time as well as providing an indication of the impact of various pollutants on the riverine biota i.e. not just the concentration of an effluent, but its actual ecological impact on the environment.

The unicellular, mostly autotrophic micro-algae known as the diatoms are gaining considerable popularity as bio-indicator organisms in many countries around the world (Prygiel *et al.*, 1999). Over the last 25 years the environmental tolerances of a large number of diatom species have been documented and these inferred tolerances have been used to construct numerical diatom-based water quality indices (Descy, 1979). These indices have proved especially useful for monitoring habitats such as canals (Prygiel and Coste, 1993) where other bio-indicator organisms are not to be found. The use of these water quality indices has been facilitated by the development of software known as "Omnidia" (Lecointe *et al.*, 1993) for both the calculation of diatom indices and archiving diatom sample information and species counts. Recent application of diatom-based water quality indices and the advantages of using these organisms for bio-monitoring in South Africa have been described by Harding *et al.* (2005), Taylor (2004), De la Rey *et al.* (2004) and Taylor *et al.* (2005).

In practice, use of diatom indices involves making a list of the taxa present in a sample, along with a measure of their abundance. The index is expressed as the mean of the optima of the taxa in the sample, weighted by the abundance of each taxon. The indicator value acts to further increase the influence of certain species (Kelly, 1998). Most of the water quality indices supported by Omnidia require species level input information. Accurate identification to species level is therefore a pre-requisite for application of the software. Harding *et al.* (2005) discuss the indices and interpretation tools in greater detail.

STUDY AREA

This brief investigation was carried out in 1998 to determine the impacts on stream integrity of a long term persistent flow of diffuse pollution from acid mine drainage. Four distinct sampling sites were located on the upper Tshoba river which receives acid drainage runoff from a defunct coal dumpsite near Hlobane (Latitude 27° 44.28'S, Longitude 30°58.20'E, some 10km to the west of the town of Vryheid). It is a small tributary at the headwaters of the Black Mfolozi river which ultimately discharges into the St Lucia estuary.

Since there are also several other coal mines (operational and defunct) in the upper Mfolozi catchment, it is of ecological interest and of management importance to determine whether self-purification from long term acid mine drainage can be detected using these biological methods. This is a necessary precautionary measure to safeguard the feeder streams of the Mfolozi River before it discharges into the estuarine area of Lake St Lucia.

Site 1: Tshoba stream. This site is located at the headwaters of the Tshoba stream, some 300m below the diffuse surface drainage from a large coal dump site. The shallow, discernible stream channel was about 5 - 10m wide at Site 1 with marginal stands of *Phragmites* and *Typha* forming a relatively small wetland at the base of the mine dump. Sample 1a: A sample was taken from the sandy substrate of the acid drainage channel upstream of the confluence with the natural headwater stream.

Sample 1b: A sample was taken from the sandy substrate of the natural stream draining the hillside, adjacent to the mine, and upstream of the confluence with the acid drain.

Site 2: Tshoba stream (50 -100m downstream of Site 1). This site was located below the confluence of inflows from 1a and 1b allowing for the first apparent visible mixing of both streams some 50-100metres downstream where the integrity of distinct channels was still visible.

Sample 2c: Acid water channel - Sandy substrate on left bank of the main stream.

Sample 2d: Partially mixed - Sandy substrate at mid- point of main channel.

Sample 2e: Alkaline channel - Sandy substrate on right bank of main stream.

Site 3: Tshoba stream (5 km downstream from the dump site). This site is beneath the bridge to the west of Vryheid on the main road to Louwsburg. Sample 3 was taken in clear water flowing over the sandy substrate on the margins and between rocks in mid-stream.

Site 4: Tshoba stream (10 km downstream from the dump site). The site is approximately 8 - 10km downstream of the mine dump - beneath a bridge on a district road. Clear water flowed over a sandy substrate between small rocks .

MATERIALS AND METHODS

Physico-chemical variables. Environmental water quality characteristics were determined for several key constituents which are typically used to characterize physico-chemical conditions in a river. Conventional analytical techniques (APHA, 1971) were followed by the accredited CSIR laboratory using automated instrumentation in the determination of pH, conductivity, suspended solids, turbidity, dissolved oxygen (Winkler), nitrates, ammonia, soluble phosphorus and a range of cations and anions (Tables 1 – 3).

Bio-monitoring protocols. Since there were no rocks at the upper sites, diatoms were sampled from the sandy substrate at all sites using a perspex corer (21mm in diameter). Several 1cm deep surface cores were removed and stored in a cooler box for immediate extraction of the living component on return to the laboratory within a day after completion of the fieldwork.

Laboratory extraction of living diatoms was accomplished by ‘floating’ coverslips on the damp sediments of each sample (Round 1991, 1993) and allowing the living cells to attach to the coverslips. Material from these coverslips was subjected to acid treatment in the manner recommended by Welsh (1964). The acid-cleaned diatom material was mounted on permanent slides for observation and counting under oil immersion with an Olympus BX51 light microscope at 1000x magnification. In this study the diatoms were identified primarily using the international reference works of Krammer and Lange-Bertalot (1986-1991), those of Hustedt (1930 - 1961) and the local reference works of Schoeman and Archibald (1988).

The diatom index scores for the Tshoba River were derived from diatom community analysis data which was then processed using Omnidia. The indices used here are known as the Specific Pollution sensitivity Index or SPI (Coste in CEMAGREF, 1982), Schiefele and Schreiner’s index or SHE (Schiefele and Schreiner, 1991), the Watanabe index or WAT (Watanabe, 1986; 1990), the Generic Diatom Index or GDI (Coste and Ayphassorho, 1991) and the Biological Diatom Index or BDI (Lenoir and Coste, 1996).

RESULTS AND DISCUSSION

Environmental water quality - variation with distance downstream

Water quality in the headwaters of the Tshoba stream at Site1 was markedly different in samples 1a and 1b - the respective feeder channels to the main stream. The drainage from the mine dump was typically very acidic (pH of 2.6) and was also contaminated with high concentrations of dissolved salts, in particular sulphate (Tables 1 – 3) giving elevated TDS and Conductivity values due mainly to high calcium, magnesium, sulphate and chloride concentrations (Tables 1- 3).

In contrast, the water sampled from the natural stream (Sample 1b) was typically alkaline (pH of 7.6) with the concentrations of most salts, except sodium and potassium lower than that of the acid drain. The water quality characteristics were taken as the ‘reference condition’ for comparison with downstream conditions. Nitrates, ammonia and soluble phosphorus concentrations of Sample 1b water were all higher than the acid drainage water of Sample 1a.

A subset of three samples (Samples 2a, 2b and 2c) was taken across the channel (left bank, mid-stream, right bank) at Site 2, some 50-100m downstream of the confluence. The configuration of the shallow stream (5cm depth) at this point and the discoloration of the stream bed indicated that proper mixing had not occurred even though the main channel was only 15-20m wide.

Concentrations of chemical constituents of the left channel were consistent with the acid drainage conditions of Sample 1a and constituents of the right channel were consistent with conditions of Sample 1b in the natural stream (Tables 1-3).

The water quality at Site 3 showed marked improvement in water quality in terms of reduced concentrations of chemical constituents. The pH value reverted to alkaline conditions and nutrient concentrations were reduced. Concentrations of cations and anions were also much reduced giving lower conductivity and TDS values. Site 4 was furthest from the mine dump where dilution from small side streams had also occurred. Concentrations of cations and anions were lower than those recorded in Sample 1b - the reference sample (Table 1). The lower nutrient concentrations and the alkaline status of the water are features consistent with the improved conditions measured upstream at Site 3.

This Tshoba river survey was made during the summer wet season of 1998 and the steady flows in the river below the mine dump contributed to the rapid recovery in water quality of the river at Site 3 and 4 before the confluence with the Mfolozi river. Some thirty years ago Archibald *et al.* (1970) also reported similar findings from their Site X12 (Tshoba stream) upstream of the confluence with the Mfolozi. (Table 1)

Diatom Community Analysis Analyses of the diatom associations are given in Tables 4 - 6.

Site 1. Sample 1a: Two dominant species showed tolerance of the harsh acidic conditions and made up almost 100% of the total count. The dominance of the acidophilic species *Nitzschia paleaformis* Hustedt (85.6%) and to a lesser extent *Stauroneis kriegei* Patrick (14.4%) was almost complete in this association (Table 4). The former has been recorded from sulphuric acid ponds associated with coal mining operations while the latter has a preference for neutral water (Krammer and Lange-Bertalot, 1986).

Sample 1b: Five dominants made up a total of 81.1% of the 17 recorded species. The species diversity and % composition of several co-dominants (*Brachysira vitrea* (Grun.) Ross in Hartley 25.2%; *Achnanthes minutissimum* (Kütz.) Czarnecki 19.2%; *Encyonopsis cesatii* (Rabenhorst) Krammer 17.7% ; *Nitzschia nana* Grun. in van Heurk 9.6% ; *Craticula buderii* (Hustedt) Lange-Bertalot 9.4% indicate 'normal' concentrations of organically bound nitrogen, well-oxygenated conditions and the circum-neutral pH of a natural stream. This association was regarded as a baseline or reference community characteristic of the water quality in the upper subcatchment of the study area. It is evident from this data that the different diatom assemblages reflect the distinctly different water quality conditions at Site 1a and 1b.

Site 2. Examination of the respective diatom associations extracted from Samples 2c, 2d, and 2e confirmed that the 'integrity' of the channel flow from Site 1a and 1b was maintained. Markedly different diatom associations developed across the stream having a width of no more than 15metres. Distinctly acidophilous diatom components still dominated on the left bank with a gradient through an intermediate midstream association to an alkalophilous diatom composition in the right channel of 'reference-quality water' (Table 4).

Sample 2c: Two dominants made up 99.0% of the population count. The diatom species association was identical to that found in Sample 1a – the acidic feeder stream from the coal mine drainage. *Nitzschia paleaformis* (88.0%) and *Stauroneis kriegei* (11.6%) remained the dominant tolerant species (Table 4).

Sample 2d: Six dominants out of 9 species made up 95% of the population count. The dominance of the acidophilous species (*Nitzschia paleaformis* – 40.0%) was reduced at the midstream site by the appearance of several other diatoms. There was a measurable shift towards a greater percentage of *Stauroneis kriegei* (30.0%) which favours circum-neutral waters. Species composition changes indicated that some mixing and dilution had occurred in mid-stream between the left and right channels (Table 4). The reduction of the acidophilous species and the concomitant increase in diversity and abundance of four other diatoms is the first indication that dilution by the natural stream has an ameliorating affect on the acid drainage within 50-100metres of the confluence. More species (9) were able to tolerate the 'mixed conditions' downstream.

Sample 2e: Seven dominants out of 21 species made up 73% of the population count. The species composition of the diatoms taken from the sample at the right hand side of the main stream is consistent with that of the alkaline conditions recorded at Site 1b upstream of the confluence with the acid mine drainage.

There was a marked reduction in the species tolerant of acidic conditions (i.e. *Nitzschia paleaformis* reduced to 4.1% and *Stauroneis kriegei* to 5.7%). The dominance was spread to a greater diversity consistent with the upstream more alkaline reference condition (*Achnanthes minutissimum* 14.6%; *Brachysira vitrea* 18.5% and *Nitzschia nana* 16.8%).

Site 3. Three dominants out of 32 species made up 58% of the total count. Higher species diversity was recorded in the count giving a broader distribution in the relative abundance of the diatom species such as *Nitzschia linearis* (Agardh) W.M. Smith (38.3%), *Caloneis molaris* (Grun.) Krammer (10.1%) and *Nitzschia denticula* Grun. (9.9%). *N. linearis* is known to favour circum-neutral water, rich in oxygen and with moderate conductivity values. (Krammer and Lange-Bertalot, 1988) (Table 4).

Site 4. Two dominants out of 20 species made up 58.0% of total count and the diatom assemblage also showed a higher species diversity with the dominants being *Achnanthes minutissimum* (50.0%) and *Gomphonema parvulum* Kütz. (7.6%). The former species is indicative of good water quality characterized by well-oxygenated conditions (Table 4).

Application of diatom-based water quality indices

The previous section has shown that stream conditions may be described by drawing inferences from the known tolerances of diatom species to water quality variables. Diatom index scores generated from diatom species abundance data (Table 4) are presented in Table 5. The diatom index scores are in agreement with the descriptive assessment of the streams. The index scores (especially BDI) indicate the extreme degradation of the acid stream, the different water quality across the width of the receiving stream as well as the gradual recovery of the receiving stream as distance increases downstream from the site of impact.

Trends in diatom index scores were compared to trends in index scores using a standard Pearson correlation matrix (Table 6). Although the sample size was small (n=7) significant correlations were found for most indices between pH, total soluble phosphorous and chloride, indicating that these variables had the most impact on the structure of the diatom communities. The correlation between pH and the diatom index scores is especially significant as hydrogen ion concentration is a very important water quality variable at sites impacted by acid mine drainage and a major determinant of the structure of diatom communities (Cholnoky, 1968).

CONCLUSIONS

Analysis of the diatom assemblages has clearly shown that the diatom-based water quality assessment technique is a valid and accurate bio-monitoring tool that can be used with confidence in delineating the impacted reaches of a river.

The diatom procedure can also detect the recovery of a river from diffuse pollution, such as that generated over time from acid-mine drainage. The findings described in this paper demonstrate the ecological and management value of using biological assemblages from a different trophic level i.e. autotrophic organisms reliant on dissolved plant nutrients at the base of the foodweb. Diatom communities, in particular, are useful for river health assessment because they respond directly to physical and chemical constituents of the water..

It was possible to demonstrate that the diatom communities responded to changing water quality brought about by acid mine drainage. There were distinct acid-tolerant assemblages in the mine drainage stream versus the alkaline water assemblages.

The findings support the need for the re-introduction in South Africa of diatoms as key biological indicators for the assessment of river health under varying conditions of water quality. The procedure is supportive of the imperatives and requirements of the National Water Act (Act 36 of 1998) which now promotes 'river health' assessments in terms of environmental water quality and in-situ bio-monitoring (Palmer *et al.*, 2002, 2003).

Accurate ecological inferences can be drawn from analysis of diatom communities and a knowledge of species tolerance for a specific quality of water. The procedure demands accurate taxonomic identifications. Both taxonomical (e.g. Prygiel and Coste, 2000) and analysis software programs (e.g. Omnidia) have now lessened previously perceived constraints and deficiencies of the past relating to time-consuming taxonomic procedures.

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Physical water quality characteristics of the Tshoba stream (CSIR survey 16/02/1998)								
Samples ►	1a	1b	2c	2d	2e	3	4	Tshoba Site X12
Water Quality Variables ▼	Acid drain	Natural	Left	Midstream	right	5km	10km	Archibald et al 1970
						downstream	downstream	
Temperature (°C)	25.2	21.3	21.7	21.7	21.7	24.5	22.3	9.8
pH value	2.6	7.7	2.6	4.3	7.0	7.1	8.1	8.3
Conductivity (mS/m)	459	277	468	289	281	178	53	31.8
Alkalinity (mg/l)		62			56	48	110	162
Total Dissolved Solids (mg/l)	5020	2360	5100	2600	22370	1430	352	202
Suspended Solids (mg/l)	27	15	21	41	16	5	26	
Turbidity (NTU)	1.1	0.4	0.3	56	0.2	2.5	24	

Table 2		Some water quality and nutrient characteristics of the Tshoba stream (CSIR survey 16/02/1998)							
Samples	►	1a	1b	2c	2d	2e	3	4	Tshoba Site X12
Water Quality Variables		Acid drain	Natural	Left	Midstream	Right	5km	10km	Archibald et al 1970
▼							downstream	downstream	
Dissolved Oxygen (mg/ℓ)		6.7	8.7		8.1		7.3	7.4	9.9
Chemical Oxygen Demand (mg/ℓ)		5	16	12	16	8.1	12	16	
NH ₄ -Nitrogen (µg/ℓ)		12	40	30	1000	110	470	40	
NO ₃ -Nitrogen (µg/ℓ)		70	2300	670	2200	2300	260	670	
Total Kjeldahl Nitrogen (µg/ℓ)			1200	1100	1100	110	800	510	
Total Soluble Phosphorus (µg/ℓ)		30	10	30	10	10	10	10	80

Table 3		Inorganic constituents of the Tshoba stream (CSIR survey 16/02/1998)							Tshoba Site X12 Archibald et al 1970
Samples ►		1a	1b	2c	2d	2e	3	4	
Water Quality Variables		Acid drain	Natural	Left	Midstream	Right	5km	10km	
▼							downstream	downstream	
Calcium	(mg/ℓ)	363	198	368	222	208	114	31	23.9
Magnesium	(mg/ℓ)	198	85	200	92	83	55	22	19.3
Sodium	(mg/ℓ)	115	350	100	338	360	213	45	23.2
Potassium	(mg/ℓ)	1.8	5.8	1.5	5.7	6.0	3.6	1.9	1.2
Sulphate	(mg/ℓ)	3100	1540	3720	1650	1530	1030	158	11.5
Chloride	(mg/ℓ)	31	8.7	31	11	9.2	12	13	8.8
Iron	(mg/ℓ)	151	1	133	1	1	1	1	

Diatom species composition data from Tshoba stream samples (CSIR survey 16/02/1998)								
Table 4	Samples ▶	1a	1b	2c	2d	2e	3	4
	Diatom Species List ▼	Acid drain	Natural Stream	Left bank	Midstream	Right bank	5km downstream	10km downstream
	<i>Achnantheidium minutissimum</i>		19.2		7	14.6	0.4	50.0
	<i>Amphora fontinalis</i>						3.7	
	<i>Brachysira vitrea</i>		25.2		2	18.5		2.3
	<i>Caloneis bacillum</i>						3.7	1.5
	<i>Caloneis molaris</i>		0.4			0.6	10.1	2.3
	<i>Capartogramma crucicula</i>						2.1	
	<i>Craticula buderii</i>		9.4			2.1		
	<i>Cyclotella meneghiniana</i>		0.8			0.6		
	<i>Cymbella affinis</i>						1.2	3.6
	<i>Cymbella aspera</i>						2.3	
	<i>Cymbella kappii</i>		1.1					
	<i>Cymbella pusilla</i>						1.9	1.5
	<i>Diploneis puella</i>						0.4	
	<i>Encyonema neogracile</i>							0.8
	<i>Encyonema silesiacum</i>						1	0.8
	<i>Encyonopsis cesatii</i>		17.7			4.8		
	<i>Encyonopsis microcephala</i>		1.7			2.1	0.2	
	<i>Fragilaria fasciculata</i>							2.3
	<i>Fragilaria nanana</i>		4.1		1	2.6		
	<i>Gomphonema gracile</i>				2			
	<i>Gomphonema parvulum</i>		2.8			2.8		7.6
	<i>Hatzschia distinctepunctata</i>						0.4	
	<i>Navicula elginensis</i>						1	
	<i>Navicula erifuga</i>						2.1	
	<i>Navicula heimansii</i>					0.3		
	<i>Navicula libonensis</i>						1.1	
	<i>Navicula pupula</i>						0.6	0.8
	<i>Navicula rostellata</i>						2.7	
	<i>Navicula schroeteri</i>		0.7			0.3	1.2	1.5
	<i>Navicula tenelloides</i>					0.3	1.2	
	<i>Navicula vandamii</i>						1.8	0.8
	<i>Navicula veneta</i>						1.6	
	<i>Nitzschia angusteforaminata</i>						0.2	
	<i>Nitzschia debilis</i>							
	<i>Nitzschia denticula</i>						9.9	1.5
	<i>Nitzschia dissipata</i>						1.2	
	<i>Nitzschia linearis</i>						38.3	1.5
	<i>Nitzschia microcephala</i>							0.8
	<i>Nitzschia nana</i>		9.4		6.8	16.8		
	<i>Nitzschia palea</i>		2.4			6.8	3.5	1.5
	<i>Nitzschia palea v. debilis</i>		0.4			3.1		3
	<i>Nitzschia paleaeformis</i>	85.5		88	40	4.1	1.6	
	<i>Nitzschia reversa</i>		0.8			6.3		0.8
	<i>Nitzschia tropica</i>		0.9		6	5.2		
	<i>Nitzschia vitrea</i>						0.4	
	<i>Rhopaloidia operculata</i>							0.8
	<i>Stauroneis kriegerii</i>	14.4		11.6	30	5.7		
	<i>Surirella angusta</i>						1.2	
	<i>Surirella tenera</i>						0.4	

<i>Synedra ulna</i>		2.8		5	1.2	0.2	3
Other species	0.1	0.2	0.4	0.2	0.9	0.4	6.8
Percentage Composition	100	100	100	100	100	100	100
No of frustules counted	532	500	517	517	579	516	132
No of species/site	2	17	2	9	21	32	20
No of dominants:Combined	2	5 (81)	2	6(95)	7(73)	3(58)	2(58)
%	(99)		(99)				

Table 5		Omnidia software Diatom Community Index scores for each site (ex data from Table 4)				
Tshoba River survey 16/02/1998						
Community Index ► Sampling Sites ▼		SPI	SHE	WAT	GDI	BDI
1a. Acid Mine drainage		11.7	10.5	0.0	2.7	1.0
1b. Natural stream		15.8	16.5	11.7	12.8	10.3
2c. Left bank acid channel		11.5	10.5	0.0	2.4	1.0
2d. Mixed midstream channel		14.5	12.1	10.7	6.2	14.3
2e. Right bank neutral channel		13.3	14.9	9.7	8.5	12.4
3. 5km downstream		12.1	12.7	10.7	8.0	10.2
4. 10km downstream		14.3	14.6	17.0	12.6	15.2
Interpretation of Index Scores		Index score	Class			
		>17	high quality			
		13 to 17	good quality			
			moderate			
		9 to 13	quality			
		5 to 9	poor quality			
		<5	bad quality			

Table 6		Correlation matrix of water quality variables (ex Tables 1, 2) and Diatom Index Scores (ex Table 5)				
Tshoba river survey 16/02/1998						
Community Index ► Water Quality Variables ▼	SPI	SHE	WAT	GDI	BDI	
pH value	0.54	0.86	0.86	0.92	0.82	
Suspended Solids	-0.36	-0.58	-0.40	-0.57	-0.33	
Conductivity	-0.04	-0.30	-0.60	-0.53	-0.56	
Total Dissolved Solids	-0.41	-0.47	-0.63	-0.65	-0.57	
Temperature	-0.57	-0.44	-0.28	-0.28	-0.32	
Chemical Oxygen Demand	0.49	0.37	0.59	0.50	0.57	
Ammonium-Nitrogen	0.61	0.32	0.67	0.49	0.72	
Nitrate-Nitrogen	0.56	0.54	0.54	0.46	0.59	
Total Soluble Phosphorus	-0.74	-0.80	-0.98	-0.95	-0.97	
Sulphate	-0.04	-0.31	-0.60	-0.53	-0.57	
Chloride	-0.68	-0.73	-0.83	-0.85	-0.79	
(NB: Bold/shaded correlations are significant at $p < 0.05$: n =7: Casewise deletion of missing data)						

CHAPTER 8

Eolimna archibaldii spec. nov. and *Navigiolum adamantiforme*
comb. nov. (Bacillariophyceae): two possibly endemic
elements of the South African diatom flora tolerant to surface
water pollution

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Eolimna archibaldii spec. nov. and *Navigiolum adamantiforme* comb. nov. (Bacillariophyceae): two possibly endemic elements of the South African diatom flora tolerant to surface water pollution

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Two little-known diatom taxa have been encountered in the saprobic and anthropogenically impacted waters of the Vaal River, South Africa. *Eolimna archibaldii*, an extremely small-celled species, is described as new to science. *Navicula adamantiformis* Archibald is transferred to the taxonomically-appropriate genus *Navigiolum* Lange-Bertalot, Cavacini, Tagliaventi and Alfinto, as no specific characters of *N. adamantiformis* coincide with *Navicula sensu stricto*.

Keywords: new combination, new species, species description, species transfer, systematics, Vaal River

Introduction

The Vaal River catchment can be regarded as the most important water supply region in South Africa. The Vaal River rises on the western slopes of the Drakensberg escarpment in the vicinity of Lake Chrissie near Breyten and, after 1 200 km, flows into the Orange River near Douglas. The latter river flows into the Atlantic Ocean at Alexander Bay. Although the Vaal River has a catchment area of 196 290 km² and is one of the major water sources in the country, its annual runoff is only 8% (4 300 m³/a) of South Africa's total annual runoff (DWA 1993, 2000). The river is highly regulated by numerous dams and small weirs, which hold back water used for irrigation, this regulation and abstraction is so extensive that there is now only sporadic flow into the Orange River (Braune and Rogers 1987).

Braune and Rogers (1987) divide the Vaal River catchment into five regions, the region of interest in this paper being the reach between Loch Vaal Reservoir (which receives effluent from the densely-populated Gauteng Province) and Bloemhof Dam (Figure 1). This reach is highly regulated to meet demands for water supply. High sulphate loads, with a corresponding contribution to total alkalinity, dominate the Bloemhof Dam catchment. The high sulphate loads and higher alkalinity are due to the contribution of Loch Vaal and to high inputs from the tributaries draining the northern part of the catchment, which are heavily polluted by intensive mining and industrial activities. There is an upward trend in salinity in this reach.

In general, the best quality water in the Vaal River catchment is still found upstream of the Vaal Dam. Below this reach the quality deteriorates markedly, due mainly to the inflow of

domestic and industrial effluent from the Johannesburg complex. No other catchment in South Africa supports as much agricultural, mining, industrial and urban development, all of which require water and which release polluted return-flows to the river. This intensive utilisation of the Vaal River places great stress on the system and has resulted in severe degradation of the river as a water resource.

Diatom assemblages from polluted waters around the world display a fairly uniform diatom flora, with similarities noted between those in Europe (Lange-Bertalot 1979), Japan (Kobayasi and Mayama 1982, 1989) and South Africa (Schoeman 1979, Taylor 2004). These floras have only 20–30 commonly-found, tolerant to extremely tolerant diatom taxa. However, the species composition, although often similar, may not always be identical in all respects, as will be demonstrated in this paper. On rare occasions, supposed endemic species may become dominant in degraded or polluted rivers in South Africa. *Eolimna archibaldii* sp. nov. and *Navigiolum adamantiforme* comb. nov. serve as examples of endemic taxa that have been found to dominate diatom assemblages in the middle Vaal River. To our knowledge, these two taxa have not been recorded from the continents of Europe, America, Asia or Australia.

Recently, several taxa have been split from *Navicula sensu lato* and grouped in more suitable genera. Lange-Bertalot *et al.* (2003) established the genus *Navigiolum*, encompassing diatoms with the following characteristics:

'Small lanceolate biraphid frustules with acutely rounded ends and a narrow rectangular girdle. Raphe branches of equal length, and sternum central and

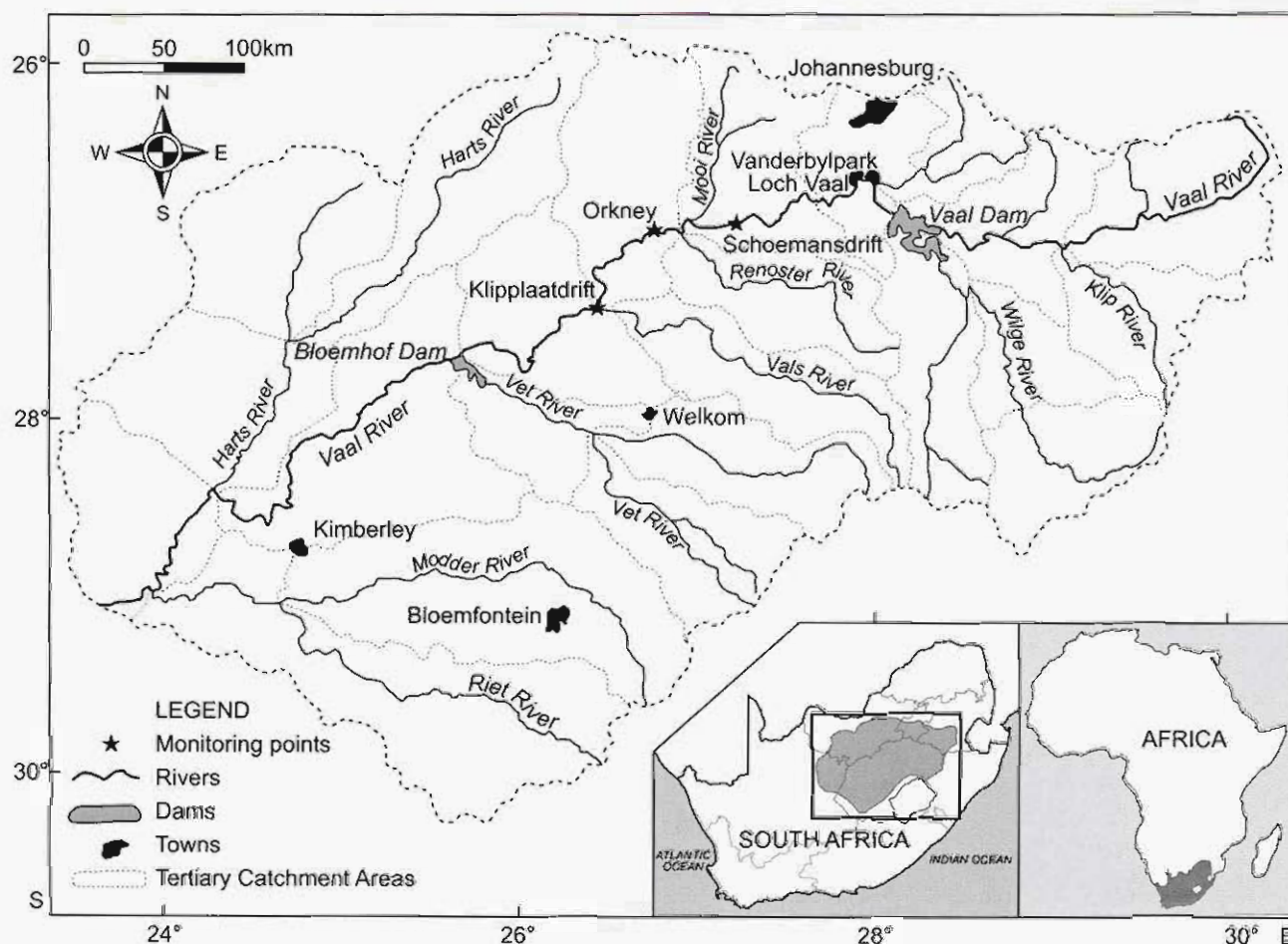


Figure 1: Map of the Vaal River catchment showing the location of the sampling sites

not raised either on the inside or outside of the valve surface. Facultative linking spines. Areolae closed by hymenes. Valve copulae open and unperforated.

This paper describes and discusses *Eolimna archibaldii* spec. nov., as well as *Navicolum adamantiforme* nov. comb., and their occurrence in waters impacted by man's activities. It also compares the diatom species composition in polluted South African rivers with assemblages in similarly polluted water courses in central Europe and Japan.

Materials and methods

Samples containing *Eolimna archibaldii* and *Navicolum adamantiforme* were collected between February and November 2002 from submerged wood and rocks at Schoemansdrift (26°55'38"S 27°10'11"E), Orkney (27°00'22"S 26°40'20"E) and Klipplaatdrift (27°20'46"S 26°25'50"E), located in the middle reaches of the Vaal River (Figure 1). Data on water temperature, oxygen concentration and turbidity were collected at the times of sampling, while chemical data — used to establish water quality at the sites, collected over the same period by the Department of Water Affairs and Forestry (DWAF) as part of their routine national chemical monitoring programme — were obtained from DWAF.

Diatom samples were collected by vigorously scrubbing 3–5 stones from the substratum with a small brush and collecting the resultant suspension in a 150 ml plastic sample bottle. A portion of each sample was oxidised by boiling in a mixture of 2:1 nitric and sulphuric acid, rinsed, and mounted in Pleurax (Hanna 1949), following standard methods (Hasle 1978). Prepared slides were examined using a Zeiss photomicroscope equipped with Nomarski DIC optics. Sub-samples in which the taxa were most abundant were mounted on aluminium stubs for SEM examination. All the SEM stubs were coated with gold-palladium and examined using an FEI 200 QUANTA ESEM at the North-West University (Potchefstroom Campus), Potchefstroom.

Canonical Correspondence Analysis (CCA), using CANOCO version 4.5 (Ter Braak and Šmilauer 1998), was used to determine the relationship between diatom species and measured environmental variables. Species name abbreviations used in the CCA diagram follow those found in the diatom database OMNIDIA version 3.1, with the exception of CKAP which is the abbreviation used for *Cymbella kappii* Cholnoky. A list of abbreviations may be found in Table 1. The basic rule for the construction of the acronyms is that the first letter indicates the genus name while the following three letters indicate the specific epithet (Lecointe *et al.*

Table 1: Abbreviations (acronyms) of diatom species presented in the CCA diagram (Figure 4) based on the OMNIDA data system (Lecointe et al. 1993)

Taxon	Acronym
<i>Achnanthes minutissimum</i> (Kützinger) Czarnecki	ADMI
<i>Achnanthes saprophila</i> (Kobayasi & Mayama) Round & Bukhtiyarova	ADSA
<i>Amphora montana</i> Krasske	AMMO
<i>Amphora pediculus</i> (Kützinger) Grunow	APED
<i>Aulacoseira granulata</i> (Ehrenberg) Ralfs	AUGR
<i>Aulacoseira granulata</i> var. <i>angustissima</i> (O.Müller) Simonsen	AUGA
<i>Cocconeis pediculus</i> Ehrenberg	CPED
<i>Cocconeis placentula</i> Ehrenberg	CPLA
<i>Craticula molestiformis</i> (Hustedt) Lange-Bertalot	CMLF
<i>Cyclotella dubius</i> (Fricke) Round	CDUB
<i>Cyclotella invisitata</i> (MH Hohn & Hellerman) Theriot, Stoermer & Håkansson	CINV
<i>Cyclotella meduanae</i> Germain	CMED
<i>Cyclotella meneghiniana</i> Kützinger	CMEN
<i>Cymbella kappii</i> Chohnoky	CKAP
<i>Cymbella tumida</i> (Brébisson) van Heurck	CTUM
<i>Cymbella turgidula</i> Grunow	CTGL
<i>Diatoma vulgare</i> Bory	DVUL
<i>Encyonema minutum</i> (Hilse in Rabenhorst) DG Mann in Round, Crawford & Mann	ENMI
<i>Eolimna minima</i> (Grunow) Lange-Bertalot	EOMI
<i>Eolimna subminuscula</i> (Manguin) Lange-Bertalot & Metzeltin	ESBM
<i>Epithemia sorex</i> Kützinger	ESOR
<i>Fragilaria capucina</i> var. <i>vaucheriae</i> (Kützinger) Lange-Bertalot	FCVA
<i>Fristulifera saprophila</i> (Lange-Bertalot & Bonik) Lange-Bertalot	FSAP
<i>Gomphonema minutum</i> (Agardh) Agardh	GMIN
<i>Gomphonema parvulum</i> Kützinger	GPAR
<i>Mayamaea atomus</i> var. <i>permitis</i> (Hustedt) Lange-Bertalot	MAPE
<i>Melosira varians</i> Agardh	MVAR
<i>Navigiolium adamantiforme</i> (Archibald) Taylor & Lange-Bertalot	NADF
<i>Navicula antonii</i> Lange-Bertalot	NANT
<i>Navicula capitatoradiata</i> Germain	NCPR
<i>Navicula cryptotenella</i> Lange-Bertalot	NCTE
<i>Navicula erifuga</i> Lange-Bertalot	NERI
<i>Navicula germainii</i> Wallace	NGER
<i>Navicula gregaria</i> Donkin	NGRE
<i>Navicula recens</i> (Lange-Bertalot) Lange-Bertalot	NRCS
<i>Navicula rostellata</i> Kützinger	NROS
<i>Navicula symmetrica</i> (Patrick)	NSYM
<i>Navicula veneta</i> Kützinger	NVEN
<i>Nitzschia acicularis</i> (Kützinger) WM Smith	NACI
<i>Nitzschia capitellata</i> Hustedt in A Schmidt	NCPL
<i>Nitzschia dissipata</i> (Kützinger) Grunow	NDIS
<i>Nitzschia fonticola</i> Grunow in Cleve & Möller	NFON
<i>Nitzschia frustulum</i> (Kützinger) Grunow	NIFR
<i>Nitzschia liebetruthii</i> Rabenhorst	NLBT
<i>Nitzschia palea</i> (Kützinger) W Smith	NPAL
<i>Nitzschia pusilla</i> (Kützinger) Grunow	NIPU
<i>Planothidium frequentissimum</i> (Lange-Bertalot) Round & Bukhtiyarova	PLFR
<i>Nitzschia constricta</i> (Gregory) Grunow	NZCO
<i>Reimeria sinuata</i> (Gregory) Kociolek & Stoermer	RSIN
<i>Rhoicosphenia curvata</i> (Kützinger) Grunow	RCUR
<i>Stephanodiscus agassizensis</i> Håkansson & Kling	SAGA
<i>Stephanodiscus hantzschii</i> Grunow in Cleve & Grunow	SHAN
<i>Stephanodiscus parvus</i> Stoermer & Håkansson	SPAV

1993). Abbreviations used for environmental variables are as follows: Temperature — Temp, dissolved oxygen — DO, turbidity — Turb, total alkalinity — TAL, dissolved inorganic phosphate — DIP, dissolved inorganic nitrogen — DIN, ratio of dissolved inorganic nitrogen to dissolved inorganic phosphate — DIN:DIP, and electrical conductivity — EC.

Observations

Navigiolium adamantiforme comb. nov. (Figure 2).

Basionym: *Navicula adamantiformis* Archibald 1966, Nova Hedwigia. Suppl. 21, p. 256, Figure 5.

Synonym: *Achnanthes adamantiformis* Archibald e.p. 1971, p. 21, Figures 1–3.

Schoeman and Archibald (1976) discussed in detail the various concepts of Archibald's taxon (*N. adamantiformis*), especially its generic position in either *Navicula sensu lato* or *Achnanthes sensu lato*. It is recorded as a very rare species, found at only four localities in the then Transvaal and Orange Free State Provinces of South Africa. A questionable locality in Lake Chad in north-central Africa can be excluded as it is based on an erroneous identification by Compère (1975).

Following the generally-accepted separation of *Navicula sensu stricto* from *Navicula sensu lato*, *Navicula adamantiformis* Archibald has been transferred to the genus *Navigiolum* Lange-Bertalot, Cavacini, Tagliaventi and Alfinito as the fine structure of this taxon matches the delineation of Lange-Bertalot *et al.* (2003) for this genus, as follows. In girdle view the cells are narrowly rectangular, all comparatively small, length below 16 µm and breadth below 6 µm. Frustules are naviculoid, central raphe system biraphid with raphe branches of equal length on both valves. External raphe slits are slightly sigmoid, while the inner raphe slits are straight with slightly deflected central endings. Potentially, they form band-like colonies in which the cells are connected by their valve faces. Valves are commonly lanceolate with acutely rounded ends. The valve face is flat, with a slightly curved junction to the mantle (Figure 2b). General tendency to biserial areolae on the valve face (*N. adamantiforme* has uniseriate areolae, Figure 2a–e) separated from a circumferential row of single areolae on the mantle (Figure 2b–c); facultative linking spines are found at the junction of the valve face and mantle (Figure 2a–c). Hymenes occluding the areolae on the outside, flush with the surface of the valve face masking the areolae pattern if they are completely intact, and not corroded. Raphe sternum central and more or less broad without projecting outside or inside the valve surface (Figure 2d–e). Raphe fissures gently sigmoid including the curving central ends and terminal fissures (Figure 2a–d). Inner raphe fissure simple, nowhere twisted, almost straight but with shortly deflected central ends (Figure 2e). The open copulae of the cell girdle seem to be unperforated.

All the fine structures of *N. adamantiformis*, including the marginal linking spines, leave no doubt whatsoever that *Navigiolum* Lange-Bertalot *et al.* 2003 is the appropriate genus for this diatom. Other presently-known naviculoid genera that possess linking spines as a facultative character are a few taxa in *Diadema* as well as *Luticola spinifera* (Bock) Denys and De Smet. Amongst the recently discovered species of *Navigiolum*, of which two are not yet described, only one other species has a wide central/axial area similar to that of *N. adamantiforme*; this is *N. spinosissimum* Lange-Bertalot, Cavacini, Tagliaventi and Alfinito from the Mediterranean island of Sardinia. However, all specimens of the latter are significantly larger (10–14 µm long, 4–5.6 µm broad, compared to 6–7 µm long and 4.5–5.4 µm broad) and have coarser striae (17–20 in 10 µm compared to 20–26 in 10 µm), and its valve outline is broadly lanceolate, never broadly rhombic lanceolate, as well as having much longer raphe branches.

Lange-Bertalot *et al.* (2003) were of the opinion that further species of *Navigiolum* might be detected in the

'reservoir' of *Navicula sensu lato*, and this has proved to be true in the case of *N. adamantiforme*. Taxa of *Navigiolum* are now also known from Algeria (unpubl. data).

Eolimna archibaldii sp. nov. (Figure 3)

Latin diagnosis: *Valvae strictae rhombico-ellipticae vel rhombico-lanceolatae apicibus cuneatis denique simpliciter rotundatis non protractis. Omnia individua minutissima comparate cum speciebus similibus. Longitudo 5.5–6.5 µm, latitudo 2.8–3.5 µm. Area axialis angustissima linearis, area centralis vix aspectabilis microscopio photonico. Striae transapicales difficulter discernendae cum illuminatione obliqua. Aspectus ultramicroscopicus externus vide Figure 2a–h. Frustula aspectu cinguli angusta ita ut typus generis et ut Eolimna subminuscule associata in praeparato. Area axialis aliquid dilatata ad mediam valvae, area centralis moderate parva circiter circularis. Raphosternum paulo elevatum super faciem valvae. Rami raphis modice curvati. Areolae uniseriatae in striis radiantes omnino, circiter 32 striae proximaliter ad 36 prope polos in 10 µm. Areolae cum foraminibus circularibus, circiter 70–90 in 10 µm.*

Typus: Praep. No. Af-299 Coll. Lange-Bertalot (FR)

Locus typicus: Vaal River at Klipplaatdrift (27°20'46"S, 26°25'50"E), Free State Province, South Africa (coll. date 15 March 2002).

Description

Valves strictly rhombic-elliptical to rhombic-lanceolate with cuneate, simply rounded, never protracted ends. Length 5.5–6.5 µm, width 2.8–3.5 µm. Axial area very narrow, linear; central area appearing barely developed. Striae difficult to discern in LM (Figure 3k–y), barely visible with oblique lighting, but impossible to enumerate. Under SEM the external raphe fissure is gently curved and located proximally in a slightly raised sternum (Figure 3c–f). The central raphe endings are slightly deflected to the primary valve side, while the terminal endings are deflected to the secondary side (Figure 3b). The small, almost circular central area is symmetrically confined by weakly radiate striae which become parallel towards the poles. The margin of the valve face is slightly raised with the mantle continuing at 90° to the valve face (Figure 3f). The mantle is perforated by a series of single poroids, in line with the striae (Figure 3f). Internally, the raphe fissure is straight with slightly deflected central endings (Figure 3i–j). The raphe terminates at the poles in small helictoglossae.

This species is named in honour of the South African diatomist REM Archibald, whose valuable contribution to the study and knowledge of South African diatoms has in the past been largely unrecognised.

Eolimna archibaldii is readily distinguished from other *Eolimna* spp., or other morphologically similar species currently included in *Navicula sensu lato*, some of which await revision or transfer to a more appropriate genus. For a description of *Eolimna* Lange-Bertalot & Schiller, see Moser *et al.* (1998).

(a) *Navicula lamii* Manguin, and its probable synonym *Navicula pascuorum* Cholnoky (Archibald 1971), is longer (8 µm on average) and is characterised by an

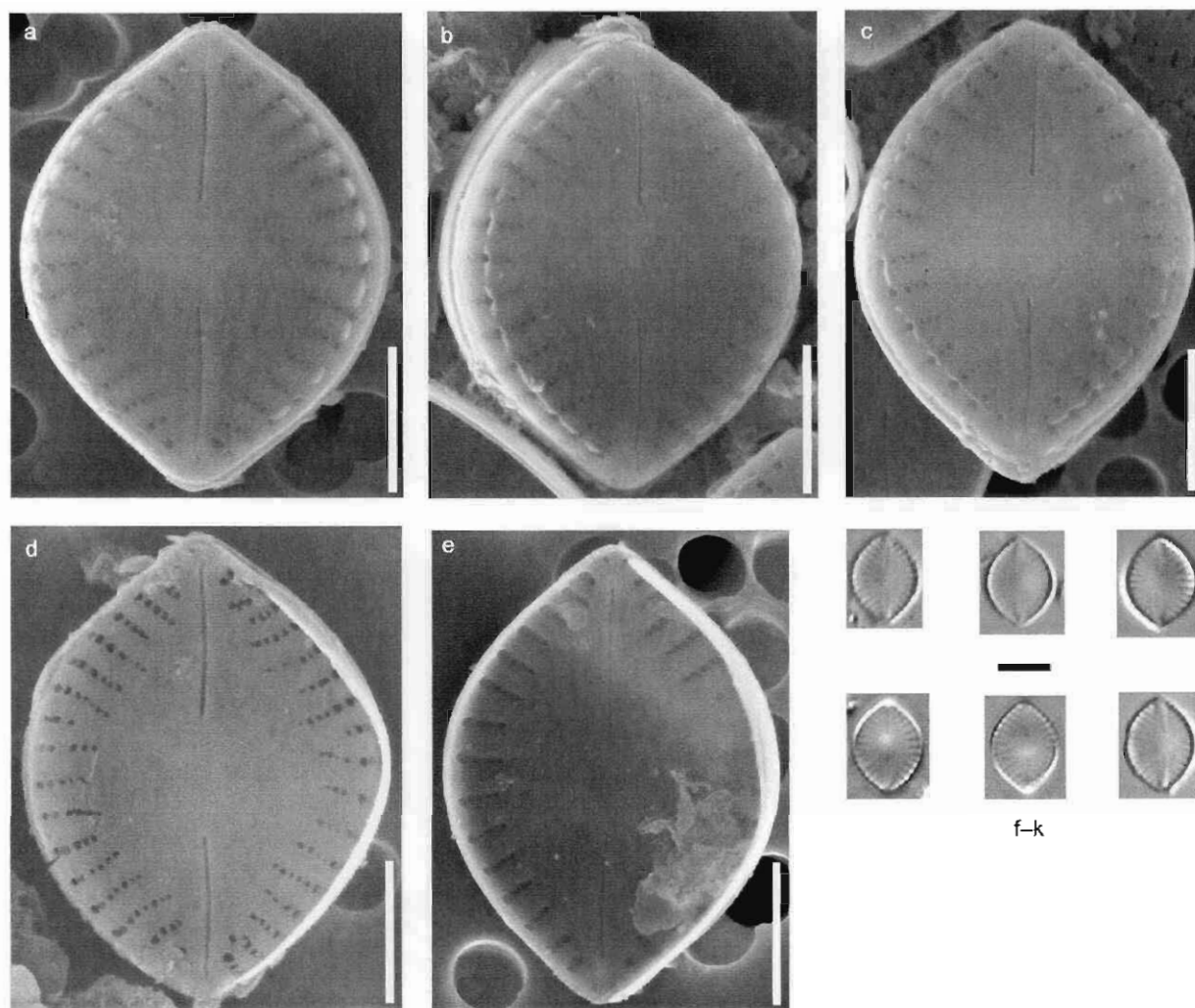


Figure 2: *Navigiolum adamantiforme* comb. nov. (a–d); external view of the valve as observed with scanning electron microscopy; (e) inner valve face as observed with scanning electron microscopy; (f–k) light microscope images. All scale bars = 2µm

- elliptical to linear-elliptical rather than rhombic to rhombic-elliptical outline. In addition, the striae of *N. lamii* are not visible under a light microscope
- (b) *Navicula* (*Fallacia*?) *indifferens* Hustedt has an elliptic-lanceolate valve outline and about 40 striae/10µm
- (c) *Navicula subminuscula* Manguin, now *Eolimna subminuscula* (Manguin) Moser, Lange-Bertalot & Metzeltin, syn. *N. frugalis* Hustedt (*sensu* Schoeman and Archibald 1976) is 7–12µm long and has 15–26 striae/10µm
- (d) *Navicula perparva* Cholnoky *non* Hustedt, also a synonym of *E. subminuscula*, is longer than 7µm and broader than 5µm, and has about 24 striae/10µm
- (e) *Navicula hecateia* Cholnoky is similar in outline to *E. archibaldii* but is, on average, longer (6.5–9µm) and slightly narrower (2.5–3µm). In addition, its striae are slightly coarser (28/10µm)
- (f) *Navicula muralis sensu* Hustedt, synonym *Mayamaea atomus* (Kutzing) Lange-Bertalot var. *permitis* (Auth) is 4–5µm broad
- (g) *Navicula seminuloides* Hustedt has 20–24 coarse striae/10µm and is longer and broader on average than *E. archibaldii*
- (h) *Navicula minuscula* Grunow has slightly radiate striae becoming strongly convergent at their proximal ends
- (i) *Navicula ventosa* Hustedt, syn. *Chamaepinnularia evanida* (Hustedt) Lange-Bertalot possesses only two areolae in each of the striae
- (j) *Navicula difficillima* Hustedt is 8–15µm long, with a linear or linear-elliptic valve shape
- (k) *Navicula pseudoventralis* Hustedt is linear-elliptic, 8–25µm long, 4–5µm broad with 18–20 striae/10µm
- (l) *Navicula mucicoloides* Hustedt is more than 8µm long, 6.5µm broad, and has c. 25 striae/10µm
- Navicula perparva* Hustedt, *Navicula seminuloides* Hustedt variety *sumatrana* Hustedt, *Navicula muralis sensu auct. non* Grunow, *Navicula vaucheriae* Petersen and *Navicula pseudomuralis* Hustedt are also morphologically similar.

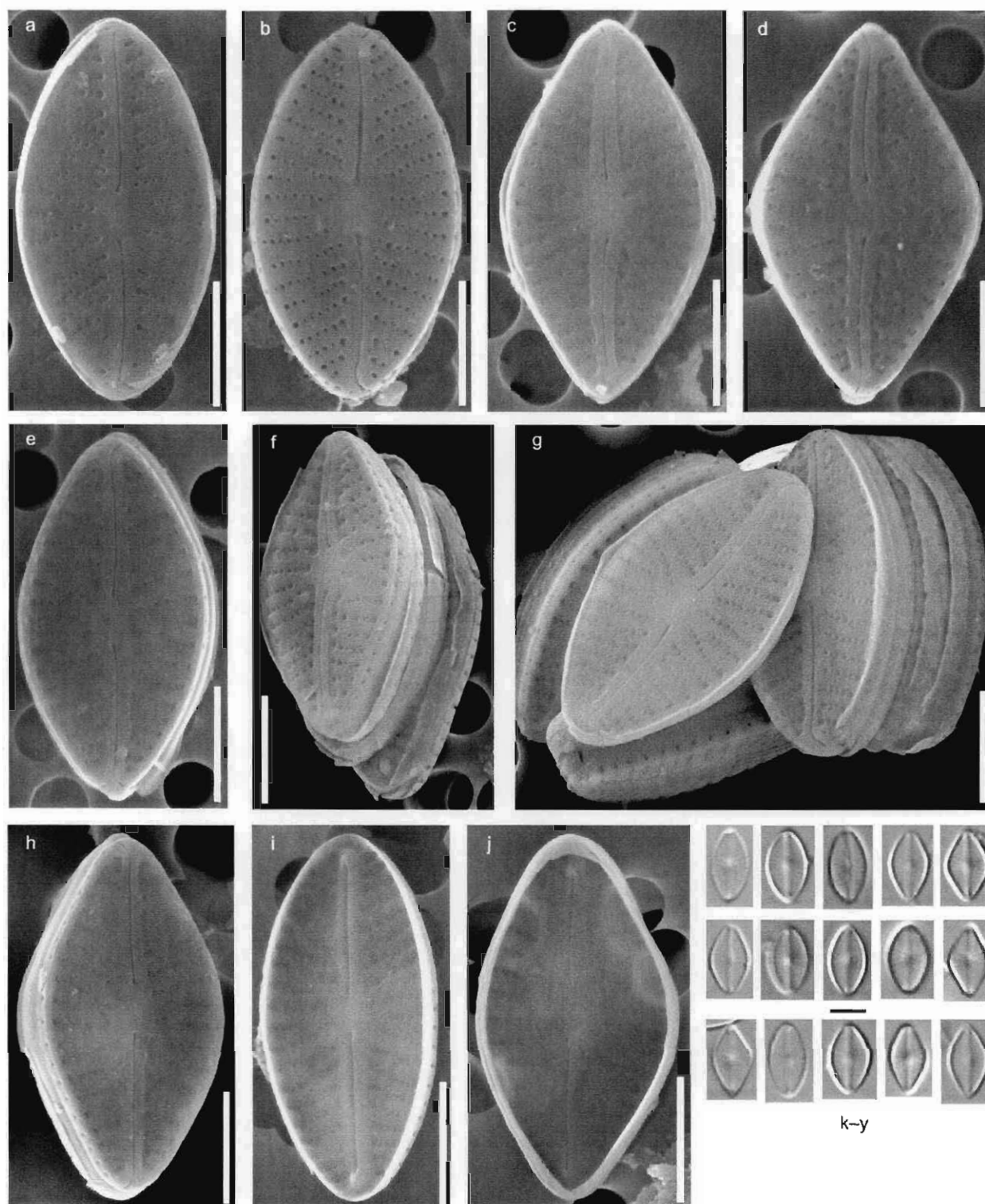


Figure 3: *Eolimna archibaldii* spec. nov.; (a–h) external view of the valve as observed with scanning electron microscopy; (i–j) inner valve face as observed with scanning electron microscopy; (k–y) light microscope images. All scale bars = 2 μm

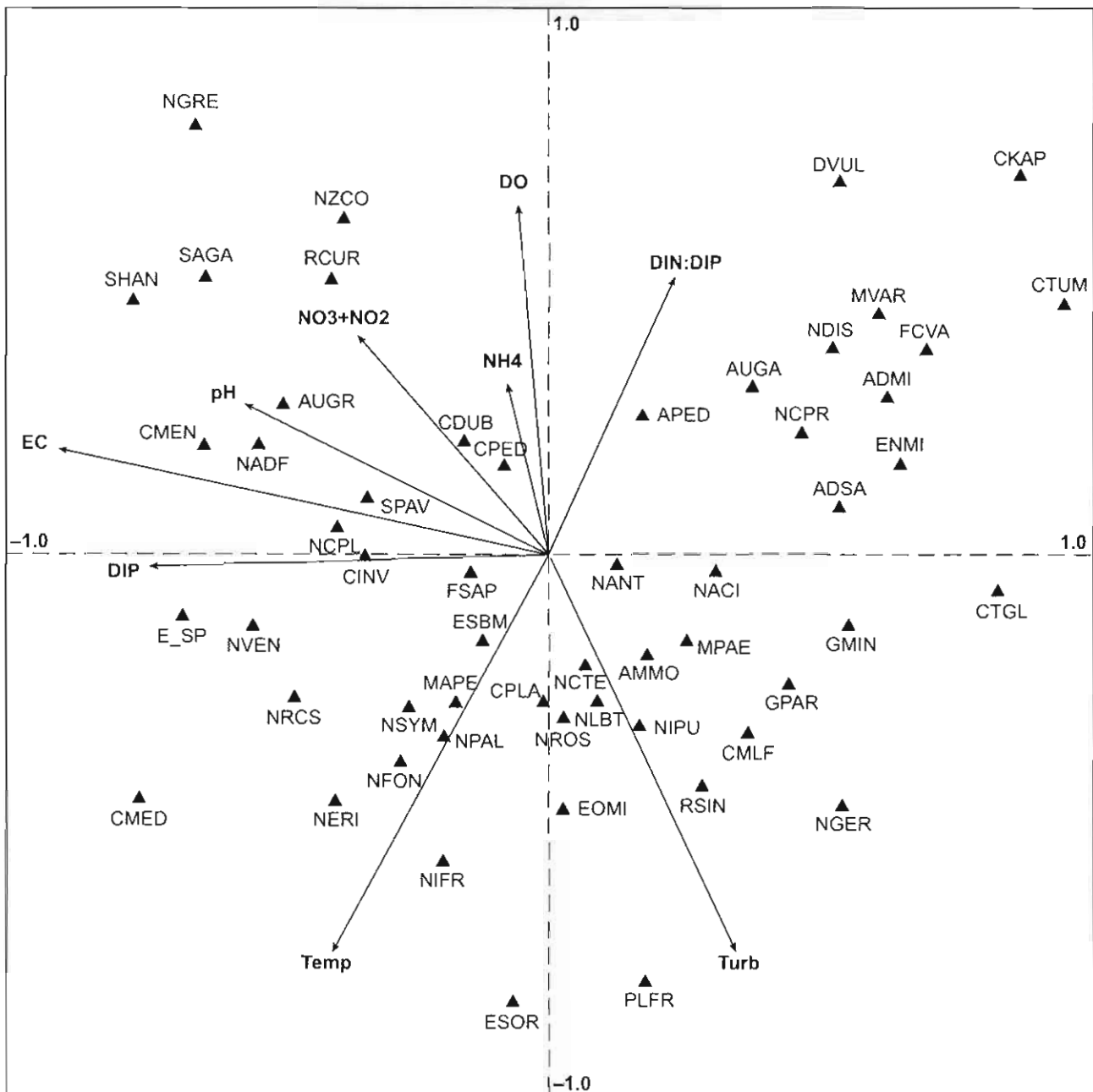


Figure 4: Canonical Correspondence Analysis (CCA) ordination diagram showing the relationship between measured environmental variables and major diatom species (species weight range from 3–100%) in the Vaal River. Explained variances of the first four axes are given in Table 3. Explanations of the abbreviations may be found in OMNIDIA version 3.1 (also see Table 1)

Discussion

Eolimna archibaldii may very well have already been observed on numerous occasions from South African habitats by workers such as Chohnoky, Schoeman or Archibald, but identified under another established *Navicula* taxon. This is borne out by comments made by Archibald (1971) who, when discussing *Navicula lamii*,

says that 'the small hyaline *Navicula* forms are very difficult to identify with certainty, the shape and the dimensions of the valve being variable, with the result that the same species can be described under different names by more than one author'. The difficulties of identification of these small species led Chohnoky, for example, to treat both *Navicula indifferens* and *Navicula pascuorum* as synonyms of *Navicula lamii* (Archibald 1971). Schoeman

(1969) illustrated and described his particular concept of *Navicula indifferens* which he encountered in South Africa. The species Schoeman discussed and illustrated as *N. indifferens* has a size range and outline very similar to *Eolimna archibaldii*. That the small, hyaline *Navicula* species referred to above are in fact different, on examination with SEM, is without doubt, and the continuous lumping or grouping of these small species under a single taxon in samples examined from South Africa may have led to the masking of many novel species, including *Eolimna archibaldii*, as described here.

Much of the South African literature concerning diatoms is directed at identifying new and rare species in a particular sample. Thus, common species receive only passing mention in the text, whilst the illustrations are limited to novel species (e.g. Archibald 1966). This is understandable when the amount of effort necessary to produce detailed line drawings is taken into account. Works of this nature may therefore have created the false impression that the majority of the diatom species encountered in South African waters are endemic to this country.

Schoeman (1982) gives a summary of diatom taxa occurring in the Jukskei-Crocodile River system, with the following stated intention that '... others working on this river system may have some benefit by having some reference to the extent of the diatom flora'. If Schoeman's checklist is examined, it is found that the majority of the taxa are encountered in both Europe and South Africa and, further, that his list of diatom taxa bears a remarkable similarity to the list of taxa encountered in the Vaal River. Diatom species occurring in close association with *Eolimna archibaldii* and *Navicula adamantiforme* include *Nitzschia palea* (Kützinger) W. Smith, *Eolimna subminuscule*, *Mayamaea atomus* var. *permitis*, *Navicula recens* (Lange-Bertalot) Lange-Bertalot, *Gomphonema parvulum* Kützinger, *Nitzschia fonticola* Grunow in Cleve and Möller, *Navicula erifuga* Lange-Bertalot and *Nitzschia capitellata* Hustedt in A. Schmidt *et al.* These species are characteristic indicators of polluted fresh waters in Europe and Japan (Lange-

Bertalot 1979, Kobayasi and Mayama 1982) as well as in South Africa (Schoeman 1979).

Canonical Correspondence Analysis (CCA) was used to demonstrate the relationships between the dominant (>5 %) diatom species of the Vaal River system and the physical and chemical variables measured in the river over the study period, and to construct an ordination diagram (Figure 4). Measured values (mean, median, minimum and maximum) for the physical and chemical variables (n = 113) in the Vaal River are presented in Table 2. These values include the calculated mean for the chemical variables, and this value can be equated with the epicentre of the CCA plot. The first four axes of the species-environment plot accounted for 72% of the total variance in the community due to measured environmental variables (Table 3). It should be noted that *N. adamantiforme* (indicated as NADF in Figure 3) and *E. archibaldii* (indicated as E_SP in Figure 4) ordinate with pollution-tolerant species on a pollution gradient in the CCA, and are associated with elevated levels of electrical conductivity (EC), dissolved inorganic phosphate (DIP) and pH.

Conclusion

Amongst the diatom communities found in polluted waters in South Africa, some species, such as *Eolimna archibaldii* and *Navicula adamantiforme*, may be endemic to South Africa, but most others are cosmopolitan.

Table 3: Results from a CCA analysis on diatom community data and measured environmental variables (n = 113)

Axes	1	2	3	4
Eigenvalues	0.42	0.30	0.19	0.17
Species-environment correlations	0.86	0.82	0.74	0.76
Cumulative percentage variance				
of species data	4.50	7.80	9.90	11.80
of species-environment relation	27.80	47.70	60.60	72.00

Table 2: Measured environmental variables in the Vaal River (n = 113)

Variable	Unit	Mean	Std error	Median	Std deviation	Range	Min.	Max.
Temperature (Temp)	°C	18.25	0.51	19.40	5.51	21.10	7.80	28.90
Dissolved oxygen (DO)	mg l ⁻¹	8.55	0.24	7.95	2.55	13.94	2.27	16.21
Turbidity (Turb)	NTU	44.44	5.33	22.55	57.42	401.56	3.44	405.00
pH		8.25	0.04	8.21	0.38	2.22	7.13	9.35
TAL	mg l ⁻¹	111.62	3.15	114.45	34.12	167.88	33.42	201.31
NO ₃ -N+NO ₂ -N	mg l ⁻¹	0.45	0.06	0.15	0.67	3.17	0.03	3.20
NH ₄ -N	mg l ⁻¹	0.05	0.00	0.03	0.04	0.24	0.03	0.27
Dissolved inorganic nitrogen (DIN)	mg l ⁻¹	0.51	0.06	0.19	0.69	3.17	0.06	3.23
Dissolved inorganic phosphate (DIP)	mg l ⁻¹	0.09	0.01	0.06	0.08	0.32	0.01	0.33
DIN:DIP		6.62	0.62	5.17	6.66	55.50	0.20	55.69
Na ⁺	mg l ⁻¹	34.52	2.29	26.69	24.80	86.02	3.05	89.07
Mg ²⁺	mg l ⁻¹	17.47	0.80	16.61	8.65	32.15	2.73	34.88
SO ₄ ²⁻	mg l ⁻¹	89.50	7.12	58.44	77.01	253.02	5.19	258.21
Cl ⁻	mg l ⁻¹	33.80	2.45	25.80	26.50	75.28	6.00	81.28
K ⁺	mg l ⁻¹	6.34	0.37	5.03	3.99	12.95	0.50	13.44
Ca ²⁺	mg l ⁻¹	37.32	1.92	31.89	20.76	68.39	9.66	78.05
Electrical conductivity (EC)	mS m ⁻¹	50.00	2.45	45.40	26.49	90.37	10.63	101.00

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CHAPTER 9

CONCLUSIONS AND RECOMMENDATIONS

CHAPTER 9 - CONCLUSIONS AND RECOMMENDATIONS

From the preceding Chapters it can be concluded that the application of diatoms for biomonitoring has achieved a growing interest in South Africa, to the extent that this technique has been used as part of the National River Health Programme (River Health Programme, 2005; www.csir/rhp). It is hoped that this technique will continue to be used to indicate water quality around the country. However, it should be immediately pointed out that much work remains to be done in South Africa, especially when examining cleaner and unpolluted waters (see Chapter 5). Only a few endemic taxa have been observed as dominant species within diatom assemblages in recent years (Taylor, 2004; Bate *et al.*, 2002), this is more than probably due to the interest in monitoring impacted and anthropogenically influenced waters, which are far more likely to be dominated by cosmopolitan, tolerant species. However, dominance of communities by cosmopolitan taxa is not always the case (see Chapter 8).

Greater attention should be paid, in the future, to determining the distribution and ecological requirements of endemic South African diatom species. As this information becomes known it may then be resolved into numerical diatom indices (e.g. Descy, 1979), or such data-sets may be used for the typology of diatom communities and the development of models to determine the deviation of diatom communities from the natural state (e.g. Tison *et al.*, 2005). In addition, further attention should be paid to both the biology and ecology of diatoms occurring in a variety of habitats impacted by different types of pollutants. It has been shown in Chapter 7 that the diatoms can be used to measure impacts (and recovery) of acid mine drainage on a river system. After further investigations diatoms could possibly be used to indicate the type and impact of pollution by South Africa's very active mining industry on water resources.

The standardisation of methodology has laid the foundation for the continued and meaningful collection and analysis of diatom samples in South Africa. Material collected in a reliable fashion can be used with great effect both at present and possibly in future by other workers.

The use of historical diatom records has been demonstrated by the re-assessment of historical diatom data collected by B. J. Chohnoky as presented in Chapter 6. The further use of historical data generated by Chohnoky, R. E. M. Archibald and F. R. Schoeman (located in the South African Diatom Collection; Harding *et al.*, 2004), now its value has been demonstrated, should be encouraged to determine the past condition of rivers and streams. This data can also serve as a valuable benchmark to determine loss of biodiversity.

The continued collection of diatom samples from around South Africa for water quality determination should be complimented with sound taxonomical investigations of these samples

to continually update and add to the knowledge of South Africa's diatom flora. Not only should the common indicator taxa, such as those illustrated in Appendix 1, be studied but also the endemic diatoms which may prove to be valuable indicators of water quality (Chapter 8).

The analysis of historical data and comparison to recent samples could be used to determine relative loss (or otherwise) of diatom species from a particular biotope and may lead to the classification of an ecosystem as endangered, in terms of the new biodiversity act (National Environmental Management: Biodiversity Act, 2004), even though traditional measures of ecosystem health used in South Africa may indicate a functional ecosystem. Such an action could be seen as one of the first steps towards meaningfully conserving microorganisms, this can only be achieved by an holistic approach to conserving all aspects of the biotope in which the organisms occur. The minister of the Environment may set indicators to measure compliance with norms and standards in the new biodiversity act. In the future, diatoms may prove to be an apt indicator for the integrity of aquatic resources and communities.

It would be perhaps fitting to close with a quotation from Cholnoky (1960) the father of diatom studies in South Africa. "...it should be pointed out that changes in one or other of the factors which have been discussed here [pH, salinity and nutrient concentrations] need not necessarily bring about the death of one or other of the algal species **so long as the changes remain within the limits occurring in nature**. On the contrary, these changes will inhibit the multiplication of some of the species originally present, and encourage that of others, so that primarily the association i.e. the percentage composition and not the flora as such, will be changed". The quotation summarises the very assumption on which diatom methods for biomonitoring is based. However, in terms of biodiversity another key point stands out. Changes within ecosystem should remain within the limits occurring in nature; otherwise these changes will result in the death and disappearance of algal species.

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