Evaluation of the applicability of diatom based indices as bioindicators of water quality in South African rivers

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Thesis submitted for the degree Philosophiae Doctor in
Environmental Sciences and Management
at the North-West University

Promoter: Dr. André Vosloo
Co-promoters: Dr. Chris Dickens
Prof. Leon van Rensburg

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Potchefstroom

DEDICATION

I would like to dedicate this thesis to my Lord Jesus Christ, who not only instructs me in fact but also in the Truth that surpass human wisdom.

*Rom: 1:20: "For ever since the creation of the world His invisible nature and attributes, that is, His eternal power and divinity, have been made intelligible and clearly discernable in and through the things that have been made. So men are without excuse." **(Amplified Bible)***

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During the studies that resulted in this document, I once again realised that there are few things in life that I can claim as being truly my own. At each step during this journey I was influenced and supported by many individuals, each contributing to my studies, personal views and interpretations of science and the world as a whole. A great many of my spiritual family in the Lord supported with prayer at times when motivation dwindled or when seemingly dead ends stared me in the face. To these friends I am truly grateful for giving me the most priceless support of all.
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ABSTRACT

Diatoms have been proven to be reliable indicators of water quality in many countries of the world particularly Europe. The potential use of diatoms as indicators of water quality in South Africa was tested in the studies in this document. This study evaluates the potential use of diatom based indices by testing it against a macroinvertebrate index (SASS 5) and evaluating the variation in the index scores of the two indices due to changes in chemical water quality and habitat. It was concluded that the diatom monitoring system performs well as bioindicator of water quality. It was also concluded that it should be used as a complementary system to the much used SASS 5 invertebrate index. This conclusion was made due to the fact that diatoms react more directly to changes in water quality than macroinvertebrates (SASS 5), and macroinvertebrates react more readily to changes in habitat than diatoms.

A further part of the study was to assess whether aut-ecological or diversity based diatom indices performed best in South African conditions. This study found that the ecological indices were more sensitive to changes in water quality than the diversity indices. The diatom based indices that performed best as water quality indicators were the specific pollution sensitivity index (SPI) and the biological diatom index (BDI). A standard method for the sampling, preparation and enumeration for diatoms to be used for index score generation is also suggested to ensure the comparability of diatom based index data to facilitate use of such biomonitoring data for management purposes.

The main focus of the study was to eliminate some of the obstacles for the use of diatoms as bioindicators of water quality in South Africa. It is believe that this aim has been accomplished in the study.

Keywords: diatoms; Bacillariophyceae; bioindicators; SASS 5; species diversity indices; water quality; aut-ecological indices.

UITTREKSEL

Daar is reeds in verskeie lande, veral in Europa, bewys dat diatome betroubare bioindikatore van waterkwaliteit is. In hierdie studie is die moontlike gebruik van diatome as indikatore van waterkwaliteit in Suid-Afrika ondersoek. Die studie evalueer die potensiële gebruik van diatoomindekse deur die resultate wat daarmee verkry is, te vergelyk met die resultate wat verkry is deur die gebruik van 'n makroinvertebraatindeks (SASS 5), en deur die verskille tussen die twee indekse as gevolg van veranderinge in chemiese waterkwaliteit en habitat te evalueer. Die gevolgtrekking word gemaak dat die diatoommoniteringsisteem goed vaar as 'n bioindikator van waterkaliteit in Suid-Afrikaanse riviere, en dat dit as 'n aanvullende sisteem tot die gewilde SASS 5 invertebraat-indeks gebruik behoort te word. Hierdie gevolgtrekking is gemaak op grond daarvan dat diatome meer direk op waterkwaliteit reageer as makroinvertebrate (SASS 5), maar dat makroinvertebrate weer tot 'n groter mater deur veranderinge in habitat beïnvloed word as diatoom indekse.

'n Verdere deel van die studie was om te bepaal of beter resultate met out-ekologiese indekse of spesiediversiteitsindekse in Suid-Afrikaanse toestande verkry word. Hierdie studie het bevind dat ekologiese indekse meer sensitief is vir veranderinge in waterkwaliteit as diversiteitsindekse. Die indekse wat hulself as goeie indikatore van waterkwaliteit bewys het, was die spesifieke besoedelingsensitiwiteitsindeks (*specific pollution sensitivity index*; SPI) en die biologiese diatoomindeks (*biological diatom index*; BDI).

'n Standaard metode vir die versameling, voorbereiding en kwantifisering van diatome tydens die saamstel van 'n indekstelling word ook voorgestel. Die doel hiervan is om die vergelykbaarheid van diatoomgebaseerde indeksdata te verseker, ten einde die gebruik van sulke biomoniteringsdata vir bestuursdoeleindes moontlik te maak.

Die hooffokus van die studie was om sommige van die struikelblokke, wat tans verhinder dat diatome as bioindikatore van waterkwaliteit gebruik word, uit die weg te ruim. Daar word vertrou dat die studie in hierdie doel slaag.

Sleutelwoorde: diatome, Bacillariophyceae, bioindikatore, SASS 5, diversiteitsindekse, waterkwaliteit; out-ekologiese indekse.

PREFACE AND PERMISSION LETTERS

The article format was chosen for this thesis. The research reported in this thesis was done in conjunction with other scientists that are listed as co-authors of the mentioned articles. The extent of involvement in each article is as follows:

1) Title: Determining the possible application value of diatoms as indicators of general water quality: A comparison with SASS 5
   Authors: PA de la Rey, JC Taylor, A Laas, L van Rensburg and A Vosloo
   Published in Water SA, July 2004, Vol. 30, No. 3, pages 325-332
   Contribution of PA de la Rey: Concept, sampling, data analysis and writing of article

2) Title: Recommendations for the collection, preparation and enumeration of diatoms from riverine habitats for water quality monitoring in South Africa
   Authors: Jonathan C Taylor, P Arno de la Rey and Leon van Rensburg
   Published in the African Journal of Aquatic Science, 2005, Vol. 30, No. 1, pages 65–75
   Contribution of PA de la Rey: Concept, desktop study, contributed to writing of article

3) Title: Can diatom-based pollution indices be used for biomonitoring in South Africa? A case study of the Crocodile West and Marico water management area
   Authors: J.C. Taylor, J. Prygiel, A. Vosloo, P.A. de la Rey & L. van Rensburg
   Published in Hydrobiologia, 2007, Vol. 592, pages 455-464
   Contribution of PA de la Rey: Concept, sampling, contributed to data analysis and general management of project

4) Title: On the use of diatom-based biological monitoring. Part 1: A comparison of the response of diversity and aut-ecological diatom indices to water quality variables in the Marico-Molopo River catchment

Authors: PA de la Rey, L van Rensburg and A Vosloo

Published in Water SA, 2008, Vol. 34, No. 1, pages 53-60

Contribution of PA de la Rey: Concept, diatom sampling and analysis, data interpretation, writing of article

5) Title: On the use of diatom-based biological monitoring. Part 2: A comparison of the response of SASS 5 and diatom indices to water quality and habitat variation

Authors: PA de la Rey, H Roux, L van Rensburg and A Vosloo

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Contribution of PA de la Rey: Concept, diatom sampling and analysis, data interpretation, writing of article.

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TO WHOM IT MAY CONCERN

22 October 2007

Dear Sir or Madam:

PERMISSION TO PUBLISH RESEARCH PAPER

The undersigned hereby give permission to Mr. P.A. de la Rey to submit the research article listed below (published in *Water SA, 2004*) as part of the degree Ph.D. in Environmental Sciences at the North-West University, Potchefstroom Campus.

> *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*
>
> Authors: PA de la Rey, JC Taylor, A Laas, L van Rensburg and A Vosloo
>
> Published in Water SA, July 2004, Vol. 30, No. 3, pages 325-332

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Yours sincerely,

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

Yours sincerely,

03.10.06

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i. 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

Yours sincerely,

le 2/10/2006

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i. A comparison of the response of diversity and aut-ecological diatom indices to water quality variables in the Marico-Molopo River catchment

Yours sincerely,

Mrs. H. Roux

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iii. A comparison of the response of diversity and aut-ecological diatom indices to water quality variables in the Marico-Molopo River catchment

iv. 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

v. A comparison of the response of SASS 5 and diatom indices to water quality and habitat variation.

Yours sincerely,

Dr. A. Vosloo

Prof. L. van Rensburg

Dr. J.C. Taylor

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GLOSSARY

Aut-ecological indices: Aut-ecological indices use the relative abundance of species in Assemblages and their ecological preferences, sensitivities, or tolerances to infer environmental conditions in an ecosystem (Stoermer & Smol, 1999).

Biodiversity: in different contexts may denote: the number of different species present in a given environment (species diversity); the genetic diversity within a species (genetic diversity); the number of different ecosystems present in a given environment (ecological diversity) (Lawrence, 1995).

Biological indicators: communities, whether plant or animal, with a narrow range of ecological tolerance that may be selected for emphasis and monitored because their presence and relative abundance serve as barometer of ecological conditions (Barbour et al., 1999).

Biological integrity: the ability of an ecosystem to support and maintain a balanced and adaptive community of organisms, having species diversity, composition and functional organization comparable to that of the natural habitats of the region (Karr & Dudley, 1981).

Biomonitoring: the use of a biological entity as a detector and its response as a measure to determine environmental conditions. This is usually done through biological surveys and toxicity tests (Barbour et al., 1999).

Biotic indices: Biotic indices are constructed when each taxon from a particular group of organisms is assigned to a sensitivity rating or 'score' based on the tolerance or sensitivity to particular pollutants. The scores of all the individual taxa at a site are summed and/or averaged to provide a value by which the integrity of the biotic community at the site can be gauged (Ollis et al., 2006).

Diatom: common name for a member of the class Bacillariophyceae, a group of algae characterized by delicately marked thin double shells of silica (Lawrence, 1995).

Ecological integrity: the ability of the physical, chemical and biological components of an ecosystem to support and maintain a balanced, adaptive community of organisms having a species composition, diversity and functional organization comparable to that of natural ecosystems within a region (Meyer, 1997).

Ecosystem health: a healthy ecosystem is sustainable and resilient, maintaining its ecological structure and function over time while continuing to meet social needs and expectations. This concept explicitly incorporates both ecological intetrity (maintaining structure and function) and human values (what society avlues in the ecosystem), (Meyer, 1997).

Ecosystem stability: ability of an ecosystem to withstand or recover from changes or stresses imposed from outside (Lawrence, 1995).

Habitat: the locality or environment in which a plant or animal lives (Lawrence, 1995).

Macroinvertebrates: any invertebrate of invertebrate larva whose size is measures in millimetres or centimetres rather than microscopic units. Such species are on of the main groups of organisms sampled in surveys of water quality (Lawrence, 1995).

Pollution: any harmful or undesirable change in the physical, chemical or biological quality of air, water or soil as a result of the release of e.g. chemicals, radioactivity, heat, large amounts of organic matter (as in sewage). Usually applied to changes arising from human activity although natural pollutants, e.g. volcanic dust, sea salt are known (Lawrence, 1995).

Resilience: ability of a living system to restore itself to its original condition after begin disturbed (Lawrence, 1995).

Species: organisms forming a natural population or group of populations that transmit specific characteristics from a parent to a offspring (Barbour et al., 1999).

Species diversity: the number and abundance of different species within a given area, which is one measure of biological diversity, a diverse environment having relatively small numbers of many different species (Lawrence, 1995).

Species evenness: uniformity in the distribution of individuals among the species encountered (Metcalfe, 1989).

Species richness: the number of different species within a given community or area (Lawrence, 1995).

Taxa: the members of any particular taxonomic group e.g. a particular species, genus, family (plural of taxon), (Lawrence, 1995).

Water Quality: Water quality is the combined effect of the physical attributes and chemical constituents of a sample of water. The idea of water quality is a human construct, implying value or usefulness, and indeed the quality of any sample of water depends on the point of view of the user. A water quality variable is any of those attributes of constituents that vary in magnitude and whose variations alter water quality (Dallas & Day, 1993).

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Chapter 1
Introduction

1. INTRODUCTION

1.1 Fresh water management

Water is one of the most important resources for life and economic growth. It is important to realise that freshwater is a finite resource with little potential for increase. More than 99% of the water on earth occurs either in the ocean or polar deposits and is not easy to utilise due to prohibitive cost of desalinisation and distribution of such water. The remaining 1%, constituting the freshwater resources of the world, collectively experience accelerating rates of quantitative and qualitative degradation. This degradation results to a large extent from both population growth and the expanding utilisation and consumption because of technological growth. Unless the demands of the rapid growth in water supply are rapidly controlled, a freshwater crisis at a global level is imminent (Wetzel, 1992).

In South Africa, the availability of fresh water is particularly limited. South Africa has been classified as a semi arid country due to a low average rainfall which is in the order of 450 mm annually. The fact that surface water is not evenly distributed across the country further exacerbates the problem. The need for the proper management of water is especially important for water stressed countries such as South Africa as it may well determine economic growth potential of the country in years to come. South Africa has long recognized that water is one of its prime limiting natural resources (Huntley et al., 1987; Department of Water Affairs, 1996a).

The fact that the decline in quality of available water is one of the major problems facing this country has been recognised by Davies and Day (1998) in their book “Vanishing Waters”. In the last ten years the potential crisis in freshwater quantity and quality has also been recognised internationally and governments throughout the world have reviewed their policies so as to achieve sustainability of water resources. This is especially true in the South African context where the government introduced the National Water Act (Act No. 36 of 1998), which dictates water resource policy and practise (Walmsley, 2000).

In the National Water Act (NWA), the Department of Water Affairs and Forestry (DWAF) takes the primary responsibility as custodians of water resources and its management in South Africa. The tenor of the democratic reform process and the underlying cornerstone of the government's water law reform process is encapsulated in a preliminary section of the NWA, which states that the National Government is the public trustee of the nation's water resources and is to "…ensure that water is protected, conserved, managed and controlled in a sustainable and equitable manner for the benefit of all persons in accordance with its constitutional mandate" (DWAF, 1996a). With respect to water quality, the mission of DWAF is to ensure the fitness of South Africa's surface water, groundwater and coastal marine resources, for water uses and for the protection of aquatic ecosystems on a sustainable basis (DWAF, 1996a). For the purpose of the current study, priority will be given to surface water resources and even more specifically lotic (riverine) aquatic ecosystems.

Water quality

According to the South African Water Quality Guidelines (DWAF, 1996b), the term *water quality* is used to describe the physical, chemical, biological and aesthetic properties of water that determine its fitness for a variety of uses and for the protection of the health and integrity of aquatic ecosystems. Many of these properties are controlled or influenced by constituents that are either dissolved or suspended in water. The guideline furthermore defines the term water quality constituent as any of the properties of water and/or the substances suspended or dissolved in it.

From the above-mentioned we can deduce that the quality of water is subjective and that it leans heavily on requirements of the user of the water resource. Traditionally water quality was used to describe water that is suitable for use for human activities such as domestic, agricultural and industrial use (Hohls, 1996). For this reason, water quality monitoring constituted mainly of chemical analysis of grab samples to assess the usability of water for the various mentioned applications. The main reason for this was that, although the behaviour of water constituents are complex, it has been studied well and can therefore be monitored fairly easily and predicted with some degree of confidence (Dallas & Day, 1993).

Even though, as mentioned in the previous section, the mandate of DWAF includes the protection of the aquatic ecosystem, the management of water resources will firstly be done in the spirit of Batho Pele (People First). This point was reiterated by Minister Buyelwa Sonjica, (Minister of Water Affairs and Forestry) in a speech at the International Conference on Water for Food & Ecosystems in 2005 on the reconstruction and development of our country. In closing she stated that: "I would like to end by saying that the South African slogan Batho Pele (People First) must be upheld, because if we put people first we will ensure sustainable utilisation and protection of our limited water resources to support social and economic activities. There is a slogan used by my Department, which encapsulates the approach we are trying to implement. It is an approach that balances economic, social and environmental needs in the use of water. It is an approach that sees the protection of the aquatic ecosystem as integral to the sustainable production of food, the sustainable development of rural communities, the future of the country. The slogan is a simple one, but a powerful one, and one that carries a message for all of us. The slogan is 'Ensuring some for all for ever, together'"(Sonjica, 2005).

The above-mentioned quote makes important points. Firstly it establishes the main beneficiary of water quality management namely man. It is therefore logical that tools (such as indices) that provide information for the management of water resources should have the suitability of water resources for the use of man as focus. High levels of dissolved and suspended constituents in water limit the use of water from a human perspective and therefore can be classified as relatively poor water quality. The opposite is true for good water quality. It is in this context that the term 'water quality' is used throughout the thesis.

The second important point that is made is that the means by which to ensure sustainability of water is by protecting the environment, especially aquatic ecosystems.

Aquatic Ecosystems

There is widespread evidence that freshwater ecosystems, and rivers in particular, are amongst the most threatened ecosystems (Ollis et al., 2006a). The NWA however recognised that in order to protect the full range of “goods and services” (e.g. provision of water, disposal of waste, supply of fish, plants and other biota) provided for humans by rivers, the entire ecosystem must be protected. Probably the most important benefit to be gained from properly functioning ecosystems is that such a system can perform a self cleansing function and, if protected, can replenish the resource (Malan & Day, 2002). In many ways the focus on ecosystem health complicated the monitoring and management of surface water resources. The main reason for this being that aquatic ecosystems are highly complex and variable and that a multitude of interrelated physical, chemical and biological factors affect the ecological integrity of such ecosystems (Ollis et al., 2006a). Many recent articles also suggest that the most effective way of protecting freshwater ecosystems and their biota is to focus policy directives and management actions on biological integrity (Ollis et al., 2006a; Karr, 1992).

1.3 Biological integrity

Karr and Dudley (1981) defined biological integrity as “the ability of an aquatic ecosystem to support and maintain a balanced, adaptive community of organisms having a species composition, diversity, and ecological functional organisation comparable to that of natural habitats within a region”. When human activities in aquatic ecosystems are minimal, the biological communities are resilient and continue to resemble those that were shaped by the interactions of their natural physio-chemical environment and are said to have biological integrity. If human activity impact heavily on an aquatic ecosystem, it may reach a point where the composition of the biological communities have been disrupted to the point where it will not be able to reach biological integrity and several wetland functions are diminished or lost (Karr, 2000).

The concept of biological integrity may also be connected to the concept of ecosystem stability. This is clear from the work of May (1976), in which it is stated that “‘ecosystem stability can be defined as the ability of a system to recover to an equilibrium state after disturbance, or simply the persistence of the system".

In many cases species diversity has been linked to ecosystem stability. The diversity-stability hypothesis asserts that species vary in their traits and that in a highly diverse (species rich) systems there will be some species than can compensate for the loss of others should disturbance occur in such a system (Pimm, 1984; Elton, 1958). Thus, species rich systems are more likely to be considered stable. Another common view of this hypothesis is that it predicts a decrease of diversity as pollution increases. The pollution intolerant species decline in abundance and the pollution tolerant species can grow rapidly without competition for space, nutrients, or other resources. This results in community abundance patterns of heavy dominance and fewer species (Van Dam, 1982). This being said, it is important to recognise that ecological systems are inherently complex, composed of many interacting biological and physical components. Predicting the behaviour of such complex systems is difficult but management and policy decisions require information on the status, condition, and trends of ecosystems (Anreasen et al., 2001).

As human activities degrade watersheds, the aquatic communities they support are modified to varying degrees (Adams et al., 1998; Onorato et al., 1998). Human activities can alter the interactions between organisms and their physio-chemical environment. When the interactions of aquatic plants and animals with their environment are disrupted, many of the functions provided by such are diminished or lost.

Ollis et al. (2006a), states that a multitude of factors affect the ecological integrity of river ecosystems and that these factors may be grouped into classes such as water quality, flow regime, habitat structure, biotic interactions and energy sources. Furthermore, according to Dallas and Day (1993), the actual species that make up any aquatic biological community are largely determined by:

i) water quality;

ii) the types of biotope availability;

iii) the degree of movement of water;

iv) historical distribution of species; and

v) other components of the biota.

It is therefore logical to assume that aquatic communities can integrate and reflect the effects of chemical and physical disturbances that occur in river and wetland ecosystems over extended periods of time. The biotic integrity or health of the biota inhabiting the river ecosystem provides a direct and integrated measure of the health of a river as a whole (Roux et al., 1999). Different taxonomic groups may therefore serve as assessment tools (Siligato & Böhmer, 2002). It has long been known that some components of the aquatic flora and fauna of streams and rivers respond in a predictable fashion to changes in the physical and chemical nature of water (Chutter, 1998). Due to the abovementioned relationships, it has been possible to construct biotic indices to assess one or more aspects of the aquatic environment. When such indices are used to assess ecological systems (for example a river) it is often referred to as biomonitoring.

1.4 Biomonitoring

It is a well established fact that important decisions should be based on sufficient data. But what should the nature of that data be, specifically in relation to South African water sources? Using indicators are an ideal means by which progress towards integrated water resource management can be monitored; providing a summary of conditions, rather like temperature and blood pressure are used to measure human health (Walmsley, 2000).

Matthews et al. (1982) define biomonitoring as “the systematic use of biological responses to evaluate (primarily anthropogenic) changes in the environment with the intent to use this information in a quality control programme”. Biomonitoring and bioassessment are based on the assumption that measurements of the response, condition and/or community integrity of biota can be used to assess the ecological integrity of an ecosystem (Ollis et al., 2006a).

Because of the difficulty of analysing for every potential pollutant in a sample of water, and of interpreting the results in terms of the severity of impact, it makes sense to turn to aquatic biota for assistance (Eekhout et al., 1996). The goal of a biological index is not to measure every possible biological attribute; doing so is indeed impossible. The goal of biological indices is to identify those biological attributes that respond reliably to human activities, are minimally affected by natural variability, and are cost effective measures (Karr & Chu, 1999). Biological monitoring can provide a low-cost, moderately sensitive mean of monitoring water quality (Gratwicke, 1999) and have been widely used to assess biological river quality (Bonada et al., 2006).

Numerous methods have been developed for the bioassessment of the integrity of aquatic systems. Some of these are based on some or other aspect of a single species, but most are based on the attributes of whole assemblages of organisms (De la Rey et al., 2004).

Several groups of aquatic fauna and flora have been used for the construction of biological indices in aquatic environments. These include fish (An et al., 2002; Kleynhans, 1999; Karr, 1981) macroinvertebrates (Chutter, 1998; Hilsenhoff, 1987; Chesters, 1980), diatoms (Lenoir & Coste, 1996; Kelly & Whitton, 1995; Coste & Ayphassorho, 1991; Coste *in* Cemagref, 1982; Descy, 1979) and vegetation (Kemper, 2001). Although some methods have been available for many years, biomonitoring has only recently become a routine tool in the management of South Africa's inland waters (Davies & Day, 1998).

There are several different approaches to use (aquatic) biological communities as bioindicators. These approaches can be broadly classified as follows (Roux et al., 1993):

- Bioassessments are based on ecological surveys of the functional and/or structural aspects of biological communities;
- Toxicity bioassays are a laboratory-based methodology for investigating and predicting the effect of compounds on test organisms;
- Behavioural bioassays explore sub-lethal effects of fish or other species when exposed to contaminated water; usually as on-site, early warning systems;
- Bioaccumulation studies monitor the uptake and retention of chemicals in the body of an organism and the consequent effects higher up the food chain; and

- Fish health studies deal with causes, processes and effects of diseases; and can form a complementary indication of overall ecosystem health.

The focus of the current study is on bioassessment indicators. Two of the most important indicator types in this category are biodiversity indices and aut-ecological indices.

1.5 Comparing species diversity/species richness indicators to biotic indices and aut-ecological indices

1.5.1 Species diversity/species richness indicators

Diversity indices attempt to combine data on abundance within species in a community into a single number. The definition of species diversity proposed by Margelef (1958) has been preferred by many other authors Washington (1984), Hulbert (1971) and Pielou (1966). According to this definition species diversity is a function of the number of a species present (species richness and abundance) and the evenness with which the individuals are distributed among the species (species evenness of equitability). Metcalfe (1989) defines species diversity indices as "the mathematical expression which use the components of community structure namely, richness (number of species present), evenness (uniformity in the distribution of individuals among species), and abundance (total number of organisms present), to describe the response of a community to the quality of the environment". The assumption underlying the diversity approach therefore is that undisturbed environments will be characterised by high diversity or richness, an even distribution of the individuals among the species, and moderate to high counts of individuals (Metcalfe, 1989; Mason et al., 1985)

The most widely used measure of diversity is the Shannon-Wiener formula (Metcalfe, 1989). Diversity indices based in the information theory (including Shannon-Wiener index) are the best known indices of diversity as well as the most commonly used (Washington, 1984).

The Shannon diversity index (*H'*) is calculated with the following formula:

$$H' = -\sum_{i=1}^{s} p_i \log_2 p_i$$

in units per individual per unit volume or area, where p_i is estimated from n_i/N as the proportion of the total population of individuals (N) belonging to the ith species (n_i) and using logarithms to the based 2. In an ecological context, *H'* measures the diversity in a many-species community (Wetzel, 2001).

Species diversity indices based on benthic diatom assemblages are regularly used to determine the impact of anthropogenic actions and pollutants on aquatic systems (Cunningham et al., 2003; Gracia-Criado, 1999; Gomez, 1999).

According to Metcalfe (1989), diversity indices are considered to have the following advantages:

1. They are strictly quantitative, dimensionless, and lend themselves to statistical analysis;
2. Most are relatively independent of sample size;
3. No assumptions are made as to the relative tolerances of individual species, which may be very subjective; and
4. They can be applied equally well to measures of biomass which are less labour intensive than counts of individuals.

In order to promote the responsible use of species diversity indices, the limitations of such indices as well as criticisms against such indices also need to be addressed. Metcalfe (1989) lists the following criticisms against diversity indices:

1. Values will vary considerably depending upon: the equation used to calculate them, the method of sample collection, the extent of identification (species diversity being greater than generic diversity), and the location and nature of the river being studied.
2. While standards have been set for the interpretation of the index values, the scales are not universally applicable. For example, not all undisturbed communities have inherently high diversity; therefore, it is not always possible to correlate certain values with ecological

damage. Furthermore, wide variations in values have been reported for unpolluted conditions.

3. In the calculation of diversity indices, individual species are reduced to anonymous numbers which disregard their pollution tolerances. It is as important to know which species are present as it is to know how many. Diversity index values cannot tell us if the community is composed of pollution-tolerant or –intolerant species. Furthermore, diversity indices are ratios of two variables and, as such, have serious statistical implications. When variables are compounded into ratios, the variances of the numerator and denominator are ignored and the resulting ratio will have greater variability than either of the two variables from which it was derived.
4. The response of a community to increasing pollution is not necessarily linear. In fact, there is evidence that moderate pollution can cause an increase in abundance without excluding species, with the result that the index values actually goes up.
5. Diversity indices have generally been applied to the extremes of the pollution scale, i.e. pristine vs. downstream of an effluent discharge. Not enough testing has been conducted in the middle range which represents most ambient waters of concern.

Another potential problem with the use of diversity indices has been discussed by Washington (1984). In his review paper he concluded that no simple answer could be given as to the relationship between diversity and ecosystem stability. In discussing the work of Connell (1978), he also stated that environmental instability may actually increase diversity above equilibrium levels. Connell's proposed the concept of the Intermediate Disturbance Hypothesis (IDH) which suggests that the diversity of species may be highest in areas which experience intermediate frequencies of disturbance.

The IDH is one of the most frequently suggested non-equilibrium explanations for the maintenance of species diversity in ecological communities (Roxburgh et al., 2004). The concept explains the contrast between the obvious variety of species existing in natural systems and the competitive exclusion principle which predicts that competition selects for the fittest species and leads to the exclusion of others (Flöder & Sommer, 1999).

Townsend et al. (1997) suggest the following explanation of how the hypothesis may work in an ecosystem:

“At one extreme, patches that are frequently and/or intensely disturbed are expected to exhibit low species richness because few species are able to colonize during the brief periods between disturbances or tolerate the high intensities of their impact. At the other end of the scale, patches in which disturbances are infrequent and/or or low intensity are expected also to be poor in species because they become dominant by competitive superior taxa. Richness should be highest at intermediate levels of disturbance because rapid colonizers and more competitive species co-occur.

Due to the potentially complex nature of the relationship between species-diversity and environmental disturbance, the suitability of diversity indices as indicators of water chemistry is debatable.

An alternative to species diversity/species richness indicators are aut-ecological indices”.

1.5.2 Biotic indices and aut-ecological indices

Biotic indices are an approach to water pollution making use of the indicator organism concept, and as such, do not represent community structure as species diversity indices do. According to Ollis et al. (2006a) biotic indices are constructed when “each taxon from a particular group of organisms is assigned to a sensitivity rating or ‘score’ based on the tolerance or sensitivity to particular pollutants. The scores of all the individual taxa at a site are summed and/or averaged to provide a value by which the integrity of the biotic community at the site can be gauged”.

Aut-ecological indices are based on the same principle: in such indices long term data gathered about the tolerances of a species are used to compile an index which can, in turn, be used to deduce environmental conditions from the species composition by taking into account the specific tolerances of the species in the community surveyed (De la Rey et al., 2004). In other words, aut-ecological indices use the relative abundance of species in assemblages, their ecological preferences, sensitivities, or tolerances to infer environmental conditions in an ecosystem (Stoermer & Smol, 1999). In the current study biotic indices and aut-ecological indices are therefore used interchangeably.

Biotic indices have been generally used in the aquatic sciences, and until 1984, they have only been applied to water pollution (Washington, 1984). These indices can be constructed to measure specific pollutants or general environmental conditions. Bonada et al., (2006) describe the advantages of biotic indices as highly robust, sensitive, cost-effective, easy to apply and easy to interpret.

As for the species diversity/richness indicators (Section 1.5.1) several limitations on the use and usefulness of biotic indices have been raised over the years. Although this has not subtracted from the widespread use of such indices, it is important to reflect on the limitations in order to gain the maximum benefit in terms of management information from the results produced by the indices.

Biotic indices are likely to be specific for one (maybe two) particular types of pollution as indicator organisms cannot be equally sensitive to all types of pollution (Washington, 1984). It is important to note that biotic indices do not measure community structure as a whole (as is the case in species diversity indices). Therefore, the index scores will not reflect impacts which the index was not designed to accommodate.

Another drawback of most biotic indices is that they are unlikely to be universally applicable because indicator organisms vary from region to region, limiting the use of such an index (Washington, 1984). Furthermore, the lack of ecological data of organism groups limits the use of biotic indices, because ecological data of the organisms is needed to construct a biotic index. In this respect, species diversity indices may be used regardless of ecological knowledge and may therefore be applied in areas where such information is scarce.

1.5.4 Aut-ecological indices as used in the current study

Aut-ecological indices use the relative abundance of species in assemblages, their ecological preferences, sensitivities, or tolerances to infer environmental conditions in an ecosystem (Stoermer &

Smol, 1999). Put in another way, aut-ecological indices make use of the niche requirements and habitat preferences of the individual species or higher taxonomic groupings. In such indices long term data gathered about the tolerances of a species are used to compile an index which can, in turn, be used to deduce environmental conditions from the species composition by taking into account the specific tolerances of the species in the community surveyed. These indices can be constructed to measure specific pollutants or general environmental conditions.

1.6 River Health Programme

Biotic indices have been found to be of great value for acquiring data on the health of water bodies and the management thereof worldwide as was explained by Section 1.5.2.

For this reason the South African Department of Water Affairs and Forestry (DWAF), as custodians of the water resources of the country, initiated the development of a National Aquatic Ecosystem Biomonitoring Programme (also called the River Health Programme or RHP) during 1995 (Roux, 1997). The RHP was designed in response to a specific information need, namely, to assess the ecological state of riverine ecosystems in relation to all the anthropogenic disturbances affecting them (RHP, 2006). The programme assessment philosophy is based on the concept of biological integrity and it makes use of biological indices, as well as indices for assessing in-stream and riparian habitats (RHP, 2006).

The main objectives of the River Health Programme can be summarised as follows (RHP, 2006):

- Measure, assess and report on the ecological state of aquatic ecosystems;
- Detect and report on the spatial and temporal trends in the ecological state of aquatic ecosystems;
- Identify and report on emerging problems regarding aquatic ecosystems; and
- Ensure that all reports provide scientifically and managerially relevant information for the national aquatic ecosystem management.

The RHP makes use of several indices, focusing on different animal groups. These include the Fish Assemblage Integrity Index (Kleynhans, 1999), the South African Scoring System (SASS) making use of macroinvertebrates (Chutter, 1998) and the Riparian Vegetation Index (RVI) (Kemper, 2001). As part of the present study, diatoms have also been included in the State of the Rivers report for the Crocodile West – Marico River catchments (Appendix A). In this report a diatom based index (SPI) was used as proxy for the water quality in river stretches. This same data was then also utilised for the compilation of manuscript three. Due to the fact that the thesis contributed to the mentioned RHP report, it was decided to include it as appendix to this document as it is seen as a valuable contribution made to the management of river health in the specific catchment.

For the sake of the rest of the present study, prominence will be given to the macroinvertebrate index (SASS 5), and the diatom indices such as the specific pollution sensitivity index (SPI) and the biological diatom index (BDI).

A recent approach to biomonitoring in South Africa is the establishment of reference conditions.

According to the European Water Framework Directive (European Commission, 2000) a reference condition is the expected background conditions (in this case of river fauna composition) with no or minimal anthropogenic stress as well as satisfying the following criteria:

a) It should reflect totally, or nearly, undisturbed conditions for hydromorphological elements, general physical and chemical elements, and biological quality elements.
b) Concentrations of specific synthetic pollutants should be close to zero of below the limit of detection of the most advanced analytical techniques in general use.

Reynoldson et al. (1997) define the reference condition as the condition that is representative of a group of minimally disturbed sites organized by selected physical, chemical, and biological characteristics. The article goes further to state that a reference condition is mainly used for comparing the biological attributes of individual test sites with a group of reference sites expected to be similar.

The reference-condition approach differs fundamentally from other approaches commonly used for water quality assessment (e.g. traditional studies using before and after, or control and impact designs) in that sites rather than multiple collections within sites, serve as replicates (Reynoldson et al., 1997). According to this concept, the degree of impairment at monitoring sites is therefore obtained from comparison to such reference conditions.

The abovementioned approach aids management of systems by alerting managers that certain impacts causes an aquatic assemblage or ecosystem to respond in some way that is outside the natural range of variation (Roux et al., 1999 as quoted by Ollis et al., 2006b). This concept of reference condition is incorporated in the River Health Programme through the introduction of Eco-classification which refers to the determination and categorisation of the Present Ecological State (PES; health or integrity) of individual biophysical attributes of the river that is being assessed, compared to the natural or close to natural reference condition. These biophysical attributes refer to the drivers (e.g. physico-chemical, geomorphology, hydrology) and biological responses (e.g fish, riparian vegetation and aquatic macroinvertebrates) of an aquatic ecosystem (RHP, 2006).

The derivation of reference conditions has however proved challenging and several papers has recently been published investigating techniques for the derivation of reference conditions for macroinvertebrates in South Africa (Ollis et al., 2006b; Dallas & Day, 2007; Dallas, 2004). Currently DWAF is busy compiling a document of reference conditions for river health programme sites across South Africa. This document should be available early 2009 (Thirion, 2007).

1.6.1 Macroinvertebrates

Macroinvertebrates are currently the most broadly used bioindicators in aquatic environments (Bonada et al., 2006; Metcalfe, 1989; Washington, 1984), and several indices have been based on this particular group. Murray (1999) concluded that invertebrate communities respond relatively quickly to localised conditions in a river, especially water quality, though their existence also depend on habitat diversity, they are common, have a wide range of sensitivities and have a suitable life cycle duration that could indicate short to medium term impacts on water quality. Metcalfe (1989) listed the following advantages for using this group:

1. They are differentially sensitive to pollutants of various types and react to them quickly; macroinvertebrate communities are capable of a graded response to a broad spectrum of kinds and degrees of stress.
2. The communities are ubiquitous, abundant and relatively easy to collect. Furthermore, their identification and enumeration are relatively easy.
3. Macroinvertebrates are relatively sedentary and are therefore representative of local conditions.
4. The organisms have life spans long enough to provide a record of environmental quality.
5. Their communities are very heterogeneous, consisting of representatives of several phyla, and the probability for reacting to a particular change in the environmental condition is, therefore high.

Ollis et al., (2006a) added that macroinvertebrates are also fairly inexpensive to collect, particularly if qualitative sampling is undertaken. In addition to having a long enough lifespan to be able to provide a record of environmental quality, the life spans of these organisms are also sufficiently short to enable the observation of recolonisation patters following perturbations.

A biotic index based on macroinvertebrates was compiled by Chutter (1998) in order to evaluate water quality changes in rivers. The South African Scoring System (SASS), as the index is known, has been revised several times and is currently in its fifth revised form called SASS 5 (Dickens & Graham, 2002).

The system is widely used in South Africa mainly due to the facts that (1) the system is rapid, affordable (especially when compared to chemical analysis) and does not require sophisticated equipment, and (2) the system is relatively easy to apply and identification to a sufficient level by trained non-specialist.

However, there are a few restrictions regarding the use of macroinvertebrates in biomonitoring and water quality assessment such as:

1. The distribution and abundance of macroinvertebrates are affected by a wide range of factors other than discernible water quality effects (e.g. flow, nature of substrate, habitat and food availability) (Dallas 2007; Dallas & Day 2007; Dickens & Graham, 2002; Chutter, 1998; Dallas, 1997);
2. They may not show responses to certain types of water quality impacts, such as some herbicides;
3. Scores may be affected by biotope availability (Dallas & Day 2007; Ollis et al., 2006b ; Chutter, 1998; Dallas, 1997);
4. Seasonality may affect SASS scores (de la Rey et al., 2008; Dallas & Day 2007);
5. The SASS method has been developed for perennial, lotic systems with low to moderate flow regimes, so it is not applicable in lentic systems of estuaries, and should be used with caution in ephemeral systems (Ollis et al., 2006b).

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

1.6.2 Diatoms

This section of the introduction repeats portions of the summary on the use of diatom indices in manuscript one, included in this study (De la Rey et al., 2004).

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 environmental management of a river system, the need to monitor the status of different taxonomic groups is vital. Diatoms provide interpretable indications of specific changes in water quality, whereas invertebrate and fish assemblages may better reflect the impact of changes in the physical habitat in addition to certain chemical changes (McCormick & Cairns, 1994).

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 autotrophic diatoms contribute significantly to the productivity of such ecosystems, they frequently form the base of aquatic food chains (Cox, 1996).

Diatoms are abundant, diverse and important components of algal assemblages in freshwater bodies. They make up 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, also extending into damp sub-aerial habitats.
- They collectively show a broad range of tolerance along a gradient of aquatic productivity, individual species have specific water chemistry requirements (Round et al., 1991; Werner, 1977).
- 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.
- They are sensitive to change in nutrient concentrations, supply rates and silica/phosphate ratios (Tilman et al., 1982; Tilman, 1977). Each taxon has a specific optimum and tolerance for nutrients such as phosphate (Bennion et al., 1996; Reavie et al., 1995; Bennion, 1994; Fritz et al., 1993; Hall & Smoll, 1992) and nitrogen (Christie & Smol, 1993), which can usually be quantified to a high degree of certainty.
- They assemblages are typically species rich. Considerable ecological information may be gained from this diversity of ecological tolerances. Moreover, the large number of taxa provides redundancies of information and important internal checks in datasets, which increase confidence of environmental inferences (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 & Lange-Bertalot, 1986-91). Species identifications are largely based on cell wall morphology.
- Diatoms can be found on substrata in streambeds even when dry, so they can be sampled at most times of the year (Stevenson & Pan, 1999).

Round (1993) lists numerous reasons why diatoms are useful tools of biomonitoring, amongst which the following bear special 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.

Although some of the following information is already part of the introductory sections of the various articles presented elsewhere in the document, it was deemed necessary to compile a thorough section on the aut–ecological diatom based indices in the introduction. This will make up the content in the following paragraphs.

A number of diatom-based aut-ecological indices are based on the weighted average equation of Zelinka & Marvan (1961) and have the basic form

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

where a_j = abundance (proportion) of species *j* in sample, v_j = indicator value and s_j = pollution sensitivity of species *j*.

Diatom indices function in the following manner: In a sample from a body of water with a particular level or concentration of determinant (e.g. orthophosphate-phosphorus), diatom taxa with their optimum close to that level of determinant will be most abundant. Therefore an estimate of the level of that determinant in the sample can be made from the average of the optima of the pollution sensitivity ('s') of all the taxa in that sample, each weighted by its abundance ('a'). 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' ('v') which is included to give greater weight to those taxa which are good indicators of particular environmental conditions. 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 pollution sensitivity 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 (de la Rey et al., 2004; Harding et al., 2004).

In 1979 Descy proposed the first true diatom index using the equation of Zelinka & Marvan (1961) on the basis of an investigation carried out on the Belgian section of the Sambre and Meuse Rivers (Prygiel et al**.,** 1999). In the following paragraphs a brief summary will be given of some of the diatom indices currently in use in several different countries for assessment of inland waters.

Using Descy's method or DES (1979) Coste (in Cemagref, 1982) proposed an index known as the Specific Pollution sensitivity Index (SPI). The SPI index is based on 189 surveys carried out during the years 1977 to 1980 at sites in the Rhone-Mediterranee-Corse basin national monitoring network. The index has been updated since 1982 in order to incorporate changes in taxonomy and new knowledge of diatom ecology.

Following the SPI, a Generic Diatom Index (GDI) was proposed (Coste & Ayphassorho, 1991) containing 174 taxa, including new genera proposed by Round et al. (1990).

The wide use of GDI and SPI in France lead to the creation of the Biological Diatom Index (BDI; Lenoir & Coste, 1996) to meet the need for an index capable of being applied to monitoring networks throughout the whole of France. The BDI was designed on the basis of 1332 biological and physicochemical surveys and includes 1028 diatom species and varieties. To maximise the usability of the BDI morphologically similar species that are difficult for the non-specialist to identify with light microscopy were combined, this reduced the number of taxa. Rare species (less than 5% of the inventory) were eliminated from the list, which resulted in 209 taxa being kept (Prygiel & Coste, 1999).

The Zelinka and Marvin equation was also used in the construction of many other diatom-based indices including 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) etc. Such indices mainly vary from the SPI and BDI in terms of species included in the calculation and the tolerances assigned to such species and are all included in the statistical package OMNIDIA version 3.1 (Lecointe et al., 1993).

The indices most used in the current study are the Biological Diatom Index (BDI) as well as the Specific Pollution Sensitivity Index (SPI). The SPI has one of the broadest species bases for the calculation of the index and therefore was a reasonable option for testing in South Africa that may not have the same environmental conditions as France where it was developed. It is also one of the most use diatom indices in Europe (Lenoir & Coste, 1996).

The BDI index was a refinement of the indices in use in France before 1996. This refinement was based on a database of 1332 surveys originating from the whole of France. This data was analysed using multivariate statistics until a number of key indicator species was identified. In all the system takes into account 209 taxa of which 57 were matched group i.e. included 2-6 morphologically similar species grouped under one name (Lenoir & Coste, 1996).

The indices have been used with success in several European countries including Poland (Kwandrans et al., 1998) Finland (Eloranta, 1994) and Portugal (Almeida, 2001).

The study of diatom flora extends back as far as the middle of the 19th century with work done by Ehrenberg (1845) and Cleve (1881). In the 1950's and 1960's Cholnoky produced work on many diatom species from South Africa (e.g. Cholnoky 1960). The potential use of diatoms as indicators of water quality was also initiated by Cholnoky from South African diatom flora. In a publication in 1968, Cholnoky applied a variation of the community analysis of Thomasson (1925 as quoted in Taylor 2004), to assess water quality using diatom community composition. Cholnoky would use the relative abundance of certain taxa to assess or track changes in specific water quality constituents (e.g. the relative abundance of *Nitzschia* spp. to assess nitrogen changes in rivers). This study was probably the precursor for the modern diatom indices as used in the current study.

In the 1970's and the 1980's, Schoeman and Archibald produced work on the systematics and ecology of South African diatoms, culminating in the work: 'The diatom flora of South Africa' (Schoeman & Archibald, 1976). In a 1976 publication, Schoeman used diatom indicator groups to evaluate changes in water quality. From this study Schoeman concluded that diatom associations may be used to assess the conditions of rivers. Schoeman also tested the 'saprobian classification system' as developed by Lange-Bertalot (1979) in a South African River system and found strong correlation between water quality constituents and diatom community composition.

As can be seen from the abovementioned, South African diatom research was developing parallel to, and even leading the way in terms of using diatoms as indicators in rivers system. Unfortunately this ceased with 1979 work of Schoeman and little work has been done on diatoms for the 20 odd years.

Diatoms, as indicators of water quality, were only again investigated in depth in South Africa by Bate et al. (2002). The investigation attempted to relate a descriptive index (Van Dam et al., 1994), based on a dataset for the environmental tolerances of diatom species found in the Netherlands, to water quality in South Africa. The environmental variables generated by the Van Dam et al. (1994) index include: pH, conductivity, oxygen requirements, trophic status, saprobian status and habitat requirements of a selected number of diatom species found in waters of the Netherlands (Van Dam et al., 1994; Taylor 2004).

Bate et al. (2002) came to the conclusion that benthic diatoms could be a useful addition to the National Biomonitoring Programme for Aquatic Ecosystems (NBPAE) as the diatoms give a time-integrated indication of specific water quality components. However, Bate and co-workers went on to state that the particular data set tested in their study, could not be transposed directly for use under South African conditions. For this reason the present thesis investigates the potential use of several other numerical, rather than descriptive, diatom indices developed in Europe

The above-mentioned background forms part of the context in which the work for this thesis was performed. The next section elaborates on the specific aims of the various studies included in the thesis.

1.7 Current project

Concerns have been expressed as to 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 (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.

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

Diatoms, although used extensively in Europe as well as other parts of the world, have not been used as bioindicators in the River Health Programme before 2005. The most recent State of the Rivers Report (River Health Programme, 2005, in Appendix A) did include diatoms as a proxy for water quality in the Crocodile (West) Marico Water Management Area. The author formed part of the team of the North-West University that gathered diatom based bioindicator data for almost 60 sites in the mentioned water management area. The inclusion of the diatom based indices was based on proven effectiveness of the diatom based indices world wide.

But how effective are these biological based indices in assessing river health and water quality? Are some of the indicators better suited to certain tasks than others? Do we need any additional biomonitoring tools or are the current ones sufficient to evaluate the state of our rivers? How comparable is the data that we do gather from some of these indices?

These questions, as well as some others, are the topics of discussion in this document. The document consists of five separate manuscripts, each of which is focused on a specific research question identified to promote understanding of some of the bioindicators used in the River Health Programme. Since diatom based indices are the least known of the mentioned biomonitoring systems, and therefore present the most questions in terms of application, a large portion of the document is spent on this particular type of index. Another objective of the studies presented in this document was the quantitative evaluation of index response to water quality and habitat availability, a theme that has been largely ignored in evaluating bioindicators.

The individual focus of the various manuscripts to gain insight into the mentioned issues will now be discussed.

Manuscript one begs the question of whether diatom based indices can be used effectively in South Africa. SASS (a macroinvertebrate based system) was used as the index to which compare the response of the diatom based indices. SASS was selected as the comparative index because in many ways, it has become the standard for the rapid bioassessment of rivers in South Africa and it yields fast and cost effective results (Dickens and Graham, 2002).

In manuscript two the importance of comparability of results from various operators in the field of biomonitoring was investigated. The paper is a review paper of diatom based monitoring techniques in terms of sampling, preparing and enumeration of diatom cells. As diatom based systems are in its infancy in terms of use in South Africa, it was recognised as an opportune time in which to suggest protocols to ensure comparability of gathered results.

The primary question of manuscript three was whether diatom based indices that were developed in other countries may be use in South Africa. This focuses on two main aspects namely do we encounter the same species in South African rivers as the ones used for bioindication in the countries in which the indices where developed and does these species have the same tolerances as in the country in which the indices was developed. The article also hints at the diatom based indices developed in other countries that may be more useful in South Africa.

The aim of manuscript four is to establish assess aut-ecological and diversity based diatom indices for their application in evaluating water quality. Both types of indices have been employed in such assessments in various international studies, and the most applicable for South African conditions needed to be determined.

The final manuscript evaluates the relationship of diatom based indices and SASS 5 with water quality and habitat integrity. The paper compares the reaction of the two types of indices to changes in habitat and water quality. This is important to ascertain as it would advise on which one index to use when evaluating river health, or if both types of indices should preferably be used as complimenting systems.

The outcome of the five papers presented in this document should provide enough motivation for the inclusion of diatoms as bioindicators in South African Rivers. It will also shed light on how accurately macroinvertebrate and diatom based indices reflect changes in water quality and habitat integrity.

This study may be viewed as additional to the work of Taylor (2006). Some of the work presented in this document was performed in conjunction with the mentioned author. The current document however also evaluates the diatom based indices against species diversity indices as well as other biotic indices (SASS 5 in particular) to establish the additional value that may be gained from the use of such indices.

The choice of the river systems used in the study is one of practical consideration. In order to evaluate the diatom index's ability to measure water quality as well as compare its performance to indices such as SASS 5, it was necessary to join programmes and available efforts. The cost of water quality analysis would have been prohibitive if such a route was not followed. This approach led to large portions of Gauteng, North West and Mpumalanga provinces to be surveyed in this study, and included water quality range from mostly natural to poor. It is believed that the studies as reported in this document as well as the thesis by Taylor (2006), provide ample proof that diatom based indices may be used with confidence in river systems in South Africa. A data disk containing all the water chemistry data as well as index data such as SASS 5, BDI, SPI and IHAS is included as part of the document in order to facilitate future inquiries into the studies.

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Chapter 2
Manuscript I

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

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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 Prozetsky 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:

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

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 (NH_4), total phosphate (total P), chemical oxygen demand (COD), five day biological oxygen demand (BOD_5), sulphate (SO_4) 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 (DO_2) 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 BOD_5 was added to this suite to provide an indication of organic load according to the analysis list of Kwandrans 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 $mg \cdot \ell^{-1}$ except for temperature (°C), electrical conductivity ($\mu S \cdot cm^{-1}$) 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 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 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

TABLE 5
Correlations of physical and chemical water qualtiy variables and water qualtiy 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	**-0.855**	**0.8133**	-0.494	-0.3569	0.0559	**-0.8945**	-0.4157	**-0.7714**	-0.3301	0.0323
	p=0.135	**p=0.000**	**p=0.001**	p=0.103	p=0.255	p=0.863	**p=0.000**	p=0.179	**p=0.003**	p=0.295	p=0.921
SASS 5	0.3369	-0.1731	0.4567	0.1069	0.3432	-0.2917	-0.3015	-0.5653	0.0731	0.0507	-0.4569
	p=0.311	p=0.611	p=0.158	p=0.754	p=0.302	p=0.384	p=0.368	p=0.070	p=0.831	p=0.882	p=0.158
ASPT	0.2248	-0.4191	0.5196	-0.2648	0.1302	-0.1039	-0.543	**-0.7463**	-0.2201	-0.3219	**-0.6066**
	p=0.506	p=0.200	p=0.101	p=0.431	p=0.703	p=0.761	p=0.084	**p=0.008**	p=0.515	p=0.334	**p=0.048**

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 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

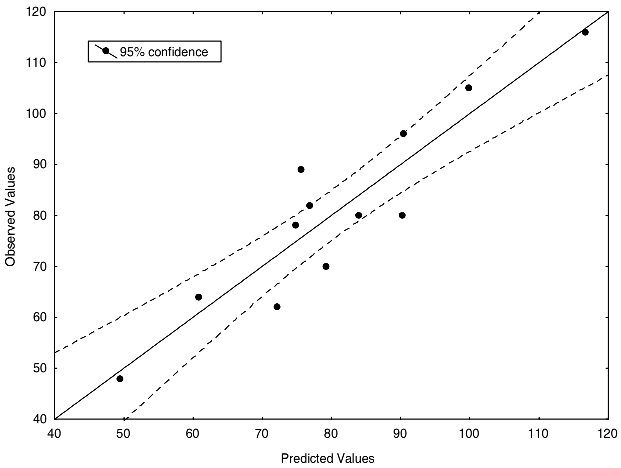

Figure 2
Predicted SASS 5 values vs. observed SASS 5 values

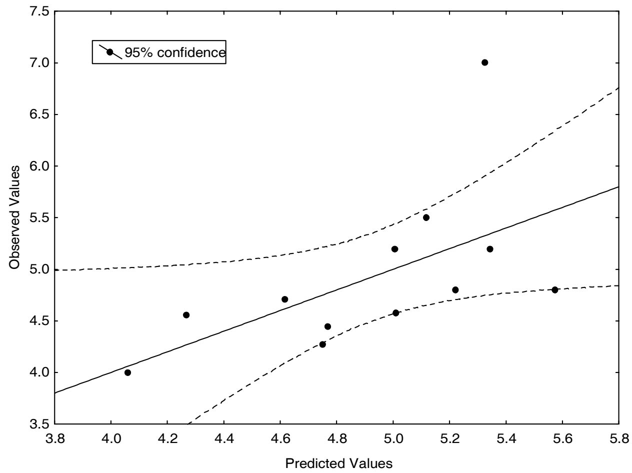

Figure 3
Predicted ASPT values vs. observed ASPT values

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 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 R^2 (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 e•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 R^2 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 R^2 (Author to check throughout the doc. whether it should be Adjusted R^2 OR adjusted R^2) 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.

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
SO4	-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

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.

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**
SO4	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

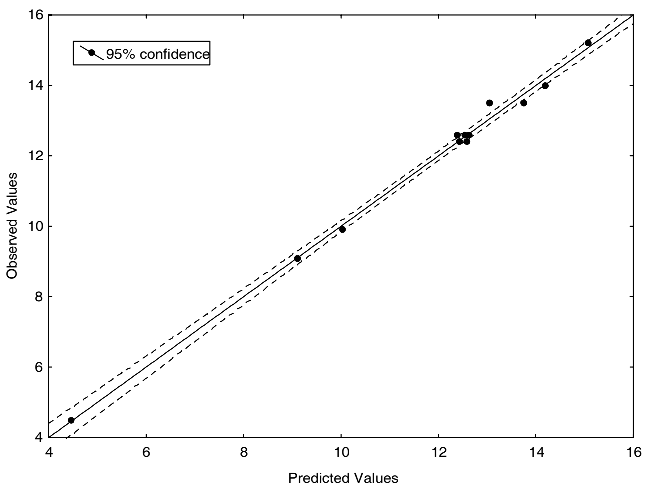

Figure 4
Predicted SPI values vs. observed SPI values

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Chapter 3
Manuscript II

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

Authors: Jonathan C Taylor, P Arno de la Rey and Leon van Rensburg
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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 monotoring. 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 *Achnanthidium* Kützing, *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 (epipsammon), 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 species composition 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 might 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 >20cm 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, 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 >20cm 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 1.6m.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 10m reach.
2. Scrub their upper surfaces with a toothbrush and rinse into a white tray.
3. Mix well and pour into 150ml 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, ≤265mm diameter) or small boulders (>256mm), 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 emergent 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 10m^2 (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 50ml 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 50ml 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 mountant.

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 mountant 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 can be 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 diatotepum 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 the first method (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 HNO_3/H_2SO_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 (HNO_3 + H_2SO_4, 2:1) and place the beakers on a hot plate. The beakers should be covered with a watch glass to prevent contamination 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 5–11 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 the first method (6–11).

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 the first method (6–11).

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 *Achnanthidium* 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 10 x 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 4
Manuscript III

Based on the article:

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

(original article in Appendix B)

Authors: J.C. Taylor, J. Prygiel, A. Vosloo, P.A. de la Rey & L van Rensburg
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References

References in the text will use the name and year system: Adam & Eve (1983) or (Adam & Eve, 1983). For more than two authors, use Adam et al. (1982). References to a particular page, table or figure in any published work is made as follows: Brown (1966: 182) or Brown (1966: 182, fig. 2). Cite only published items; grey literature (abstracts, theses, reports, etc) should be avoided as much as possible. Papers which are unpublished or in press should be cited only if formally accepted for publication.

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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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This paper has not been submitted elsewhere in identical or similar form, nor will it be during the first three months after its submission to *Hydrobiologia*

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 receiving water bodies by the addition of chemical compounds, colloidal material and suspended solids, often resulting in changes in salinity, nutrient status and turbidity. 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 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 well-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 (98%) of benthic diatoms present in the heavily impacted Vaal River 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 topic 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* (*Achnanthidium*), *A. standeri* was described by

Cholnoky, and is found in abundance in certain mountain streams (Cholnoky, 1957). The occurrence of these endemic species 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).

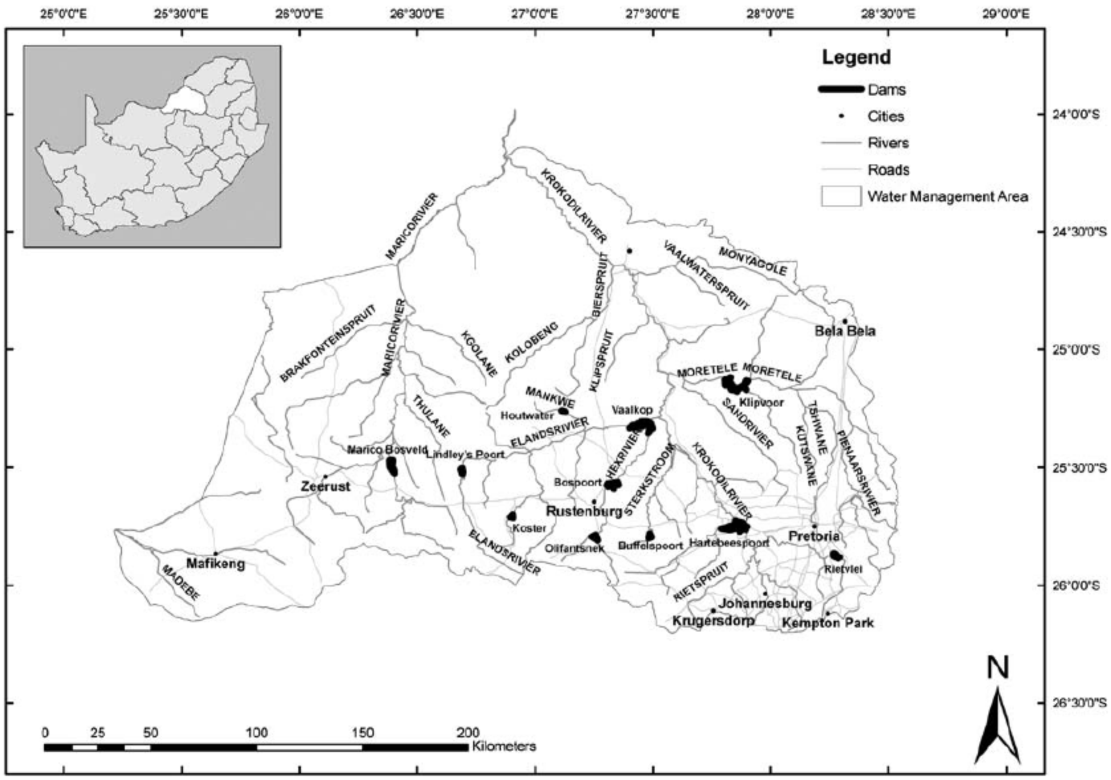

Fig. 1 Map showing location of some of the larger rivers in the Crocodile West and Marico water management area

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

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 3). Measured values (mean, median, minimum and maximum) for the physical and chemical variables (n = 50) 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. Only species that constitutes more than 1% (weight range) of the diatom community was included in the CCA.

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.

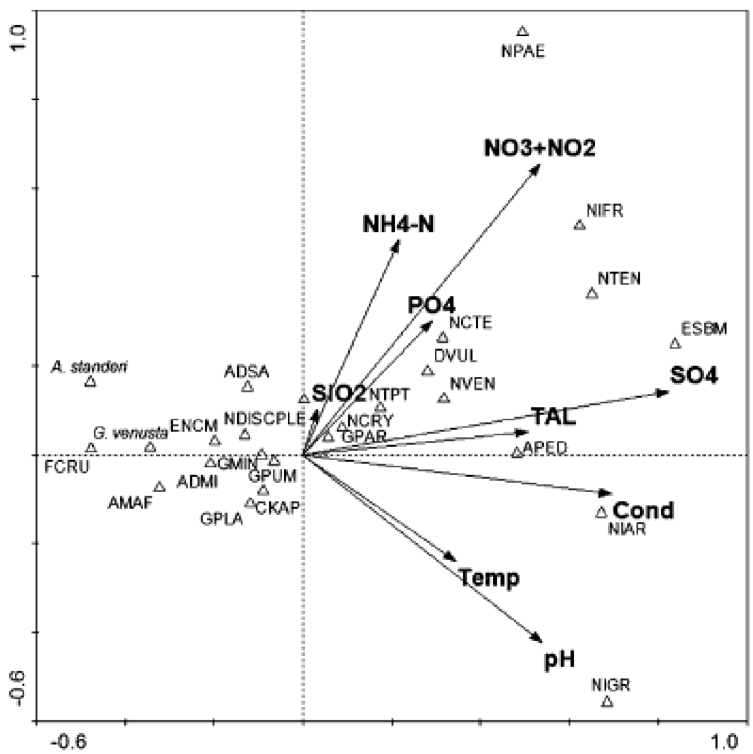

Figure 2. CCA biplot showing the relationship between measured environmental variables and some diatom species in the Crocodile West and Marico water management area. Species with a weight range of 1-100% are shown. Acronyms are presented in Table 4

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 environmental tolerances in widely separated geographic areas. It is clear from Figure 2 that those species commonly associated with poor water quality in Europe e.g. *Eolimna subminuscula* Lange-Bertalot, *Nitzschia frustulum* (Kützing) Grunow ordinate on the right hand side of the diagram together with elevated levels of water quality variables. Another typical example is *Diatoma vulgaris* Bory, which commonly occurs in worldwide 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. *Achananthidium minutissimum*, *Achnanthidium affine* (Grunow) Czarnecki, *Encyonopsis microcephala* (Grunow) Krammer and *Fragilaria capucina* var. *rumpens* (Kützing) 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.

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

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* (Kützing) 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 different environmental requirements, *A. pyrenaicum* 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 environmental requirements are different.

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 lead then 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.

Table 4. Acronyms used in the CCA biplot (Fig. 2)

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

Conclusions

It is concluded that the index approach is 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 unique South African diatom index.

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Chapter 5

Manuscript IV

Based on the article:

On the use of diatom-based biological monitoring.
Part 1: A comparison of the response of diversity and autecological diatom indices to water quality variables in the Marico-Molopo River catchment

(original article in Appendix C)

Authors: PA de la Rey, L van Rensburg and A Vosloo
Publiched in Water SA, 2008, Vol. 34, No 1, pages 53-60

Water SA

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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.

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On the use of diatom-based biological monitoring
Part 1: A comparison of the response of diversity and aut-ecological diatom indices to water quality variables in the Marico-Molopo River catchment

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Abstract

Two main approaches have been followed in using diatoms as bio-indicators in the past few decades namely species diversity indices and aut-ecological indices. This study, based on 102 water quality and epilithic diatom samples from the Crocodile Groot-Marico catchment in South Africa, evaluated both types of indices by establishing how well they reflect changes in water quality. It was found that less of the variation in diversity indices could be attributed to changes in water quality variables than was the case for the aut-ecological indices. Furthermore it was found that species diversity indices tend to be higher at intermediate levels of pollution, rather than at low levels of pollution.

Introduction

Southern Africa is a subcontinent notorious for its unpredictable rainfall. South Africa is a semi-arid country, and the decline in the quality of available water is one of the biggest problems currently facing the country (Davies & Day, 1998). For this reason the integrated management of water resources has enjoyed high priority in the National Water Act as well as in research and management actions by both government and non-government organizations.

Freshwater is vital to human life and societal well-being, thus its utilisation for consumption, irrigation, and transport have long taken precedence over other commodities and services provided by freshwater ecosystems (Baron et al., 2002).

Walmsley et al. (2000) state that indicators are an ideal means by which progress towards integrated water resource management can be monitored, in that they provide a summary of conditions, rather like temperature and blood pressure are used to measure human health. Using the same analogy, it is important to be able to distinguish between indicators which can truly be linked to health and are not just features of the system which do not have relevance to the question posed. For the aforementioned reason it is important to be able to test indicators quantitatively in order to assess how closely they can be linked to the health of aquatic ecosystems, in the case of water resource management, and/or water quality. Diatoms have been shown to have narrow tolerance ranges for many environmental variables and respond rapidly to environmental change, making them ideal indicators (Reid et al., 1995).

Several kinds of indicators have been proposed over the years to evaluate ecosystem health. Two main groups of indicators using diatom community data will be discussed in this paper namely diversity indices and aut-ecological indices.

Species Diversity/Species Richness Indicators

Ecosystem stability can be defined as the ability of a system to recover to an equilibrium state after disturbance, or simply the persistence of the system (May, 1976). The diversity-stability hypothesis asserts that species vary in their traits and that in a highly diverse (species rich) system, there will be some species than can compensate for the loss of others should disturbance occur in such a system (Pimm, 1984; Elton, 1958). Thus, species rich systems are more likely to be considered stable. Another common view is that this theory predicts a decrease of diversity as pollution increases. The pollution intolerant species decline in abundance and the pollution tolerant species can grow rapidly without competition for space, nutrients, or other resources. This results in community abundance patterns of heavy dominance and fewer species (Van Dam, 1982).

It is on the basis of the two abovementioned hypotheses that Species Diversity indices enjoy widespread use in ecology and, more specifically, aquatic ecology. Diversity indices are related to community structure and are not specific to any type of contamination. Species diversity indices consist mainly of three measures namely: species richness (related to the number of species), the evenness (how evenly the individuals are distributed between the species) and a combined measure called the diversity index such as the Shannon Diversity index (Shannon & Weaver, 1949).

Species Diversity indices based on benthic diatom assemblages are regularly used in the study of water resources. In such instances these indices are mainly used to determine the impact of certain actions and pollutants on aquatic systems (Cunningham et al., 2003; Gómez 1999; Gracia-Criado et al., 1999). An alternative to Species Diversity/Species Richness indicators are aut-ecological indices.

Aut-ecological Indices

Aut-ecological indices use the relative abundance of species in assemblages, their ecological preferences, sensitivities, or tolerances to infer environmental conditions in an ecosystem (Stoermer & Smol 1999). Put in another way, aut-ecological indices make use of the niche requirements and habitat preferences of the individual species or higher taxonomic groupings. In such indices long term data gathered about the tolerances of a species are used to compile an index which can, in turn, be used to deduce environmental conditions from the species composition by taking into account the specific tolerances of the species in the community surveyed. These indices can be constructed to measure specific pollutants or general environmental conditions. A number of diatom-based aut-ecological indices are based on the weighted average equation of Zelinka & Marvan (1961) and have the basic form

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

where a_j = abundance (proportion) of species *j* in sample, v_j = indicator value and s_j = pollution sensitivity of species *j*.

The method mainly utilizes the distribution of species along a water quality gradient in terms of sensitivity to pollution as well as broadness of the species distribution along the water quality gradient. This equation is used in many diatom-based indices including the Descy's index or DES (Descy, 1979), 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) etc. Such indices mainly vary in terms of species included in the calculation and the tolerances assigned to such species.

Diatom indices constructed by this approach have been tested with success in South Africa (Taylor et al., 2007; De la Rey et al., 2004) and in many countries in Europe and the rest of the world (Sabater, 2000; Kelly, 1998; Reavie et al., 1995; Zeeb et al., 1994; Hall & Smol, 1992).

Rationale

This research paper consists of two parts. This paper (Part 1) tests the performance of two approaches to the use of diatoms as bio-indicators namely diversity indices and aut-ecological indices. The second paper (Part 2) compares the performance of diatom-based indices with that of a macro-invertebrate index (SASS 5) in terms their ability to indicate changes in water quality variables.

The purpose of the current paper (Part 1) is to compare diversity indices and aut-ecological indices as measures of aquatic ecosystem health by comparing their response to water quality variables. This will enable an informed decision as to which of the two approaches will be most appropriate when using diatoms as bio-indicators in river and stream ecosystems in South Africa.

Since water quality is one of the main environmental factors affecting the ecology in rivers and streams, it can be used as a measure of the applicability of indicators for integrated water resource management. Diatoms are appropriate for the purpose of this study as they provide interpretable indications of specific changes in water quality (McCormick & Cairns, 1994).

Materials and Methods

Sampling Localities

Thirty three sites were identified in the Groot Marico and Molopo River systems for the study (Figure 1, A3 map available in Appendix D of thesis). The identified sites form part of the River Health Program of the North West Province, South Africa. Samples were collected in April, June, September and November 2005 at the sites, as water levels permitted (at times certain samples could not be collected due to low water levels), resulting in a total of 102 separate data points with diatom data and same-day water quality data. These sites were chosen to represent a wide range of water quality (see water quality summary in Table 1) as well as the wide range of diatom index scores recorded (SPI: 1.3-19.7 out of a possible 20).

TABLE 1
Descriptive statistics of water quality variables

	Mean	Median	Min	Max	Standard Deviation
Ca (mg/ℓ)	32.18	30.06	1.49	82.25	23.78
Cl (mg/ℓ)	14.98	4.99	2.00	145.57	24.12
F (mg/ℓ)	0.18	0.15	0.05	0.61	0.14
K (mg/ℓ)	2.17	0.73	0.15	11.28	2.91
Mg (mg/ℓ)	24.27	18.52	1.02	65.79	18.52
NH_4 (mg/ℓ)	0.68	0.02	0.02	16.78	2.43
NO_3+NO_2	0.38	0.09	0.04	7.52	1.02
Na (mg/ℓ)	11.91	3.45	1.00	109.31	18.05
PO_4 (mg/ℓ)	0.21	0.02	0.01	4.90	0.70
SO_4 (mg/ℓ)	15.18	5.70	2.00	113.27	20.22
Si (mg/ℓ)	6.26	5.94	1.27	11.20	2.16
pH	8.16	8.23	7.31	8.66	0.30
DO (mg/ℓ)	6.55	6.94	1.81	11.48	2.49
Temperature (°C)	17.98	18.60	7.70	29.52	4.18
Turbidity (NTU)	12.49	6.77	0.00	75.10	14.34
EC (mS/m)	39.21	31.10	2.66	113.70	28.57
TAL (mg/ℓ)	168.73	146.83	4.00	497.00	121.00

The Marico River originates south of Groot Marico town and feeds into the Limpopo River in the north. The origin of the Marico River falls within the Groot Marico dolomitic aquifer compartment. The two primary sources of this river are the Grootfontein dolomitic eye, that gives rise to Kaaloog-se-loop and an eye on the farm Renosterhoek 343JP that feeds the Rietspruit. Secondary sources include Draaifontein tributary that is of a mixed dolomitic nature and the Sterkstroom tributary that is fed by springs of a non-dolomitic nature. Other tributaries of the Marico River include the Klein Marico River. This tributary is seasonal and is thus not a major flow contributor to the Marico River. The 'Molemane-se-Loop' is of a dolomitic nature originating in the Molemane Nature Reserve. Major dams in this sub-catchment include the Marico-Bosveld Dam in the upper catchment and the Molatedi Dam further downstream. The upper reaches of this sub-catchment are not densely populated (RHP, 2005).

The Molopo River originates east of Mafikeng and feeds into the Vaal River in the south west. The origin of the Molopo River falls within the Groot Marico dolomitic aquifer compartment. The primary source of this river is the Molopo dolomitic eye. Major dams in this sub-catchment include Cooke's Lake, Montshioa Dam, Lotlamoreng, Modimolo Dam (Setumo) and the main dam in the system is the Disaneng Dam close to the Botswana Border.

From the results in the study, it is clear that the water quality deteriorates downstream, especially as it flows through the towns of Groot Marico, Zeerust and Mafikeng. Downstream water quality impacts may also occur from impoundments (dams and weirs) as well as from agricultural activities.

Indices

Diatoms were collected, prepared and enumerated according to the protocol as set out in Taylor et al. (2005). Only epilithic diatoms were sampled for the study due to the fact that this is the community of preference for the most diatom indices as well as being the community that yields the best coefficients when multiple regressions are performed with water quality variables (Hodgkiss & Law, 1985). Diatom identification was according to the nomenclature of Krammer & Lange Bertalot (1986-1991) and the Specific Pollution sensitivity Index (SPI), Biological diatom index (BDI), SPI was chosen as it has the broadest species base; BDI showed the best overall correlation to water quality variables in studies performed recently on the Vaal River (Taylor et al., 2007).

Species Diversity Index, Species Evenness and No. of Species were calculated using the OMNIDIA software package (Lecointe et al., 1993). For the biodiversity indicator (Species

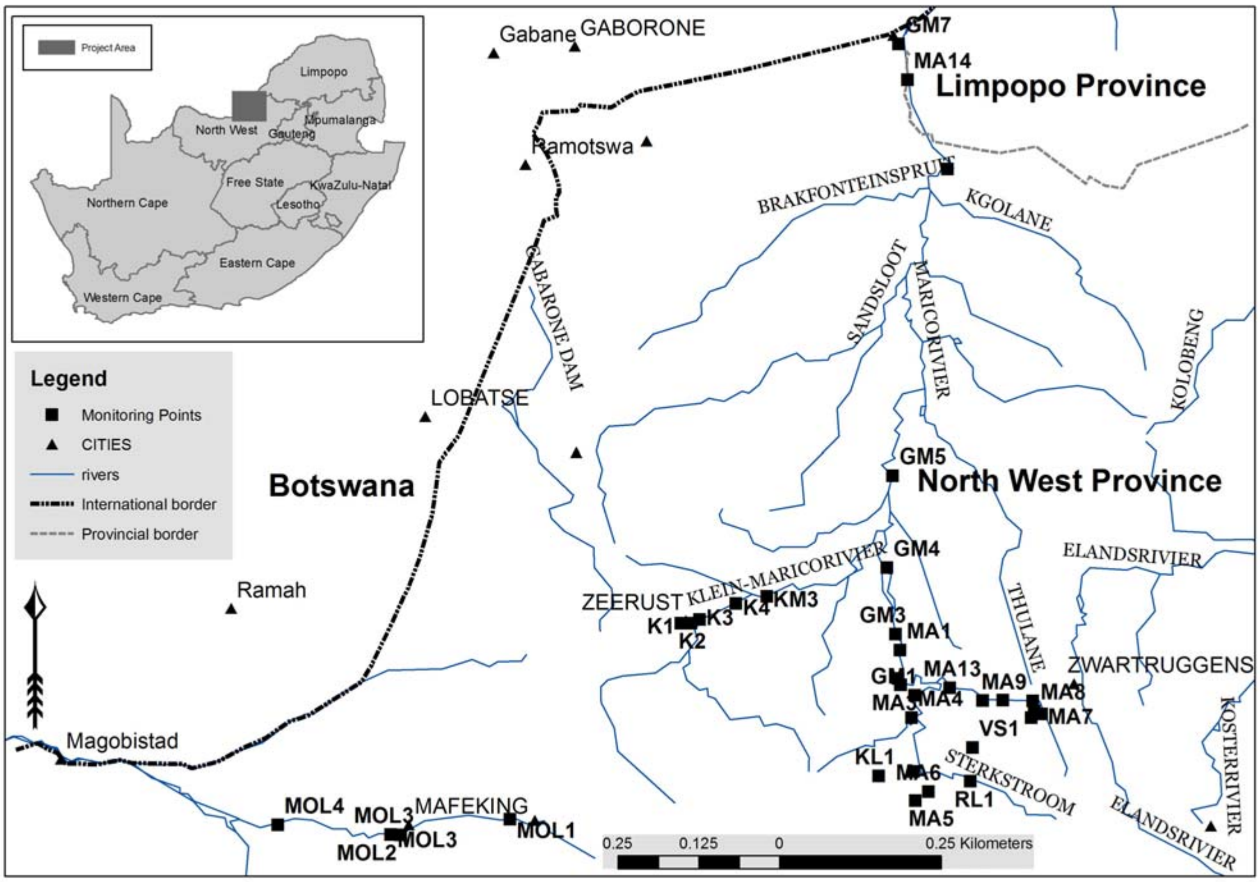

Figure 1

The Groot Marico and Molopo River systems (North West Province, South Africa) showing the location of the study area diversity, Species Evenness and No. of Species) calculations, the same number of cells that were counted for the aut-ecological index calculation was used (400 cells per slide). It is a well known fact that there is a relationship between diversity indices and sample size (Seber, 1986, Lewins & Joanes, 1984). Since the same number of cells was counted on each slide, the data are comparable between samples as well as between indices.

Water quality

Instream water quality measurements for pH, dissolved oxygen (mg/ℓ), electrical conductivity (mS/m) as well as temperature (°C) were done at each locality by means of an YSI 556 MPS Multimeter. Water samples were taken concomitantly with biological samples at every site. Sampling bottles used were obtained form the laboratory of Resource Quality Services (Department of Water Affairs and Forestry) after it has been cleaned. Samples were kept cool but not frozen.

The water sample was preserved by means of an $HgCl_2$ ampoule broken into each sample and delivered to Resource Quality Services (Department of Water Affairs and Forestry, Roodeplaat) for analysis. The following water quality variables formed part of the analysis:

Calcium (mg/ℓ Ca), Chloride (mg/ℓ Cl), Fluoride (mg/ℓ F), Potassium (mg/ℓ K), Magnesium (mg/ℓ Mg), Ammonia (mg/ℓ NH_4-N), Nitrate and Nitrite (mg/ℓ NO_3+NO_2), Sodium (mg/ℓ Na), Ortho-Phosphate (mg/ℓ PO_4-P), Sulphate (mg/ℓ SO_4), Silica (mg/ℓ Si), Total Alkalinity (mg/ℓ TAL) and Turbidity (NTU, nephelometric turbidity units).

Due to the fact that turbidity changes if stored, samples turbidity samples were taken separately and measured as soon as it reached the laboratory.

Due to the fact that the samples were preserved by $HgCl_2$, the laboratory subtracted the Cl_2 concentration resulting from the preservative before the final Cl_2 results were used for statistical analysis.

Statistical analysis

Multiple regressions and correlation analysis were performed using the STATISTICA software package (Release 7, Stat Soft. Inc., United States of America), while Principle Component Analysis (PCA) was performed using CANOCO for Windows (Version 4.51, Biometris-Plant Research International, The Netherlands). Before analysis, all the data were standardized by subtracting the sample mean and dividing the result by the sample standard deviation (Hair et al., 1998). This was done to ensure that all the variables could contribute equally to the regressions and correlations by removing the scale factor. For the regressions the forward stepwise method was chosen to eliminate variables that do not contribute to the regression. For the purpose of the multiple regressions the Electrical Conductivity and Total Alkalinity were left out of the analysis as these variables contributed to multi-colinearity in the data, due to high correlation with other water quality variables (e.g. Ca, Cl, F, K, Mg, Na, SO_4). The R^2 value was used, instead of the R value, as it is a stricter measure of the predictability of multiple regressions.

Results

Diversity indices

Table 2 represents the results for the multiple regression analysis for water quality and diatom species diversity. The R^2 for the regression was 0.215. This shows that approximately 21.5% of the variation in the diatom species diversity could be attributed to the measured water quality variables. The beta values in the table are the regression coefficients while the p-values indicate whether a specific variable contributed statistically significant to the regression. Water quality variables that contributed significantly ($p<0.05$) to the regression were fluoride and the pH of the water. The fact that changes in fluoride and pH concentrations influences diatom community structures have been noted by several authors (Joy & Balakrishnan, 1990; Ares et al., 1983; Lewin, 1962).

TABLE 2 Regression summary for Shannon species diversity with water quality (italicised values significant at $p<0.05$)			
	R^2= 0.215		
N=102	Beta	Std.Err. of Beta	p-level
Intercept			*<0.001*
F (mg/ℓ)	*0.385*	*0.131*	*0.004*
pH	*-0.347*	*0.109*	*0.002*
SO_4 (mg/ℓ)	0.175	0.125	0.164
DO (mg/ℓ)	0.101	0.100	0.313

The results for the regression performed to explain species evenness with water quality is provided in Table 3. The R^2 value for prediction significance of water quality for this variable was only slightly lower than for the species diversity at 20%. Once again the significant contributors to the regression were pH and fluoride.

TABLE 3 Regression summary for Pielou species evenness with water quality (italicised values significant at $p<0.05$)			
	R^2= 0.201		
N=102	Beta	Std.Err. of Beta	p-level
Intercept			*<0.001*
F(mg/ℓ)	*0.430*	*0.104*	*<0.001*
pH	*-0.211*	*0.105*	*0.048*
PO_4 (mg/ℓ)	0.183	0.093	0.051

The regression performed for the number of diatom species and water quality (Table 4) indicates that considerably more of the variation in the values of this index can be explained by the measured water quality variables. Just over 32% of the variation in the number of species encountered in samples could be attributed to water quality variables and Ca, Mg, Si, and K were the significant contributors in the regression model.

TABLE 4 Regression summary for the number of diatom species with water quality (italicised values significant at p<0.05)			
	R^2= 0.321		
N=102	Beta	Std.Err. of Beta	p-level
Intercept			*< 0.001*
pH	-0.239	0.123	0.056
F (mg/ℓ)	0.151	0.219	0.494
Na (mg/ℓ)	-1.462	0.845	0.087
Temperature (°C)	-0.090	0.102	0.379
Ca (mg/ℓ)	*-1.072*	*0.270*	*< 0.001*
Mg (mg/ℓ)	*0.802*	*0.310*	*0.011*
Si (mg/ℓ)	*0.319*	*0.149*	*0.036*
SO_4 (mg/ℓ)	0.251	0.174	0.153
K (mg/ℓ)	*0.592*	*0.287*	*0.042*
Turbidity (NTU)	-0.198	0.115	0.089
Cl (mg/ℓ)	0.740	0.735	0.317

Archibald (1971) stated, in his studies on South African diatom species diversity, that clean water samples displayed diatom diversity scores between that of moderately enriched and polluted samples. This result was supported by findings of Van Dam (1982) by demonstrating that the highest number of diatom species occurred in moorland pools which were moderately affected by acidification as opposed to the low diversity found in very acidic pools. That diversity tends to be high at 'medium water quality' and lower at either 'good' or 'poor' water quality suggests a quadratic relationship of species diversity to disturbance may be more appropriate than a linear one. To test whether this tendency is also followed in the current data set, a quadratic or second degree polynomial regression was fitted on the data. This was done by the use of, for example Ca as well as $(Ca)^2$ (a quadratic term), as terms in the regression with species diversity indices (see e.g. Hair et al., 1998 or Neter et al., 1985). If the results are better than those for the linear regression it would show that the relationship is therefore rather quadratic than linear. This observation would indicate that the use of diatom species diversity in a strictly linear fashion as tool to evaluate water quality is not optimal.

Tables 5, 6 and 7 present the second degree polynomial regressions that were performed, for species diversity, species evenness and number of species, respectively, with water quality.

TABLE 5 Polynominal regression summary for Shannon species diversity with water quality (italicised values significant at p<0.05)			
	R^2= 0.546		
N=102	**Beta**	**Std.Err. of Beta**	**p-level**
Intercept			*<0.001*
F*F (mg/ℓ)	*0.658*	*0.167*	*<0.001*
Ca*Ca (mg/ℓ)	0.375	0.255	0.146
Temperature (°C)	*0.223*	*0.101*	*0.030*
Ca (mg/ℓ)	*-0.947*	*0.377*	*0.014*
F (mg/ℓ)	-0.253	0.229	0.273
Si (mg/ℓ)	0.103	0.166	0.537
DO* DO (mg/ℓ)	0.007	0.097	0.945
PO_4 (mg/ℓ)	0.720	0.552	0.196
K*K (mg/ℓ)	*-1.773*	*0.447*	*<0.001*
Mg (mg/ℓ)	0.255	0.532	0.633
Mg*Mg (mg/ℓ)	-0.378	0.265	0.158
SO_4*SO_4 (mg/ℓ)	*-1.075*	*0.290*	*<0.001*
SO_4 (mg/ℓ)	*1.648*	*0.499*	*0.001*
Turbidity*Turbidity (NTU)	*0.670*	*0.219*	*0.003*
Turbidity (NTU)	*-0.601*	*0.193*	*0.003*
DO (mg/ℓ)	0.210	0.119	0.080
K (mg/ℓ)	*0.916*	*0.388*	*0.021*
PO_4*PO_4 (mg/ℓ)	-0.141	0.524	0.789
NH_4*NH_4 (mg/ℓ)	*0.878*	*0.371*	*0.020*
NH_4 (mg/ℓ)	-1.014	0.547	0.068
Cl*Cl (mg/ℓ)	0.391	0.217	0.075

As can be seen from the R^2 values for the regression, which includes second degree polynomial terms of water quality variables, much more of the variation in the diversity data was explained than with the linear regressions. The R^2 for species diversity increased from 21.5% to 54.5% and the R^2 for the species evenness increased from 20% to 40% when including the second degree polynomial terms. The number of species showed the least amount of improvement in the R^2 that increased from 32% to 43%.

TABLE 6

Polynomial regression summary for Pielou species evenness with water quality (italicised values significant at $p<0.05$)

	R^2= 0.404		
N=102	**Beta**	**Std.Err. of Beta**	**p-level**
Intercept			*<0.001*
Si*Si (mg/ℓ)	*0.416*	*0.143*	*0.005*
Ca*Ca (mg/ℓ)	*0.212*	*0.098*	*0.034*
F*F (mg/ℓ)	*0.532*	*0.138*	*<0.001*
Temperature (°C)	*0.185*	*0.087*	*0.036*
DO* DO (mg/ℓ)	-0.086	0.085	0.312
PO_4 (mg/ℓ)	*0.771*	*0.225*	*0.001*
K*K (mg/ℓ)	*-0.737*	*0.223*	*0.001*
Turbidity*Turbidity (NTU)	*0.257*	*0.118*	*0.032*
NH_4 (mg/ℓ)	-0.604	0.382	0.117
NH_4*NH_4 (mg/ℓ)	0.298	0.270	0.272

TABLE 7

Polynomial regression summary for the number of diatom species with water quality (italicised values significant at $p<0.05$)

	R^2= 0.430		
N=102	**Beta**	**Std.Err. of Beta**	**p-level**
Intercept			*<0.001*
F*F (mg/ℓ)	*0.341*	*0.153*	*0.029*
pH	-0.193	0.161	0.233
K*K (mg/ℓ)	-0.497	0.364	0.176
Ca*Ca (mg/ℓ)	*0.710*	*0.262*	*0.008*
Ca (mg/ℓ)	*-1.778*	*0.386*	*<0.001*
Mg (mg/ℓ)	*1.946*	*0.482*	*<0.001*
Mg*Mg (mg/ℓ)	*-0.638*	*0.281*	*0.026*
Si*Si (mg/ℓ)	-0.211	0.181	0.246
Na (mg/ℓ)	*-1.153*	*0.413*	*0.006*
Si (mg/ℓ)	0.242	0.172	0.163
PO_4*PO_4 (mg/ℓ)	0.106	0.209	0.614
K (mg/ℓ)	*0.831*	*0.414*	*0.048*
Cl*Cl (mg/ℓ)	0.492	0.308	0.115
Temperature (°C)	-0.148	0.100	0.142
pH*pH	-0.135	0.109	0.220
DO (mg/ℓ)	-0.113	0.107	0.295

Aut-ecological/ biotic indices

The regression summary for the biotic indices BDI and SPI is presented in Table 8 and Table 9. For both these indices water quality contributed to about 80% of the variation in the data. For the SPI the significant contributors were Na, Si, pH, PO_4, Ca, Cl and SO_4. The significant water quality contributors to the BDI were much the same as for the SPI with the addition of NO_3+NO_2, Mg and F. When second degree polynomial regressions were fitted for the BDI and SPI, the R^2 value for the regressions changed marginally from near 80% to 84% (not shown). The increase in the R^2 value is probably due to a minor degree of "overfitting" due to the addition of variables to the model in the case of the second degree polynomial regression (Hair et al., 1998). This change in R^2 value is small when compared to the changes encountered in the diversity related regressions (see above).

As indices are usually applied in a linear fashion (high value indicating good environmental condition, low values indicating poor environmental conditions), a linear response of index scores to water quality variables is a desirable attribute of such indices. Since the linear regressions of SPI and BDI with water quality variables are high (approximately 80%) and little is gained from the addition of polynomial terms, it is suggested that the linear relationship of SPI and BDI with a water quality gradient is sufficient for use of the index in a linear fashion in river systems.

TABLE 8
Regression summary for the SPI with water quality (italicised values significant at p<0.05)

	R^2= 0.796		
N=102	**Beta**	**Std.Err. of Beta**	**p-level**
Intercept			1.000
Na (mg/ℓ)	*-1.978*	*0.524*	*<0.001*
Si (mg/ℓ)	*-0.297*	*0.093*	*0.002*
pH	*0.305*	*0.068*	*<0.001*
NO_3+NO_2	0.249	0.150	0.101
K (mg/ℓ)	-0.231	0.175	0.191
PO_4 (mg/ℓ)	*0.368*	*0.173*	*0.036*
NH_4 (mg/ℓ)	-0.054	0.171	0.752
Ca (mg/ℓ)	*-0.344*	*0.172*	*0.049*
Cl (mg/ℓ)	*1.108*	*0.449*	*0.015*
SO_4 (mg/ℓ)	*-0.195*	*0.097*	*0.048*
F (mg/ℓ)	0.186	0.137	0.179
Mg (mg/ℓ)	0.229	0.190	0.231

TABLE 9 Regression summary for the BDI with water quality (italicised values significant at $p<0.05$)			
	$R^2 = 0.810$		
N=102	Beta	Std.Err. of Beta	p-level
Intercept			1.000
Na (mg/ℓ)	*-2.032*	*0.458*	*<0.001*
Si (mg/ℓ)	*-0.287*	*0.080*	*0.001*
NO_3+NO_2 (mg/ℓ)	*0.305*	*0.083*	*<0.001*
K (mg/ℓ)	-0.265	0.152	0.084
pH (mg/ℓ)	*0.178*	*0.064*	*0.006*
Ca (mg/ℓ)	*-0.571*	*0.152*	*<0.001*
Cl (mg/ℓ)	*1.159*	*0.387*	*0.004*
PO_4-P (mg/ℓ)	*0.305*	*0.096*	*0.002*
Mg (mg/ℓ)	*0.360*	*0.178*	*0.046*
F (mg/ℓ)	*0.229*	*0.113*	*0.047*
SO_4 (mg/ℓ)	-0.174	0.089	0.054

Principle Component Analysis (PCA)

A principle component analysis was performed to visually represent the response of the two types of indicators with water quality variables. The results of this analysis are shown in Figure 2 and Table 10. From the figure we can see that the main drivers in the water quality in the catchment are Na and Cl (correlates with the first ordination axis). The BDI and SPI indices (representing the aut-ecological indices) are strongly affected by the main drivers of the water quality in the catchment and show a strong negative response to increasing salt loadings (Na and Cl). The length of the vectors representing the various indices in Figure 2 also indicates a much stronger effect of water quality variables on the BDI and SPI than on the diversity index measures (as the vectors of the former are longer than those of the latter).

Diversity and evenness as shown in Figure 2 seem to increase in the same direction as most of the water quality variables. This might be caused by the fact that diversity seems to be higher at sites with intermediate values for the water quality parameters measured in the study.

The number of species (Figure 2) was associated with increased oxygen in the water and negatively associated with the chemical variables that denote possible organic loading of the water system (NH_4, PO_4, NO_2+NO_3). However, the relationship of the number of species to water quality variables was low, with R^2 values of 0.320 and 0.430 (Tables 4 and 7).

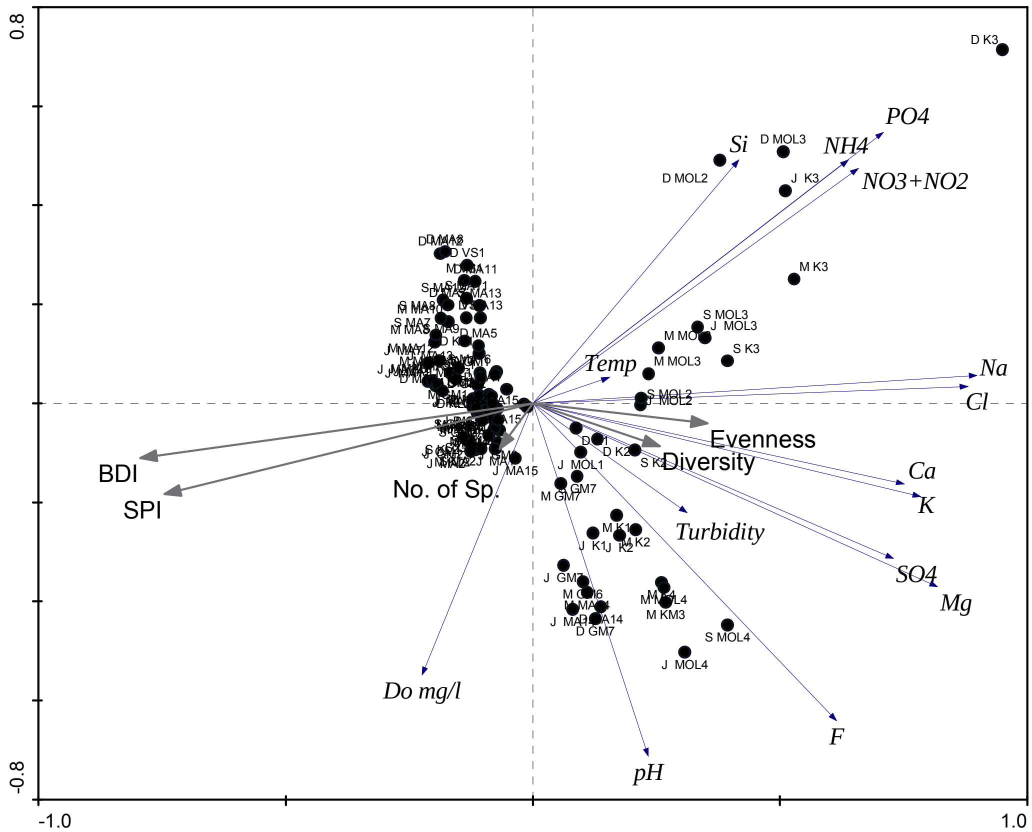

Figure 2

Principle Component Analysis (PCA) indicating chemical variables and diatom-based indices as vectors and sites as dots (Site names are denoted by a letter denoting the month of sampling, followed by the site name as indicated in Figure 1)

TABLE 10 **Results for PCA performed for sites in the Marico- and Molopo Rivers** **(Biological and habitat indices were used as supplementary data in the ordination)**					
Axes	**1**	**2**	**3**	**4**	**Total variance**
Species-environment correlations	0.803	0.378	0.331	0.143	
Cumulative percentage variance:					
of species data	40.6	57.8	70.5	78.8	
of species-environment relation	81.1	88.7	93	93.6	
Sum of all canonical eigenvalues					0.323

Discussion

From the results it is clear that both types of diatom-based indicators (diversity and aut-ecological) used were significantly influenced by water quality variables. From the three different diversity indices calculated, the number of species showed the strongest response to changes in water quality variables, although this relationship is fairly weak (Figure 2). Of all the indicators tested in the study, the species diversity and the species evenness showed the weakest response to water quality variables (Tables 2 and 3).

From the second degree polynomial multiple regressions, it can be seen that the highest species diversity and evenness as well as number of species occur in moderately impacted water as suggested by Archibald (1971) and Van Dam (1982). A high degree of dominance may therefore be expected at both clean water and polluted water sites. It would seem logical to conclude that moderately impacted water can harbor species that can be dominant in either good or polluted water, as observed by Van Dam (1982). This species distribution is also in line with what the Intermediate Disturbance Hypothesis (IDH) as postulated by Connell (1978) would suggest. The IDH is one of the most frequently suggested nonequilibrium explanations for the maintenance of species diversity in ecological communities (Roxburgh et al., 2004).

Townsend et al. (1997), suggest the following explanation of how the hypothesis may work in an ecosystem:

"At one extreme, patches that are frequently and/or intensely disturbed are expected to exhibit low species richness because few species are able to colonize during the brief periods between disturbances or tolerate the high intensities of their impact. At the other end of the scale, patches in which disturbances are infrequent and/or or low intensity are expected also to be poor in species because they become dominant by competitive superior taxa. Richness should be highest at intermediate levels of disturbance because rapid colonizers and more competitive species co-occur."

Due to the fact that the species evenness does not exhibit a strong linear association with water quality, it would be logical to conclude that, in the case of diatoms, a high level of dominance in the population (as would be represented by a low evenness index in this study) cannot be equated to polluted or less favorable conditions. It would be more consistent with the data to expect that diatoms have well defined niches and that taxa best suited to water quality conditions at a specific point in time will become dominant. This is borne out by Cholnoky (1960) who stated that: "…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*" (own italics).

This would also suggest that specific diatom taxa will be dominant in certain (and most) water quality conditions. Representatives of many species are always present in low numbers in the population and can become dominant when water quality is suitable.

The data is also consistent with postulations from Kelly (1998) who discussed the concept that diatoms are 'subcosmopolitan', i.e. they occur anywhere in the world if 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. Finlay (2005) also states that it is now clear that distribution patterns of protists are quite different from those of macroscopic organisms – e.g. the recent discovery of the ubiquity-biogeography transition, where organisms smaller than about 1 mm occur worldwide wherever their required habitats are realized. However, some diatoms may be more susceptible to desiccation etc. and thus may not be so easily distributed. This would appear to be the case when (possibly) endemic diatoms such as *Achnanthes standerii*

Cholnoky are found *en-masse* in certain rivers and streams around South Africa but have never been reported from outside our borders. Whether diatoms such as these are in fact true endemics or if their distribution in simply governed by the factors such as local geology and climate, which may not be found elsewhere, remains a topic for further investigation.

In comparison to the diversity indices, the BDI and SPI as representatives of aut-ecological indices displayed a significantly better relationship with measured water quality variables as shown by the multiple regression results. It is also important to note that the relationship of the BDI and SPI with water quality did not increase significantly when the quadratic functions were added. The implication of this would be that the aut-ecological indices may be applied in a linear fashion in contrast to diversity indices.

The abovementioned results indicate that aut-ecological indices based on diatoms are more useful in biomonitoring programs of rivers and streams than diversity indices; a point strongly supported by the high R^2 values of the linear multiple regressions for SPI and BDI in Table 8 and 9.

Conclusions

The current study and the results presented in the different sections of the current study warrant the following conclusions:

- Diversity measures based on the abundance of diatoms appear to show a relationship to water quality variables although that relationship is not linear.

- The results from the linear and second degree polynomial regressions show that diatom species diversity (especially as reflected by the Shannon Species Diversity Index used in this study) tends to be higher in moderately impacted water.

- Due to the highly significant relationship of aut-ecological diatom indices with water quality, these indices are deemed more relevant and reliable for use in rivers and streams to inform decision making in integrated water resource management.

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Chapter 6
Manuscript V

Based on the article:

On the use of diatom-based biological monitoring.
Part 2: A comparison of the response of SASS 5 and diatom indices to water quality and habitat variation

(original article in Appendix E)

Authors: J.C. Taylor, J. Prygiel, A. Vosloo, P.A. de la Rey & L van Rensburg
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Water SA

Author Guidelines

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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.

On the use of diatom-based biological monitoring
Part 2: A comparison of the response of SASS 5 and diatom indices to water quality and habitat variation

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Abstract

Due to the fact that South Africa is a water-scarce country, integrated water resource management based on sound information is essential. Bio-indicators have provided valuable information for water resource management in recent years and have enjoyed increasing popularity. Bio-indicators especially stepped to the forefront with the realization that aquatic eco-systems are not only a source of water but also deliver several goods and services, as well as being essential for industrial growth and quality of life of many South Africans. This study aimed to quantitatively test two kinds of biomonitoring tools namely diatom-based (SPI and BDI) and macro-invertebrate based (SASS 5) in order to assess (1) their applicability in South African River systems and (2) if any additional information can be gained by using the two tools in tandem. The results showed that diatom indices are affected more by changes in water quality than SASS 5, while SASS 5 displayed a higher dependency on habitat quality, as measured by IHAS, than the diatom indices. It is therefore suggested that the two indices be utilized as complementary indicators for integrated assessment of river health.

Introduction

Species of flora and fauna 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). Aquatic communities (e.g. fish, riparian vegetation, macro-invertebrates) can integrate and reflect the effects of chemical and physical disturbances that occur in river ecosystems over extended periods of time.

Walmsley et al. (2000) stated that bio-indicators are ideal means of monitoring aquatic ecosystems, leading towards integrated water resource management, and that bio-indicators provide a summary of conditions ‘rather like temperature and blood pressure are used to measure human health’.

The South African Department of Water Affairs and Forestry (DWAF), as custodians of the water resources of the country, initiated the development of a National Aquatic Ecosystem Biomonitoring Programme (also called the River Health Programme or RHP) during 1995 (Roux, 1997). Examples of such indicators include the Fish Assemblage Integrity Index (Kleynhans, 1999), the Riparian Vegetation Index (Kemper, 2001) as well as the South African Scoring System, better known as SASS (Chutter, 1998). Although some methods have been available for many years, biomonitoring has only recently become a routine tool in the management of South Africa’s inland waters (Davies & Day, 1998). The SASS biomonitoring system has gained a large body of support as a rapid and fairly accurate system of evaluating water quality in streams and rivers, and is currently in its 5^{th} revised form namely SASS 5 (Dickens & Graham, 2002).

Recently diatom-based indices such as the Specific Pollution Index (SPI) and Biological Diatom Index (BDI) have come into the spotlight as potential additions to more established bio-indicators such as SASS 5. Several papers have been published in the last few years exploring the potential use of diatoms as bio-indicators such as Taylor et al. (2007b), De la Rey et al. (2004) and Harding et al. (2005). A standard protocol for assessment using diatoms has also been published (Taylor et al., 2005) to facilitate comparability of diatom index results. The value of diatoms as indicators has been recognized to the point that it has been included in the state of the rivers report for the Crocodile (West) – Marico River Water Management Area (Taylor et al., 2007c; River Health Programme, 2005).

In a recently published article, Ashton et al. (2005) called for a shift in thinking from a point where water is seen as simply a commodity to recognizing that it is an integral part of a larger ecosystem, and that such an ecosystem approach demands an understanding of the various components of the hydrological cycle as well as the interrelationships of these various components. For this reason it is believed important to understand how different bio-indicators respond to the various changes in the aquatic ecosystem.

With the abovementioned in mind, it is also important to evaluate biological indices in terms of their relationship to habitat characteristics. In this study, the Integrated Habitat Assessment System version 2 (McMillan, 1998) was used as indicator of habitat condition. This assessment focuses on sampling habitat, especially habitat that can be utilized by invertebrate fauna, as well as other stream characteristics, such as water quality, which may be modified by anthropogenic or natural impacts.

Currently IHAS is the most widely used method for invertebrate habitat assessment in South Africa (Ollis et al., 2006) and was therefore used as indicator of habitat in the current study.

This assessment focuses on sampling habitat, especially habitat that can be utilized by invertebrate fauna, as well as other stream characteristics, such as water quality, which may be modified by anthropogenic or natural impacts. Two different facets of in stream habitat are assessed by the system. Firstly the sampled habitat is assessing in three categories namely Stones In Current, Vegetation and Other Habitats. These three categories amount to a maximum score of 55. The second section evaluated the stream characteristics such as flow tempo, depth, breadth etc. This section has a maximum score of 45 which, when counted with the previous section, gives a total IHAS score of 100. The habitat condition is therefore easily interpreted as a percentage.

This paper represents the second part of a study that aims to evaluate the efficacy of diatom-based indices in river systems in South Africa. The paper follows from Part 1: A comparison of the response of diversity and aut-ecological diatom indices to water quality variables in the

Marico-Molopo River catchment, which concluded that aut-ecological indices should be preferentially used as they respond in a linear fashion to environmental water quality gradients.

The current paper aims to compare the relationship of the SASS 5 invertebrate index and diatom indices to chemical water quality and habitat availability. There are several questions that the current paper strives to answer. Firstly whether there is a significant difference in the response of SASS 5 and diatom-based aut-ecological indices to changes in stream habitat and water quality. If the two indices respond similarly, and to the same extent, to water quality variables, there would be no additional benefit to be found in using both indices for monitoring changes and impacts in rivers. Secondly the present paper aims to evaluate the dependency of index response on variation in habitat and seasonal changes. The answers gained from such analysis can assist in the application and interpretation of results gained when using the various bio-indicators evaluated in this paper.

Materials and Methods

Sampling Localities

For information on the sampling localities please refer to Part 1: A comparison of the response of diversity and aut-ecological diatom indices to water quality variables in the Marico-Molopo River catchment.

Indices calculations

Macroinvertebrates were collected using the SASS 5 methodology. The SASS score, Average Score Per Taxon (ASPT) and Number of Taxa (No. of Taxa) were calculated according to standard methods as set out in Dickens & Graham (2002) and Chutter (1998).

Diatoms were collected, prepared and enumerated according to the protocol as set out in Taylor et al. (2005). The diatom identification was according to the nomenclature of Krammer and Lange Bertalot (1986-1991). For the current paper the aut-ecological method for evaluating water quality by means of diatoms were used. This choice is based on the results obtained from Part 1 of the study. A description of these indices is given in Part 1 of the study. For the current study the Specific Pollution sensitivity Index (SPI) (Coste *in* Cemagref, 1982) as well as Biological Diatom Index (BDI) (Lenoir & Coste, 1996), indices were calculated for the various sampling localities using Omnidia v.3.1 software (Lecointe et al., 1993). The reasons for the selection of these two indices are that SPI has the broadest species base and that BDI showed the best overall correlation to water quality variables in studies performed recently on the Vaal River (Taylor et al., 2007b). More details are given in Part 1 of this paper.

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The in-stream habitat was evaluated by means of the Integrated Habitat Assessment version 2 (IHAS) (McMillan, 1998). This evaluation was done for every sampling site on every sampling occasion. IHAS endeavours to numerically express the availability of in-stream habitat in terms of quantity, quality and diversity. In the system both the sampling habitat (stones, vegetation, gravel, sand and mud) and the general river/stream condition is evaluated and a total habitat score calculated.

As diatoms were only sampled from the "Stones in Current" biotope, the SASS biotopes (Stones in Current and Stones out of Current, Marginal- and Submerged Vegetation as well as Gravel Sand and Mud) were also scored individually to facilitate comparability of the responses of the indices to habitat and water quality variables. It is a well known fact that macroinvertebrates are influenced by habitat availability (Ollis et al , 2006a; McMillan, 1998; Dallas, 1997; Karr & Dudley, 1981). Due to this fact, IHAS is mainly used in this study to facilitate interpretation of macroinvertebrate-based data as compared to diatom-based data, rather than as a definitive measure of stream quality. This study does therefore not directly focus on the reliability of IHAS as an indicator of habitat conditions influencing invertebrates.

Water quality

For details on the water quality analysis for the study please refer to Part 1 of the study.

Statistics

Details on the methods used for the statistical analysis of the data are given in Part 1 of the study. However, it was felt that an abbreviated overview is justifiable due to minor alterations in statistical methods applied in Part 1.

Again, multiple regressions and correlation analysis were performed using the STATISTICA software package (Release 7, Stat Soft. Inc., United States of America), while Principle Component Analysis (PCA) was performed using CANOCO for Windows (Version 4.51, Biometris-Plant Research International, The Netherlands). Before analysis, all the data were standardized. For the purpose of the multiple regressions the Electrical Conductivity and Total Alkalinity were left out of the analysis as these variables contributed to multi-colinearity in the data. In addition to the above analysis, predicted vs observed graphs of certain regression analysis are also shown. Such graphs were obtained from the STATISTICA software package. Since multiple regression endeavours to construct a linear relationship between explanatory variables (water quality variables) and a dependant variable (index scores) predicted vs. observed graphs indicates the strength of this relationship. The scatter plots show the relationship between the predicted values of the dependant variable (i.e. the multiple regression) and the observed (actual) value of the dependant variable. If the dots in the scatterplot are close to the linear line, it suggest that the regression fits the data very well.

Another set of multiple regressions was performed to investigate whether season has an influence on the performance of the different indicators used. Season is a categorical dependent variable and was transformed into multiple (dummy-) coded dependent variables (see Hair et al., 1998) for the analysis. In the current study, direct comparison of seasonal response of the bio-indicators was prevented because data from all four sampling periods could only be obtained for 17 of the sites. This was mainly due to varying flow at the identified sites in the different seasons which hindered SASS 5 sampling in many instances. It was therefore deemed preferable to include season as variable in the multiple regression of the combined data set.

Results and Discussion

The results are presented and discussed in three sections. Firstly the correlations between the different bio-indicators will be investigated. This was done to establish whether there is any value in using more than one bioindicator. High correlation between the indicators will show that they respond in a similar fashion to environmental variables, showing that little additional information can be obtained from the extra effort, time and money spent in acquiring the data for the additional indicator. This section will also be used to determine whether bio-indicators sampled from the same biotope are more alike in response to water quality and habitat quality than bio-indicators from other biotopes.

The second section examines the correlation of the biological indices to specific components of water quality in order to ascertain whether the tested bio-indicators respond differently to water quality variables. Whereas the previous section focussed on whether the bio-indicators are correlation with one another, the focus in this section is therefore rather which water quality and habitat variables influence specific bio-indicators.

Section three entails exploration of the response of bio-indicators to water quality and habitat data by means of multiple regressions. This enables quantification of the influence of water quality and habitat on the different bio-indicators.

1. Correlation between different bio-indicators

Table 1 shows the correlation of the different bio-indicators between one another. The correlations between SASS 5 and the No. of (invertebrate) Taxa with the diatom-based indices (BDI and SPI) are reasonably high at 62% and 66% respectively. From the three invertebrate indices calculated according to the SASS 5 protocol, the ASPT displays the highest correlation with the diatom indices at approximately 70% correlation. The SASS 5 score exhibited slightly lower correlations with the diatom indices than the ASPT, while the No. of Taxa displayed the lowest correlation with the diatom-based indices.

An analysis was also performed to compare the macroinvertebrate index scores for the different biotopes mentioned in the SASS 5 protocol. When comparing the scores generated from the individual biotopes (S - stones in current biotope, V - vegetation biotope and GSM - gravel sand and mud biotope) with the diatom-based indices, there was little difference in the correlation values. The stones in current biotope however, did show a slightly higher correlation with diatom-based indices than the other two biotopes. The total SASS scores however still showed a higher correlation with the diatom indices than any of the individual biotopes.

Although there is a fairly high correlation between the diatom-based indices and the invertebrate-based indices (62%-71%; Table 1), there is still a significant amount of difference in the response of the indices to changes in their environment (29%-38%; Table 1). It is therefore useful to further investigate the relationships of the different indices to individual components of their environment (for instance water quality and habitat). This is explored in the following sections.

TABLE 1 Significant correlation between different bioindicators		
Correlations are significant at $p < 0.05$ N=102		
	SPI	BDI
SASS 5	0.66	0.62
ASPT	0.71	0.69
No. of Taxa	0.65	0.63
SASS (S)	0.63	0.59
ASPT (S)	0.79	0.76
No. Taxa (S)	0.60	0.57
SASS (V)	0.56	0.56
ASPT (V)	0.73	0.72
No. Taxa (V)	0.48	0.49
SASS (GSM)	0.57	0.54
ASPT (GSM)	0.76	0.73
No. Taxa (GSM)	0.53	0.52
(S) Stone biotope; (V) Vegetation biotope; (GSM) Gravel, Sand and Mud biotope		

2. Correlation of bio-indicators with water quality and habitat variables

The correlation of biological indicators with various water quality variables and habitat (IHAS) is presented in Table 2. From the table it is clear that all the bio-indicators (invertebrate-based as well as diatom-based) correlate well with water quality and habitat variables. The water quality variables that show the lower correlation with the bio-indicators (diatom-based and SASS 5) are pH, dissolved oxygen and water temperature. In general, it is also of interest that, diatom indices display a stronger correlation to almost all water quality variables than do the invertebrate indices. The water quality variables which display the highest correlation to the diatom-based indices are variables reflecting the salinity of the water like Na, Cl and EC. Note that in Part I, Na and Cl were also significant contributors in the regressions of water quality with SPI and BDI, but not in the regressions of water quality with species diversity and evenness. This is probably due to the weak regression results (low R^2 values) found for species diversity and evenness. The highest correlation of SASS with water quality variables is also with the ionic components of water quality such as Cl, EC, SO_4 and Na. This may be because the sampling area is dominated by agricultural activities which may lead to changes in salinity and this may in turn be the dominating determinant of water quality.

SASS indices display a higher correlation to the IHAS index than the diatom-based indices. This correlation is as high as 60% in the case of the SASS 5 score, while the correlation of IHAS and diatom indices is about 30%. It is interesting to note that the ASPT showed slightly better correlations with water quality variables and a lower correlation with IHAS. Table 1 also shows that ASPT is more closely correlated with the diatom-based indices than the SASS5 score or the Number of Taxa. This shows that the ASPT score is more likely to be influenced by water quality than habitat availability. This finding corresponds with the findings of Dallas (1997) who states that the ASPT score is relatively constant between biotopes, suggesting that sites that have different biotopes and habitats available for habitation by aquatic fauna can be compared on the basis of ASPT so that the extent of the impairment of the water quality can be established.

In their evaluation of the relationship of IHAS with SASS, Ollis et al. (2006b) suggested that the results obtained showed weak correlation between the two indices. This statement was mainly based on the results obtained from SASS 4 and IHAS scores. The study did however show strong correlations (up to 60%) when Total IHAS scores for Mpumalanga and the Western Cape were correlated with SASS 5 scores. This is approximately the same correlation found in the current study (Table 2) which also used SASS 5 as apposed to SASS 4. This correlation however does not indicate cause and effect but merely correlation (Hair et al. 1998). The results from the multiple regression are more indicative of the effect of habitat (as represented by IHAS) on SASS. This evaluation is dealt with in the next section.

TABLE 2
Correlation of bioindicators with water quality and habitat variables

Shaded correlations are significant at $p < 0.05$
N=102

	SASS 5	ASPT	No. of Taxa	SASS (S)	ASPT (S)	No. Taxa (S)	SASS (V)	ASPT (V)	No. Taxa (V)	SASS (GSM)	ASPT (GSM)	No. Taxa (GSM)	SPI	BDI
Ca (mg/ℓ Ca)	-0.28	-0.41	-0.28	-0.26	-0.47	-0.25	-0.28	-0.33	-0.28	-0.23	-0.44	-0.23	-0.46	-0.57
Cl (mg/ℓ Cl)	-0.51	-0.53	-0.53	-0.47	-0.58	-0.46	-0.46	-0.54	-0.43	-0.45	-0.60	-0.45	-0.73	-0.75
EC (mS/cm)	-0.43	-0.54	-0.42	-0.40	-0.61	-0.38	-0.39	-0.47	-0.37	-0.36	-0.59	-0.33	-0.65	-0.74
F (mg/ℓ F)	-0.25	-0.29	-0.17	-0.26	-0.37	-0.18	-0.19	-0.22	-0.14	-0.16	-0.33	-0.07	-0.34	-0.39
K (mg/ℓ K)	-0.47	-0.51	-0.44	-0.45	-0.55	-0.40	-0.38	-0.49	-0.31	-0.38	-0.54	-0.33	-0.62	-0.63
Mg (mg/ℓ Mg)	-0.39	-0.50	-0.37	-0.38	-0.57	-0.33	-0.36	-0.40	-0.33	-0.32	-0.53	-0.28	-0.54	-0.62
NH_4 (mg/ℓ NH_4-N)	-0.37	-0.43	-0.41	-0.36	-0.51	-0.38	-0.33	-0.45	-0.31	-0.35	-0.49	-0.38	-0.58	-0.59
NO_3+NO_2 (mg/ℓ NO_3+NO_2-N)	-0.37	-0.45	-0.41	-0.33	-0.45	-0.34	-0.34	-0.45	-0.35	-0.33	-0.49	-0.36	-0.47	-0.47
Na (mg/ℓ Na)	-0.54	-0.57	-0.55	-0.51	-0.62	-0.50	-0.48	-0.56	-0.44	-0.46	-0.63	-0.46	-0.77	-0.78
PO_4-P (mg/ℓ PO_4-P)	-0.36	-0.41	-0.40	-0.33	-0.44	-0.35	-0.33	-0.43	-0.32	-0.34	-0.48	-0.38	-0.50	-0.51
SO_4 (mg/ℓ SO_4)	-0.44	-0.47	-0.40	-0.41	-0.51	-0.36	-0.41	-0.42	-0.38	-0.38	-0.49	-0.34	-0.45	-0.50
Si (mg/ℓ Si)	-0.25	-0.36	-0.28	-0.24	-0.44	-0.25	-0.24	-0.35	-0.24	-0.21	-0.42	-0.22	-0.44	-0.47
TAL (mg/ℓ TAL)	-0.34	-0.47	-0.33	-0.33	-0.55	-0.30	-0.32	-0.38	-0.30	-0.28	-0.51	-0.25	-0.53	-0.64
pH	0.09	0.08	0.08	0.06	0.12	0.02	0.06	0.18	0.02	0.13	0.15	0.12	0.09	-0.06
Do (mg/ℓ DO)	0.15	0.21	0.13	0.10	0.33	0.05	0.17	0.25	0.14	0.17	0.28	0.17	0.30	0.28
Temp (°C)	0.11	0.09	0.16	0.12	-0.08	0.19	0.13	0.02	0.18	0.15	0.01	0.18	-0.13	-0.11
Turbidity (NTU)	-0.36	-0.25	-0.36	-0.37	-0.29	-0.35	-0.32	-0.27	-0.29	-0.27	-0.29	-0.25	-0.36	-0.32
IHAS (%)	0.60	0.40	0.61	0.61	0.40	0.59	0.57	0.44	0.53	0.50	0.39	0.50	0.31	0.27

(S) Stone biotope; (V) Vegetation biotope; (GSM) Gravel, Sand and Mud biotope

3. Response of bio-indicators to water quality and habitat

Table 3 presents the multiple regressions performed for each of the indicators with water quality (indicated with WQ in Table 3), habitat (IHAS) as well as a combined multiple regressions for each indicator with both water quality and habitat as predictors. Multiple regressions were also performed for the different biotopes (S, V and GSM) that make up the SASS 5, ASPT and No. of Taxa scores.

TABLE 3
Results for regression performed for bioindicators with (1) habitat and water quality (2) water quality and (3) habitat

Dependent variable	Independent variables used	R^2	Significant contributors
SASS 5	WQ & IHAS	0.651	IHAS, Na, SO_4, Cl, pH, PO_4, Mg, F, K
	WQ	0.491	Na, SO_4, pH, Temp, Cl, Turb
	IHAS	0.365	IHAS
ASPT	WQ & IHAS	0.623	IHAS, Mg, pH, Temp, K, F
	WQ	0.598	K, Temp, pH, Mg, PO_4, F, SO_4
	IHAS	0.157	IHAS
No of Taxa	WQ & IHAS	0.651	IHAS, Na, Cl, SO_4, F, Mg
	WQ	0.505	Na, Temp, pH, SO_4, Cl, Turb, F
	IHAS	0.367	IHAS
SPI	WQ & IHAS	0.799	Na, Si, pH, PO_4, Ca, Cl
	WQ	0.796	Na, Si, pH, PO_4, Ca, Cl, SO_4
	IHAS	0.093	IHAS
BDI	WQ & IHAS	0.813	Na, Si, NO_3+NO_2, pH, Ca, Cl, PO_4, Mg
	WQ	0.810	Na, Si, NO_3+NO_2, pH, Ca, Cl, PO_4, Mg, F
	IHAS	0.071	IHAS
SASS (S)	WQ & IHAS	0.617	IHAS, Na, Cl
	WQ	0.461	Temp, Turb, pH, SO_4
	IHAS	0.371	IHAS
ASPT (S)	WQ & IHAS	0.732	IHAS, pH, Mg, PO_4, NH_4
	WQ	0.706	Si, pH, Mg, PO_4
	IHAS	0.163	IHAS
No of Taxa (S)	WQ & IHAS	0.578	IHAS, Na, Temp, Cl
	WQ	0.437	Na, Temp, Cl, Turb, SO_4
	IHAS	0.349	IHAS
SASS (V)	WQ & IHAS	0.519	IHAS, SO_4, F
	WQ	0.394	Na, SO_4, Turb, Cl
	IHAS	0.320	IHAS
ASPT (V)	WQ & IHAS	0.587	IHAS, pH, SO_4, F, K
	WQ	0.551	pH, SO_4, F, K, PO_4, Si
	IHAS	0.190	IHAS
No of Taxa (V)	WQ & IHAS	0.429	IHAS, Na
	WQ	0.351	Na, SO_4, Turb
	IHAS	0.281	IHAS
SASS (GSM)	WQ & IHAS	0.470	IHAS, pH, K
	WQ	0.405	Temp, pH, SO_4
	IHAS	0.249	IHAS
ASPT (GSM)	WQ & IHAS	0.673	IHAS, pH, Mg, Temp
	WQ	0.664	Si, pH, Mg, Temp
	IHAS	0.149	IHAS
No of Taxa (GSM)	WQ & IHAS	0.446	IHAS, Na, Temp
	WQ	0.365	Na, Temp, pH, SO_4
	IHAS	0.247	IHAS

Significance chosen at a p-value $\leq$ ***0.05.***
IHAS denotes habitat scores. WQ denotes water quality variables.
(S) Stone biotope; (V) Vegetation biotope; (GSM) Gravel, Sand and Mud biotope

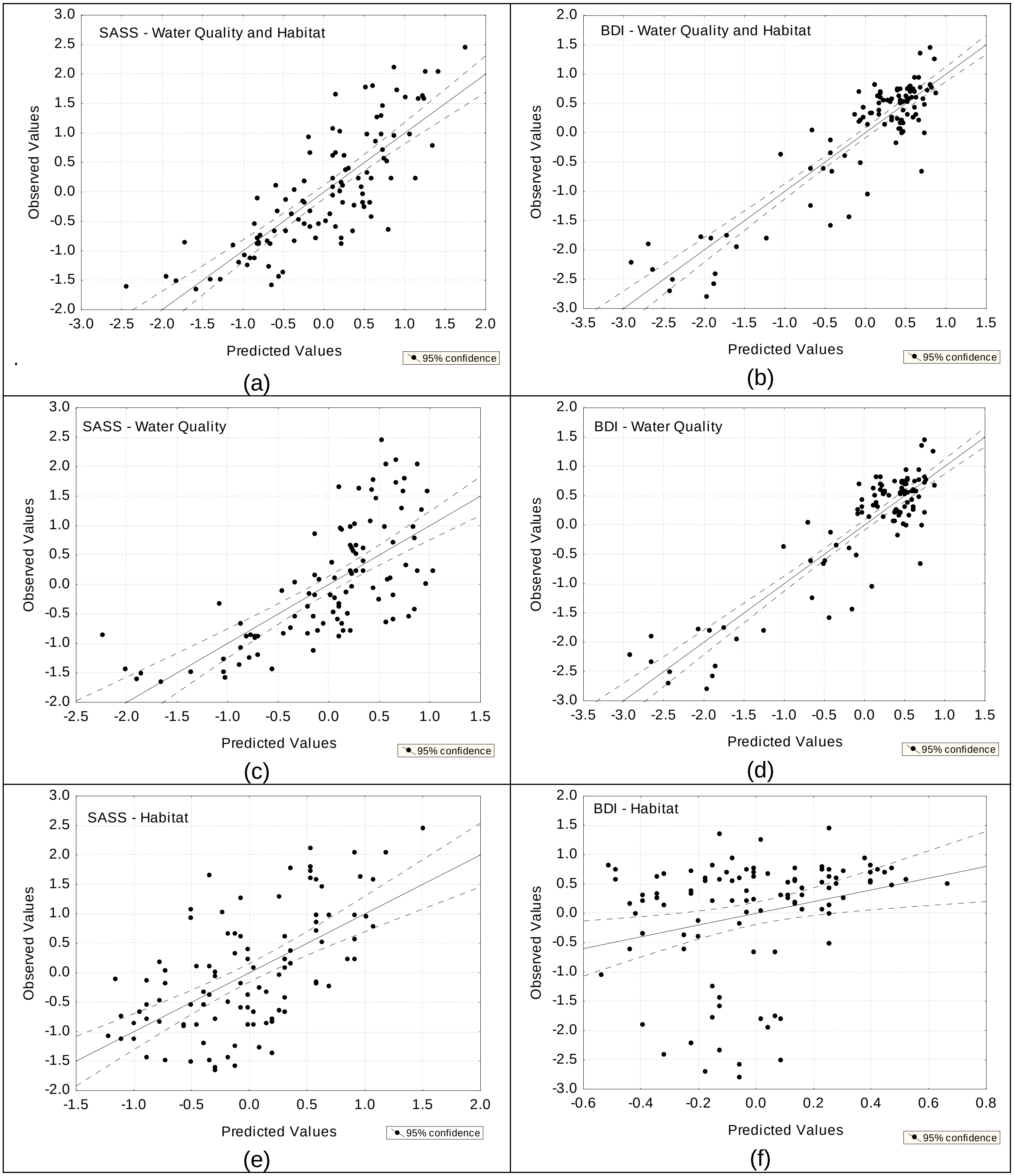

Figure 1

Representation of regression results (predicted against observed graphs) of SASS 5 (left) and BDI (right) using water quality and habitat (top), only water quality (middle) and only habitat (bottom) as independent variables.

From Table 3 one may observe that the habitat score (IHAS) influences SASS 5, ASPT and No. of Taxa more strongly than the diatom-based indices (SPI and BDI). Only 6-8% of the variance explained by the multiple regression for diatom-based indices could be attributed to IHAS, whereas habitat explained 35% of the variance in the SASS 5 and the No of Taxa, and 15% to the ASPT score. This is a much lower value than the 60% found in the correlation results (Table 2). The apparently conflicting results can be explained by the fact that, in many instances, lower IHAS scores correspond with sites that also displayed poorer water quality in terms of the measured variables. This will have the effect that the correlation values will be higher but does not necessarily reflect causality. The results from the multiple regression on the other hand do indicate a 'cause and effect' relationship between IHAS and SASS scores (or any other variable used in the multiple regression e.g. ASPT, BDI etc). In the current data set therefore, water quality contributes to approximately 50% of the variation in the SASS score, while the habitat as reflected/indicated by IHAS contributes approximately 36%.

These above-mentioned results are illustrated in Figure 1. From the results indicated in Table 2 as well as Figure 1 it is clear that there is little difference in the predictive power of the linear model for the BDI index if water quality alone is used, or when habitat and water quality are used as independent variables (panels b and d) due to the similarity of the R^2 values and the similarity of the graphs. It is also clear from the graphs that habitat has better predictive power when used for SASS 5 than for BDI (panels e and f).

Table 3 also shows that the chemical variables that influenced the diatom-based indices were Na, Si, pH, PO_4, Cl, Ca, NO_3+NO_2, Mg, F and SO_4. Overall, chemical variables in water influenced the diatom indices more strongly than was the case for the invertebrate indices, while the habitat seemed to exert little influence on the diatom index scores in the combined analysis (R^2= 0.071 to 0.093). There was very little difference in the amount of variation explained by water quality variables for the SPI and BDI respectively. In both cases about 80% of the variation in the diatom index scores could be explained by water quality variables. The main difference between the indices (SPI/BDI) is the number of species accommodated in the system. BDI utilises approximately 209 species while the SPI can accommodate a larger number of taxa (approximately 1700 species; Coste *in* Cemagref, 1982). SPI is very sensitive to changes in water quality and provides high correlations with chemistry (e.g. De la Rey et al., 2004) but it has some disadvantages. SPI is regularly updated to take into account taxonomical research results and it is sometimes unclear which version is used. The list of taxa is also dependent on the skills of the operator, on the flora used, and on the time spent on analysis. Since the BDI index employs only 209 important indicator taxa, it facilitates more rapid identification than the SPI. Problems using the BDI may occur in cases where samples contain dominant diatom species that are not used by the index. However, this was not encountered in the current study.

Although the BDI and SPI responded adequately in the current study, these indices need to be adapted for South African conditions by the addition of endemic species. The groundwork for such an adaptation has been laid by a Water Research Commission (WRC) report (Taylor et al., 2007a) that describes about 400 species dominant in South African rivers along with ecological information and gives an account of some endemic species not included in European indices. Another WRC project is currently in progress, the ultimate goal of which will be to formulate a unique diatom index for South Africa and will include the more common diatom species endemic to South Africa (Taylor, 2006).

The important chemical variables that influenced the invertebrate indices were Na, Cl, SO_4, Mg, F and K (Table 3). The temperature of the water also significantly influenced all three of the invertebrate indices.

The ASPT component of the SASS 5 scores (Table 3) showed the strongest response to water quality variables and the weakest response to the habitat scores. Of all the scores generated in the different biotopes, the ASPT in the stones biotope showed the strongest response to water quality and habitat scores in comparison with the other indices and other biotopes. In all biotopes the ASPT was the component of SASS 5 that showed the most reliable response to water quality and habitat variation. This is in agreement with the findings of Dickens & Graham (2002) who stated that the ASPT appears to be a more consistent and repeatable measure of river health.

TABLE 4
Multiple regression results for the tested bioindicators with water quality, habitat and season*
*(season used as a categorical variable)

Dependent variable	Independent variables used	R^2	Significant contributors
SASS 5	WQ & IHAS	0.651	IHAS, Na, SO_4, Cl, pH, PO_4, Mg, F, K
	WQ & IHAS & Season	0.701	IHAS, Season, DO, Temp., pH, Mg
ASPT	WQ & IHAS	0.623	IHAS, Mg, pH, Temp., K, F
	WQ & IHAS & Season	0.627	IHAS, Mg, pH, Cl, Temp., Season, K
No of Taxa	WQ & IHAS	0.651	IHAS, Na, Cl, SO_4, F, Mg
	WQ & IHAS & Season	0.681	IHAS, Na, Season, Cl, Temp., pH, Mg
SPI	WQ & IHAS	0.799	Na, Si, pH, PO_4, Ca, Cl
	WQ & IHAS & Season	0.799	Na, Si, pH, PO_4, Ca, Cl
BDI	WQ & IHAS	0.813	Na, Si, NO_3+NO_2, pH, Ca, Cl, PO_4, Mg
	WQ & IHAS & Season	0.813	Na, Si, NO_3+NO_2, pH, Ca, Cl, PO_4, Mg

As can be seen in Table 4, season contributed statistically significantly ($p<0.05$) to the stepwise multiple regressions of SASS, ASPT as well as No. of Taxa.

The variation of macroinvertebrate indicator scores over season has been noted in several papers (Maloney & Feminella, 2006; Maul et al., 2004; Townsend et al., 1987). Gratwicke (1999) stated that SASS scores improved with the rainy season (January to March) but deteriorated in the dry season. Dallas (2004) found significant differences in ASPT and No. of Taxa between seasons in the Western Cape, with higher ASPT values recorded in winter and spring, while the No. of Taxa was higher for summer than winter. The same study did not find any significant differences between SASS, ASPT and No. of Taxa values when compared among seasons when samples from Mpumalanga were analysed. For the current study, no statistically significant differences were recorded between samples from different seasons, although SASS and No. of Taxa scores were higher in spring and summer than for autumn and winter (analysis not shown). The ASPT values were fairly constant between seasons which are also indicated from the multiple regressions in Table 4. The diatom-based indices

(Table 4) were not affected significantly by seasonality and water quality remains the only significant factor influencing variation in these indices.

From the presented data it would seem that both indices perform well as bioindicators in all seasons, although the diatom-based indices seems to be more stable, in terms of their potential to reflect water quality in rivers, than SASS. This is due to the slight effect season has on SASS scores.

Principle Component Analysis

A principle component analysis was performed on the data and the results are presented in Figure 2 and Table 5. The aim of the analysis was to help contextualize the performance of the two types of indices (macroinvertebrate and diatom-based) in terms of the catchment in which the study was performed.

From the PCA it is clear that the main drivers for water quality in the catchment are sodium and chloride (associated with the first ordination axis), while dissolved oxygen and pH were the strongest contributors to the second ordination axis. The two main groups in the figure are associated according to geographical occurrence in the catchments. Group 1 in Figure 2 includes sites associated with the headwaters of the Groot Marico while group 2 represents sites in the different towns as well as sites lower down in the river system. This observation corresponds with the general hypothesis that rivers show a downstream increase in salinity as discussed for example by Pillsbury (1981) for North American rivers.

It is also clear from Figure 2 that the various indices also responded negatively to PO_4, NH_4 and NO_2+NO_3 (these elements may indicate organic loading). The IHAS score also responded negatively to these variables and this phenomenon might be explained by increased sedimentation as well as algae growth on rocks that occur in eutrophic river systems. This can have a negative effect on the ‘stones in current’ and ‘stones out of current’ biotopes, reducing the surface area of rocks on which macroinvertebrates can colonize, thus reducing the IHAS scores.

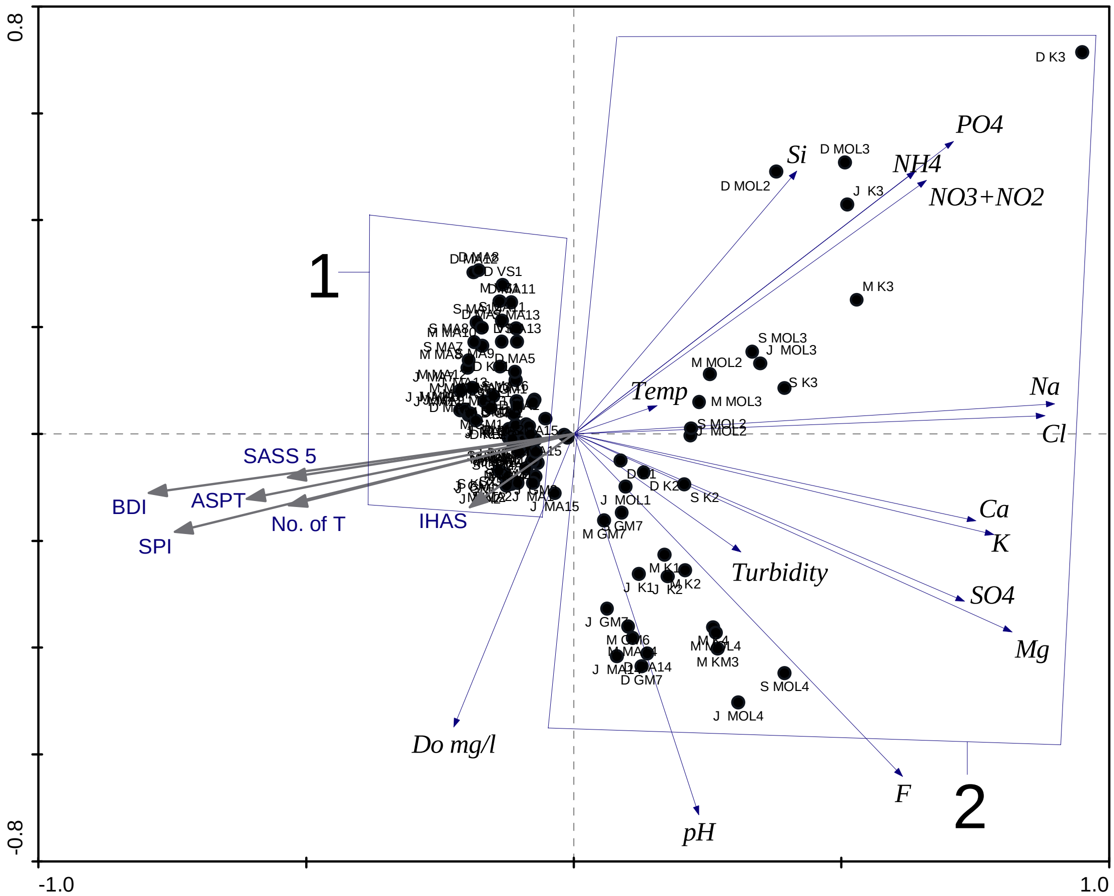

Figure 2

Principle Component Analysis (PCA) indicating chemical variables and diatom- and macroinvertebrate-based indices as vectors and sites as dots. 1 denotes upstream sites and 2 denotes downstream sites (Site names are denoted by a letter denoting the month of sampling, followed by the site name as indicated in Figure 1 in Part 1)

TABLE 5

Results for PCA performed for sites in the Marico- and Molopo Rivers

(Biological and habitat indices were used as supplementary data in the ordination)

Axes	1	2	3	4	Total variance
Species-environment correlations	0.803	0.414	0.368	0.472	
Cumulative percentage variance:					
of species data	40.6	57.8	70.5	78.8	
of species-environment relation	75.4	83.9	88.9	94.2	
Sum of all canonical eigenvalues					0.347

Although the different indices seem to react negatively to temperature (Figure 2), this effect might be due to the fact that the sites showing high levels of PO_4, NH_4 and NO_2+$NO_{3,}$ represents sites with shallower water levels as the rivers at these sites run through the towns of Mafikeng and Zeerust (see Figure 1 in Part 1). The temperature effect is therefore not to be mistaken for a seasonal effect on the indices. Such an explanation of the data concurs with the results from the regression analysis (Table 4) indicating a low level of influence of season on the various indices.

Interestingly, all of the biological indices respond in a similar fashion to the chemical variables in the water suggesting that both types of indices respond to the main water quality drivers in a given system, corresponding with the results from the correlation analysis in Table 2. However, due to the longer vectors of the diatom-based indices, we can also conclude from the figure they are more strongly influenced by water quality than the macroinvertebrate-based indices. This is in agreement with the results from the multiple regression analysis in the previous section.

This finding may however not necessarily indicate that diatoms are more sensitive to changes in water quality than macroinvertebrates, but may also reflect on the way in which the index is calculated. The diatom based indices as used in the current study are a reflection of the relative abundance of species found at a particular site, while the macroinvertebrate scores mainly utilise presence and absence to calculate the SASS 5 scores. Since the presence or absence of a single individual in a sample may alter the SASS 5 score, this promotes more variability in the scores than would be true for the diatom based indices. The advantage of a presence/absence type index such as SASS 5 is that analysis is more rapidly obtained than is the case for diatom based indices which utilise relative abundance.

Conclusions

Both invertebrate- and diatom-based indices showed significant correlations to water quality variables. The different indices reacted to similar water quality variables, and no conclusions could be made as to which water quality variables most strongly influence diatoms or invertebrates.

The diatom-based indices showed a stronger response to general water quality than did the invertebrate indices and did not respond to changes in season. The most probable reason for the stronger association of diatoms with water quality constituents is due to the fact that habitat does not influence the diatom index scores as much as is the case for SASS.

The invertebrate indices showed a stronger relationship to changes in habitat scores than did the diatom-based indices. Season also influenced macroinvertebrate indices more than the diatom-based indices, although the total effect of seasonality on the various indices was found to be low.

The ASPT was less influenced by habitat and more by water quality than the other two SASS indices. The study therefore confirms the work of Dallas (1997) who states that the ASPT score is relatively constant between biotopes, suggesting that sites that have different biotopes and habitats available for habitation by aquatic fauna can be compared on the basis of ASPT

This study shows that diatoms can be used to indicate short to medium term changes in general water quality that might not be detected when only using invertebrate indices. On the other hand, diatoms are not able to indicate habitat degradation and since this is an important component of the functioning of healthy rivers, invertebrates cannot be excluded from the biomonitoring of rivers and streams.

Figure 3 represents a conceptual model reflecting the positioning of diatoms and SASS 5 as indicators in water resource management.

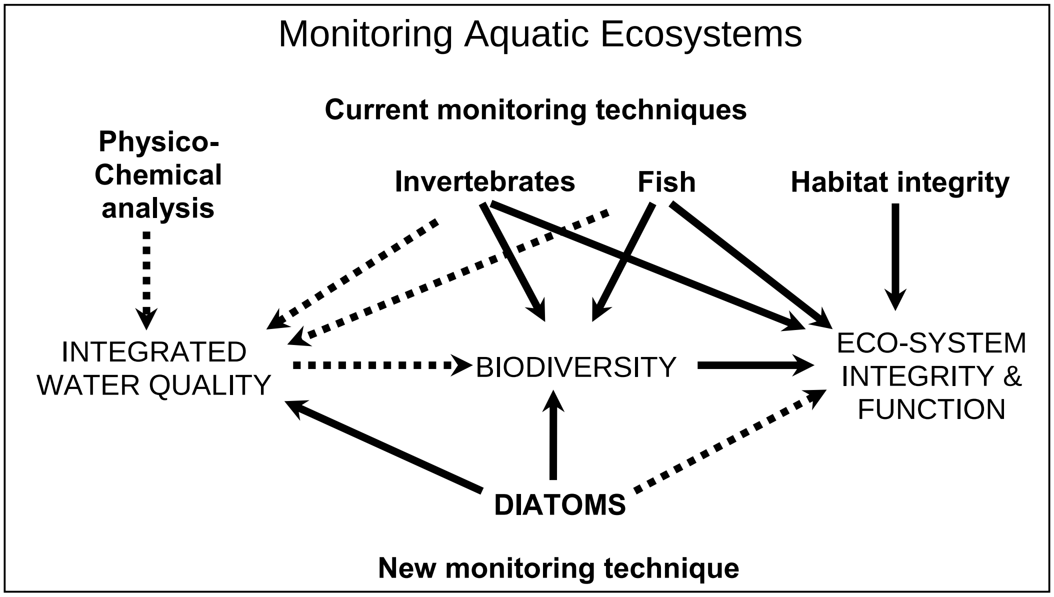

***Figure 3**: Schematic representation of the relationship between parameters used to monitor the environment and what they indicate. Solid arrows represent a strong relationship while dotted arrows represent a weak relationship (Taylor et al., 2006).*

The concept communicates the relationship between biological indicators and what they may tell us about the environment. Macroinvertebrates, because of habitat affinity, food requirements, reproductive cycles etc. have a stronger relationship to the functioning and the ecological integrity of their direct environment and thus may be used as indicators of these parameters. On the other hand, the diatoms (as micro-organisms and primary producers) are directly influenced by chemical water quality. This is because diatoms need nutrients for growth and reproduction and are physiologically influenced by changes in salinity, pH and other key water quality variables (Taylor et al., 2006).

It is therefore recommended, based on the results of this study that diatoms and SASS 5 can, and should, be used as complementary techniques in the biomonitoring of rivers and streams, in the North West Province and the entire country.

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Chapter 7
Summary and Conclusions

7. SUMMARY AND CONCLUSIONS

This thesis endeavours to evaluate whether diatom based indices, as used in many countries over the world, represent significant added value over and above the techniques used in riverine biomonitoring in South Africa. Due to the abovementioned goal, it was necessary to construct a series of field and laboratory tests that would be able to answer most of the possible questions for the introduction of the proposed technology for use in South Africa. Some of the questions were raised by the international diatomalogical science community while others are anticipated questions that could be raised by aquatic specialists within the country.

The potential questions that were addressed include the following:

- Can certain numerical diatom indices developed in other countries be successfully transferred to South African conditions?
- Do diatom species from South Africa have the same morphology and environmental tolerances that species as the species from which the indices were constructed?
- What are the best methods for obtaining diatom data for index calculation?
- What diatom based indices should be used?
- How does the index compare with indices already available in South Africa for the assessment of water quality?
- Is there any additional value in the use of diatom based indices over and above that of the existing indices?

This section aims to consolidate the main findings of the five papers presented in this document as well as to demonstrate the coherent nature of the planning, execution and results of this body of research. The manuscripts presented in this thesis are assessed on an individual basis in the next section, and it will explain the role of each paper in achieving the original aims set out for this thesis.

7.1 Manuscript I: 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

The first manuscript in this thesis (De la Rey et al., 2004) assessed the possible application value of diatom related bioindices. In this article, the strength of the association of water quality and habitat variables with diatom based indices was evaluated. From the outset it was realised that it would be necessary to test the effectiveness of the diatom index in representing changes in the mentioned variables to that of SASS 5 as the standard in biomonitoring in South Africa.

Due to the aim of the study (to obtain the ***possible*** application value of the indices) only a small scale study was planned within a single river (the Mooi River, North-West Province). Furthermore, it was reasoned that if the diatom based indices explained the same amount of the variation in water quality and habitat as SASS 5, that the system would not be tested any further due to the fact that the SASS 5 protocol was already well established and that little value would result from just adding an additional system.

The results from this study were very promising and indicated that, not only can the diatom systems be transferred fairly successfully to South Africa, but that it was more sensitive to changes in water quality than the SASS 5 system. Since the study was only performed on a very limited basis, it was subsequently repeated and expanded on a larger scale to ensure the credibility of the method. This was done, amongst others, in Manuscript V.

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

Due to the fact that variations exist on the methods for the use of diatoms as bioindicators, it was necessary to establish a dependable method for the sampling, preparing and enumeration of diatom samples. Since there is little recent diatom data available from South Africa it is important that newly generated data is comparable in order to test and refine diatom based monitoring in the South African context. The establishment of a standard protocol will ensure this comparability.

One of the chief contributions that the article makes is that, although it gives the preferred preparation method, it also details methods for the preparation of diatom samples with fairly low cost infrastructure. This is an important benefit because it allows for diatom samples to be taken, prepared and mounted by people that do not have access to very high levels of technology or laboratories. The prepared samples can then either be sent to institutions with the expertise to properly identify such a sample or identification can be done on light microscopes able of 1000X magnification which is also relatively affordable.

This article is an important contribution to the introduction of diatom based indices in much the same fashion as the Dickens and Graham (2002) article was for the adoption of the SASS 5 protocol. The article not only consolidates the relevant international literature (that might not be accessible to local scientists that is interested in the field) but also provides valuable guidance towards methods that have proven consistent by trail and error.

7.3 Manuscript 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

Manuscript three tackles various issues for the introduction of diatoms as bioindicators in South Africa. The first issue is whether diatom based indices developed in other countries can be used in South Africa. It was found that the indices performed as well in the researched catchments as in the country of development and it would be fair to transfer such indices to the South African context.

Concerns have been raised by members of the diatom scientist community to the transfer and comparison of biomonitoring data between the Northern and Southern Hemispheres. Although some species have the same morphology, 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). A converse concept was proposed by Kelly et al. (1998), namely that diatoms are “sub-cosmopolitan” meaning that they occur anywhere in the world where a certain set of environmental conditions exist. At present no studies exist that compare South African diatom species with that of European species genetically. The present study did however ascertain that species occurring in the studied catchments that are morphologically similar to species encountered in Europe, display similar preferences for water quality variables. From the mentioned data it would seem that the sub-cosmopolitan view of diatom distribution is probably the correct one and therefore systems developed overseas can be transferred to South Africa.

The study also highlighted that some caution needs to be taken in applying diatom based indices developed in other countries to South Africa. This refers to the problem encountered with species that might be endemic to South Africa due to unique water quality circumstances. If such species is dominant at a site, diatom based indices calculated from such a community might over - or underestimate the water quality of such a site. Although this seems to be an isolated problem, it highlights the need to adapt some of the indices to contain species that occur frequently in South African waters, but not elsewhere in the world.

Finally the paper indicates that the two indices of preference are the SPI (Specific Pollution Sensitivity Index, Coste *in* Cemagref, 1982) and the BDI (Biological Diatom Index, Lenoir & Coste, 1996). These were chosen due to consistent high correlation with water quality variables.

7.4 Manuscript IV: On the use of diatom-based biological monitoring. Part 1: A comparison of the response of diversity and aut-ecological diatom indices to water quality variables in the Marico-Molopo River catchment

Manuscript four tackles the issue of whether aut-ecological diatom indices or species diversity indices are better capable of indicating differences in water chemistry. This was deemed necessary due to the fact that, although aut-ecological indices are very popular in Europe, diversity indices are still being used especially in some studies conducted in the United States of America.

Similar to the case of the protocol article (manuscript two), the idea behind an article like this is to establish a preferred method for use in diatom based biomonitoring to provide guidance to future workers in the field, as well as to ensure that gathered data is useful and comparable.

The study showed that both species diversity and aut-ecological indices responds readily to changes in water quality parameters. However, it was also found that diversity measures, based on the abundance of diatoms, appear to show a relationship to water quality variables that is not linear. The results from the linear and second degree polynomial regressions show that diatom species diversity (especially as reflected by the Shannon species diversity index used in this study) tends to be higher in moderately impacted water.

Due to the highly significant relationship of aut-ecological diatom indices with water quality, these indices are deemed more relevant and reliable for use in rivers and streams to inform decision making in integrated water resource management.

7.5 Manuscript V: On the use of diatom-based biological monitoring. Part 2: A comparison of the response of SASS 5 and diatom indices to water quality and habitat variation

The manuscript entails a broad comparison of the responses of the different diatom related and macroinvertebrate related (SASS 5) indices. This study is also the broader data set evaluation to confirm the results from manuscript one.

One of the questions that could be raised on the fact that diatoms are more sensitive to water quality than macroinvertebrates, is the question of whether this effect might be due to the habitat it is sampled from. Or differently stated, will SASS 5, if sampled from the rock biotope only, be able to perform just as well as the diatom related indices in the prediction of water quality changes? The study found that the stone biotope was slightly better in indicating changes in water quality than the other biotopes used for the SASS 5 protocol, but that the total SASS 5 score was still more robust than any of the individual biotopes.

The main aim of the article however, was to evaluate whether SASS 5 and diatom based indices respond differently to changes in water quality and habitat. The study found that the two indices did not respond to different components of water quality. It seems from the data that the main drivers of water quality in a river system influence both types of indices. The strength of the response did however vary between the two types of indices. Diatom based indices responded stronger to water quality variables than did the invertebrate index, confirming the findings of manuscript one, but this time on a much bigger data set.

Probably the most important observation made from the study was that, although diatoms responded strongly to water quality variables, it did not show a strong response to habitat variation. SASS 5 showed a much stronger response to habitat variability than did the diatoms, leading to the conclusion that the two systems should probably be used as complimentary systems in biomonitoring efforts in South Africa.

7.6 Final conclusions

One of the main issues tackled in the study is whether diatom indices developed in European countries may be used successfully in South Africa. A potential shortcoming of the current study is the fact that the study areas cover only summer rainfall areas and does not represent samples from the South Western parts of the country. Although it may be prudent to wait for similar studies in the South Western Cape to verify the applicability of the diatom based indices, there are several factors that show that transfer of the system untested areas in South Africa would not be unfounded.

In manuscript three we saw that species from South Africa that is morphologically similar to species form the northern hemisphere display the same water quality preferences. This fact supports the sub-cosmopolitan distribution concept proposed by Kelly (1998). This is also strengthened by the high degree of correspondence between changes in water quality constituents and changes in index scores as exhibited in manuscript one, three, four and five presented in this document. One could therefore argue that the transfer of the system from summer to winter rainfall areas should not pose a significant problem. The BDI and SPI indices as used in the manuscripts of the current study has also been used in several European countries with different environmental conditions which included more acid water in Finland (Eloranta 1994) and should therefore be useful as indicators in the South Western Cape.

Another question that remains to be answered is why the systems tested during the current study preformed so well when the studies of Bate et al. (2002) found that the qualitative index of Van Dam et al. (1994) could not be transposed directly for use under South African conditions. This is certainly an important question. A possible explanation may be found in the methodology as utilised in the Bate study. Instead of using attached diatoms in rocks or plant material as prescribed for diatom sampling in Europe, the study used species from sediment (epipelic species). The problem with the above-mentioned is that eppipelic species is far less known than eppilithic species in terms of ecological tolerance. Another possible explanation is that microhabitats may be available in the epipelon that is not available for the epilithon (and visa versa). The study furthermore only utilised the motile diatom species (migrating onto coverslips) and therefore mainly excluded the attached groups of the *Fragilaria* and *Achnathidium* groups. These are large taxa to exclude and therefore may also have skewed the results sufficiently to justify the conclusion made by the Bate study that, although the diatoms show promise as bioindicators and could be a useful addition to the National Biomonitoring Programme for Aquatic Ecosystems (NBPAE), it could not be transposed directly for use under South African conditions.

We can summarise the other main findings of this thesis as follows:

- Diatom based indices are valuable tools for evaluating riverine water quality in South Africa;
- The methods for using diatoms in biomonitoring in South Africa are available and can aid in the comparability of data from different scientists in this field of expertise;
- Aut-ecological diatom indices such as the BDI and SPI can be used with success in South Africa due to the fact that similar species react similarly to environmental variables as that of European species;
- Aut-ecological indices like BDI and SPI are the preferred method, above diversity indices like Shannon species diversity and Pielou species evenness, when using diatoms as bioindicators of water quality in South Africa;
- Although diatom indices are good indicators of changes in water quality, it cannot give an indication of habitat related degradation of rivers, while SASS seems to be more sensitive to changes in habitat; and

- Diatom indices can be used as a complimentary riverine bioindication system alongside SASS 5.

It is believed that the study provides a handy summary to aid in the use and interpretation of diatom and macroinvertebrate based index data. From the data discussed in the current study, the following suggestions are made for the use of certain indicators in this study:

1. Diatom-based indices

From the results of the current study, diatom species diversity does not seem to be a suitable indicator of water quality for use in the South African context and preference should be given to the aut-ecological indices such as the SPI and BDI as utilised in this study.

It is suggested that aut-ecological diatom indices be used as bioindicators in situations where water quality evaluations are to be done. Due to the fact that diatoms, like other bioindicators, retain a memory (in terms of indicating present and recent past water chemistry conditions) of environmental conditions, it is preferable to chemical monitoring in longer term studies. Aut-ecological diatom based indices also give reflection of the combined effect of chemical variables on the ecosystem. Aut-ecological diatom based indices, although more affordable than full chemical analysis, are not rapid techniques and involve laboratory preparation and enumeration by means of microscope. Diatom taxonomy is also more complex than that of invertebrates (using the SASS 5 protocol that only requires identification to family level) and therefore requires more training for operators to produce reliable results. Advantages of aut-ecological diatom based indices include that they are more sensitive to changes in water quality than SASS 5 as well as the fact that the index scores are affected to a much lesser extent by habitat and seasonality than SASS 5. The aforementioned indicates that aut-ecological diatom based indices should be the preferred index in cases where the focus is more specifically on changes in water quality.

The lack of response to habitat may indicate that aut-ecological diatom based indices may be used in areas where habitat availability prohibits the use of SASS (e.g. concrete channels), as well as in other aquatic ecosystems such as wetlands.

Another advantage of using diatoms as bioindicators is that, since diatom preparations can be stored for many years, previous results may be reassessed long after the initial survey. This makes diatoms particularly suitable for long term monitoring programmes and for recording community (and therefore water quality) changes over time.

Two aut-ecological indices performed particularly well in this study namely the Specific Pollution Index (SPI) and Biological Diatom Index (BDI). Since the BDI makes use of fewer species than SPI, it will probably be the long term answer for diatom based indices in South Africa. However, since certain species are not included in the BDI, it may in isolated instances give a skewed impression of water quality. It is therefore recommended that until such time as the BDI may be updated to include such species, the SPI is the index that should be utilised for diatom based water quality monitoring in South Africa.

2. SASS 5

SASS 5 has proven itself to be reliable indicator of the water quality of rivers in South Africa. It is particularly useful as a rapid bioassessment system and circumstances in which impact needs to be assessed in short periods of time. Another advantage of the systems is that it makes use of higher taxonomical levels (mostly family) than diatoms and are therefore easier to master. In this study it was found that up to 30% of the index scores was determined by habitat availability. This may be a drawback of the system in areas where habitat availability is variable. However, it may also be and advantage of the system when seeking to answer questions on ecosystem health, in which habitat availability is an important determinant. One of the major drawbacks of the system is that it only functions in lotic systems and may not be used in other aquatic habitats such as dams and wetlands. Since the system only makes use of the presence or absence of a species to calculate the various scores utilised in the system, it is also not suitable to track long term changes in the community composition. Also the preservation of samples takes up a lot of room and is not as permanent as is the case for diatoms. Re-evaluation of data is therefore not feasible.

In view of the all the above mentioned, it is believed that the study gives a good scientific basis for the implementation of diatoms as indicators of water quality in South Africa. Diatoms were found to be reliable indicators of water quality in South African River systems and have been able to answer foreseeable objections against its use. The addition of a method guideline (manuscript two) serves to make generated diatom index scores comparable between regions and operators, therefore increasing its value as tool for biomonitoring in South Africa.

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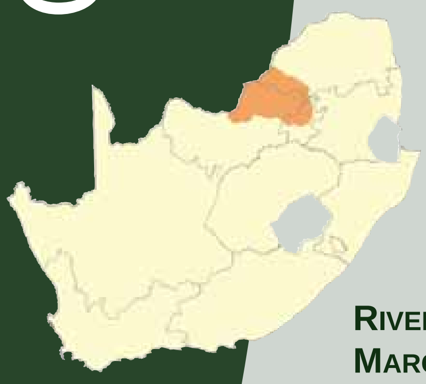
Appendix A
State-of-Rivers Report

Crocodile (West) Marico

Water Management Area

STATE-OF-RIVERS REPORT: MONITORING AND MANAGING THE ECOLOGICAL STATE OF RIVERS IN THE CROCODILE (WEST) MARICO WATER MANAGEMENT AREA



RIVER HEALTH PROGRAMME
MARCH 2005

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University of Venda (Univen)
South African National Botanical Institute (SANBI)
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State-of-Rivers Report Number 9

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March 2005

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Introduction

The Relationship between People and the Natural Environment

We live in an era where most aspects of the structure and functioning of Earth's ecosystems cannot be understood without accounting for the strong influence of humanity. Social and ecological systems are co-evolving at both local and planetary levels. Despite tremendous improvements in technological, economic and material well-being - at least in some parts of the world - people in all parts of the world rely on the capacity of the biosphere to support and sustain social and economic development. Freshwater is the bloodstream of the biosphere's capacity.

Two broad paradigms influence the management of natural resources, namely a development paradigm whose goal is to put water to work for people and another that advocates water for the benefit of all, including ecosystems and the physical environment. Under the first paradigm, social well-being is considered to follow directly from economic development. Ecosystems are essentially harvested to support an economic sector for the production of social value.

A coordinated effort to bring the development paradigm together with environmental concerns was initiated in the 1980's with the introduction of the concept of 'sustainable development'. Sustainable development in respect of water resources seeks to ensure that future generations can meet their own water needs while promoting socio-economic development and improved quality of life for all in the current generation. This can only be achieved through utilising water resources within the ability of these ecosystems to satisfy society's needs now and in the future.

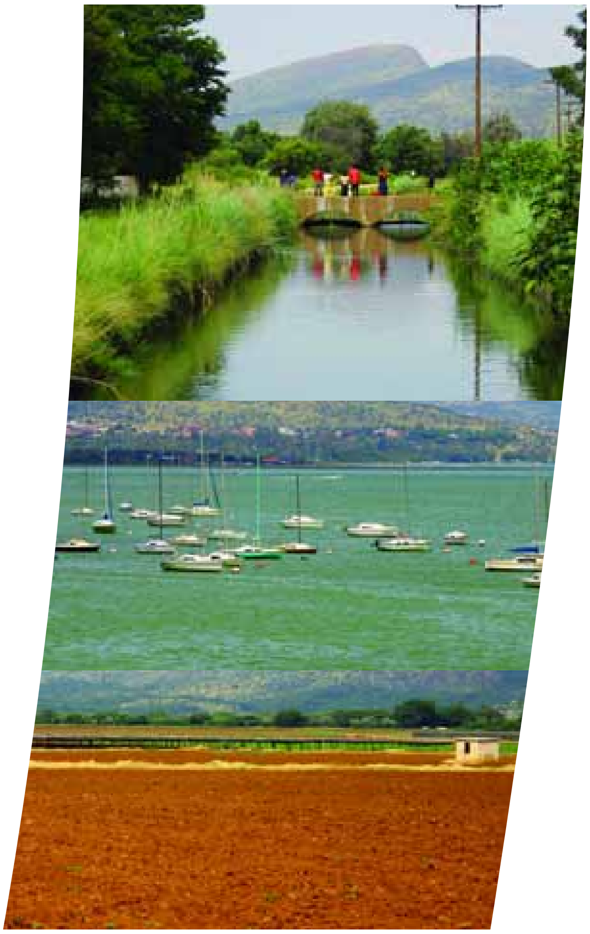
Rivers in both rural and urban settings are complex, multifunctional ecosystems that have developed their own self-sustaining balance. Modification of a particular function over another may cause an imbalance that, in the case where it persists, may eventually lead to degradation of the aquatic environment and ecology. There is a great diversity of ways that rivers have been modified depending on the needs of their adjacent communities. In the extreme, some urban streams have gradually been turned into canals for transporting waste. Some have been covered, turned into sewers and 'forgotten'. With the recent increase in environmental awareness, even urban streams are being revisited and their aesthetic and environmental values appreciated. Rehabilitation of rivers, whereby the state of the river is improved in terms of physical characteristics, chemical quality, ecological diversity and aesthetic appearance, is receiving increasing attention.

It is acknowledged that, due to both social and ecological complexity, a pathway to sustainability cannot be charted in advance but must rather be navigated through processes of learning and adaptation. Therefore, sustainable development should not be seen as a destiny but as a journey based on an ethos that shapes the behaviour of individuals, institutions and nations. There is a risk that the material success of humans can lead to them being mentally disconnected from nature - a belief system of human progress as independent of nature. However, a critical element of this journey of sustainable development is to be aware of the ways in which ecosystems respond to disturbances, to learn from the feedbacks that the environment provides, and to adapt our actions in ways that would improve the harmony between the healthy functioning of ecosystems and society's developmental aspirations.

The River Health Programme

The River Health Programme (RHP) was initiated in 1994 in response to the need to monitor, assess and report on the ecological state of river ecosystems based on their biological condition in relation to all the human-induced disturbances affecting them. The Department of Water Affairs and Forestry, as the legal custodians of water resources in South Africa, has played the leading role in initiating and designing the RHP.

During the initial few years, the emphasis was on research and development of the basic monitoring protocols. From 1996, the programme became operational when a number of provincial implementation teams started applying the RHP design. Today, the RHP is a co-operative venture with participants from many government and non-government organisations, including provincial government departments, local authorities, universities, conservation agencies and private sector organisations. All of these organisations have a stake in collecting data and making information available on the state of the rivers in their areas of responsibility. Through collaborating and combining their resources, a joint implementation team can achieve more than would be possible for any of the organisations on their own.



3

State-of-Rivers Reporting

Introduction

The overall goal of communicating natural resource information should be to change the behaviour of the recipients of the information. In the case of the RHP, the program must:

(1) Provide information to inform ecologically sound management of rivers in South Africa; and
(2) Inform and educate the people of South Africa regarding the health of their rivers.

Changed behaviours relate to the degree to which water resource managers incorporate river health information in their decision-making processes. Similarly, a positive change in civil society's perception and appreciation of rivers would testify to effective communication. To achieve these goals, RHP practitioners had to rethink the formats used for packaging information as well as the strategies used for disseminating information. Out of this emerged the State-of-Rivers (SoR) reporting concept.

SoR reporting is aligned with the Pressure-State-Response (PSR) framework that was developed by the Organisation for Economic Co-operation and Development (OECD). According to this framework, social and economic activities exert **pressure** on an ecosystem, and as a consequence, the **state** of that ecosystem changes. These states can result in **responses** (policies and management actions) from society that ultimately aim at mitigating undesirable impacts through directly managing pressures and indirectly influencing the state of ecosystems.

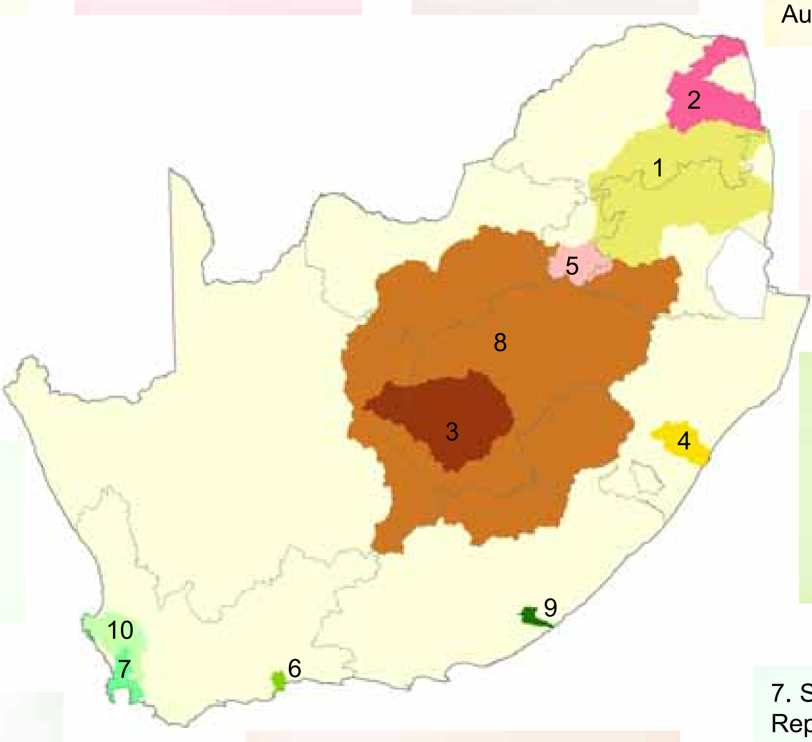
Existing State-of-Rivers Reports and Products

1. State-of-Rivers Report: Crocodile, Sabie-Sand & Olifants River Systems
March 2001

2. State-of-Rivers Report: Letaba & Luvuvhu River Systems
December 2001

3. State-of-Rivers Poster: Modder River
December 2001

4. State-of-Rivers Report: uMngeni River and Neighbouring Rivers and Streams
August 2002



5. State-of-Rivers Poster: Ecological State of the Southern Gauteng Rivers
March 2003

6. State-of-Rivers Report and Summary Poster:
The Hartenbos and Klein Brak River Systems
March 2003

10. State-of-Rivers Report: Berg River System
March 2005

9. State-of-Rivers Report: Buffalo River System
November 2004

8. State-of-Rivers Report: Free State Region River Systems
March - May 2004
State-of-Rivers Posters:
River Health Free State Region
River Health Freistata
River Health Vrystaatstreek
River Health Free State

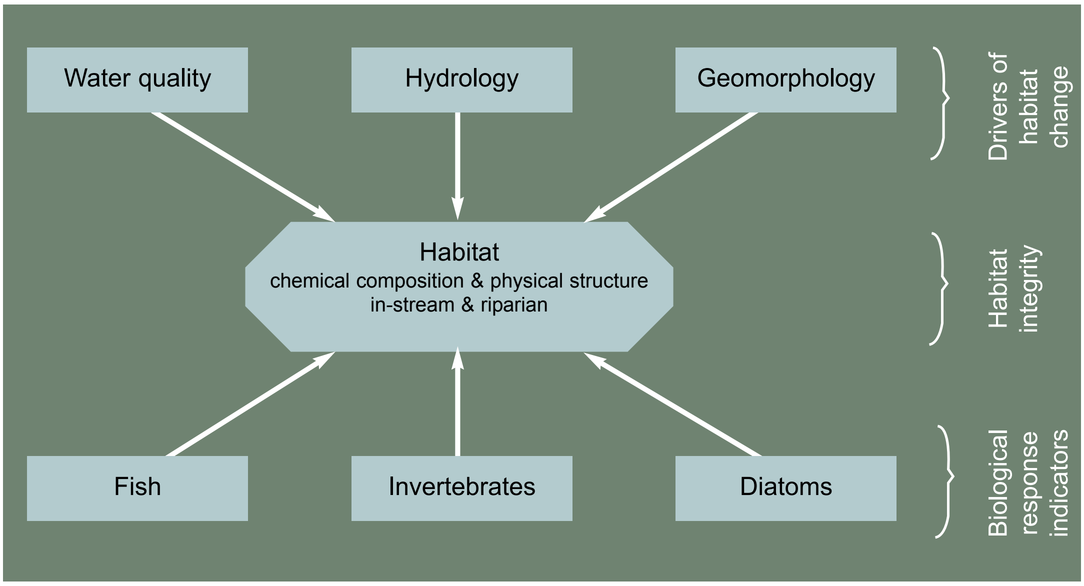
7. State-of-Rivers Report: Diep, Hout Bay, Lourens and Palmiet River Systems
August 2003

The 2004 Crocodile (West) Marico River Survey

The RHP has been operational in the Gauteng and Limpopo provinces for a number of years, with several river surveys having been conducted in these provinces. At the beginning of 2004, the Department of Agriculture, Conservation, Environment & Tourism (DACET) of the North West Province indicated that they would like to champion the launch of the RHP in their province. The RHP teams of Gauteng and Limpopo, together with DWAF: RQS, agreed to take part in a river survey and SoR reporting exercise for the Crocodile (West) Marico WMA, and in the process transfer some of their know-how to the new North-West team. River surveys took place during a total of eight weeks spread out between April and August of 2004. This report is intended to be a summary report of the major findings from these biomonitoring surveys. It is not a technical report detailing the results of the surveys undertaken. For technical information please contact the RHP champions for each of the provinces comprising this Water Management Area and DWAF Resource Quality Services.

ECOLOGICAL STATUS ASSESSMENT

The **ecological status (EcoStatus)** of a river refers to its overall condition or health, i.e. the totality of the features and characteristics of the river and its riparian areas, which manifests in its ability to support a natural array of species. This ability relates directly to the capacity of the system to provide a variety of goods and services.



For this report, data was collected primarily on habitat integrity and the biological response indicators shown in the above figure. To achieve this, available water quality and flow data as well as an assessment of the geomorphological state of rivers were used in a qualitative way by experts in order to determine the habitat template to which aquatic biota would respond. The integrated response of the habitat to modifications and the response of the biota to this, determines the health of the surveyed rivers. The outcome of this overall assessment will be referred to as the EcoStatus and comprises six indicators, namely:

- Instream Habitat Integrity
- Riparian Zone Habitat Integrity
- Riparian Vegetation Integrity
- Fish Assemblage Integrity
- Macro-invertebrate Integrity
- Water Quality (as indicated by diatoms)

Ecostatus Indices

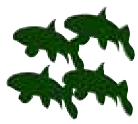
The RHP makes use of a suite of ecological indicators that have specifically been selected for their ability to integrate the impact of multiple disturbances on the state of rivers.

- ***Instream Habitat Integrity*** - This encompasses considerations of the severity of impacts on instream features such as the modification of the volume of water, a change in the flow regime (i.e. natural flow patterns), bed and channel modification, water quality, alien water plants, alien fauna that influences habitat directly and waste disposal. All of these impacts are considered in terms of their impact on the natural instream habitat features that would be expected for a particular type of river.

- ***Riparian Zone Habitat Integrity and Riparian Vegetation Integrity*** - This considers the severity of impacts on riparian features such as the modification of the volume of water, a change in the flow regime (i.e. natural flow patterns), channel modification, water quality, reduction in vegetation and invasion by alien plants. All of these impacts are considered in terms of their impact on the natural riparian habitat features that would be expected for a particular type of river.

Instream Habitat,
Riparian Zone Habitat and
Riparian Vegetation Integrity

- ***Fish Assemblage Integrity*** - Fish are relatively long-lived and are good indicators of the longer-term changes in the condition of river habitats. These changes may be in response to alteration in river flows, changes in river structure or changes in the chemical composition of the water. Fish biologists assess the characteristics of a fish assemblage that occur in a specific reach - for example the number of species found, their respective sensitivity to various forms of disturbances, preferences to particular environmental conditions, different age classes and the general health and condition of fish (i.e. tumours, lesions etc.) - to arrive at an overall expression of health.

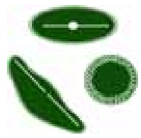


Fish Assemblage Integrity

- ***Macro-invertebrate Integrity*** - Aquatic macro-invertebrates include beetles, mussels, snails, crabs, worms and insect larvae. These organisms have relatively short life cycles therefore are good indicators of changes in water quality and habitat conditions over the short term.

Macro-invertebrate Integrity

- ***Water Quality*** - In this study diatoms were used to support the assessment of water quality. Diatoms are unicellular algae with their cell walls made of silica. A typical diatom community consists of a myriad of species, each with its unique shape. Each species has a specific water quality preference and tolerance. After sample collection in the field, dominant diatom species are identified in a laboratory with the aid of a microscope. Where the water quality preferences of dominant diatom species are known, conclusions can be drawn regarding the water quality at a particular site.



Water Quality

Introduction

The ecological importance and sensitivity (EI&S) of the various river reaches were also determined in this survey. EI&S provides an indication - from an ecological perspective - of whether a river should receive a high level of protection or not. The assessment of a river's EI&S relies on various measures, where:

- Ecological importance refers to the diversity, rarity or uniqueness of the habitats and biota. Consequently, it reflects how important the protection of these ecological attributes are, from a local, national and even international perspective.
- Ecological sensitivity refers to the ability of the ecosystem to tolerate disturbances and to recover from certain impacts.

Through integrating the above measures, the following EI&S categories can be assigned to a river:

EI&S Category	Description
VERY HIGH	A high or very high EI&S indicates that there is strong ecological motivation for awarding a high level of protection to the associated river, and such rivers should ideally be maintained in a natural or good river health category.
HIGH	
MODERATE	A low/marginal or moderate EI&S denotes that a river has relatively lower conservation value and that such a catchment is more suited to development than one where a river has a higher EI&S.
LOW / MARGINAL	

River Health Categories

A river health categorisation is used to provide a simplified user-friendly key to a much more intricate and complex process of assessing the EcoStatus of a river. Each river health category relates to a level of ecosystem health, which in turn relates to the potential of the river to support a particular range of ecosystem services. The river health categories and their relation to the water resource classification system as proposed by the Department of Water Affairs and Forestry are presented in the table below:

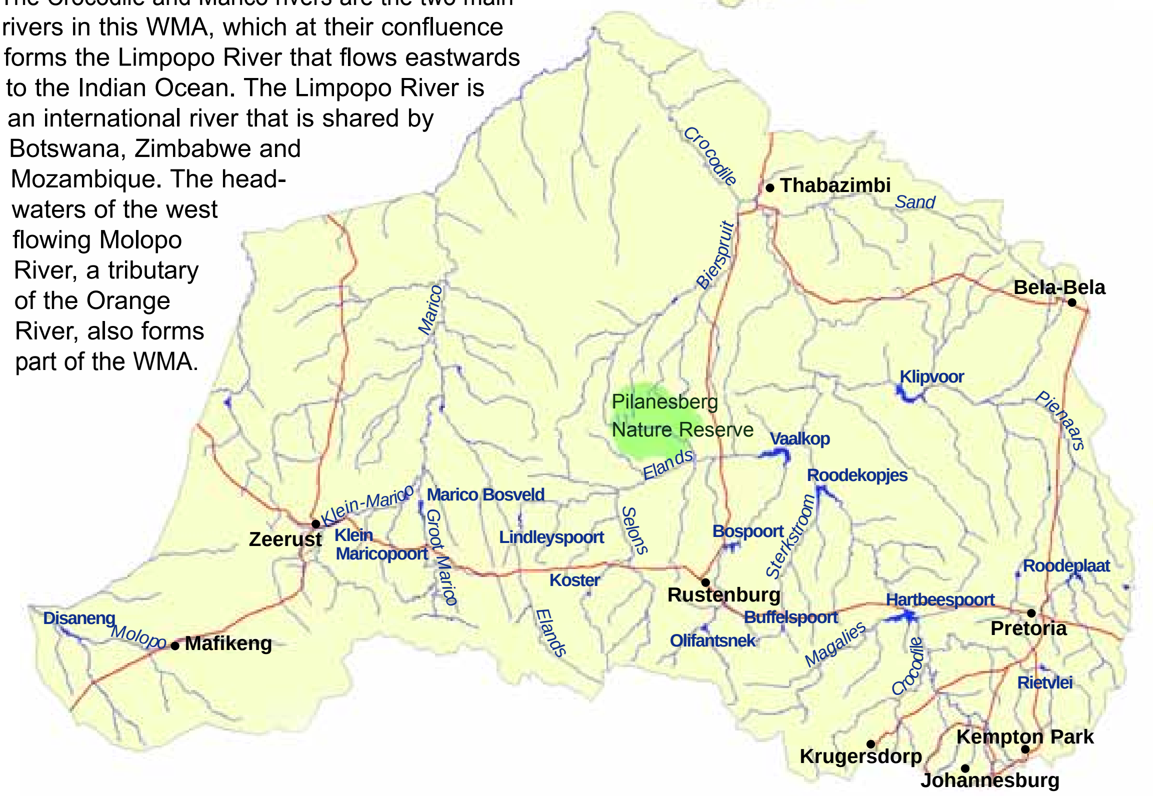
River Health Categorisation		Water Resource Classification System (National Water Resource Strategy, 2004)	
Category	**Description**	**Proposed Class**	**Description**
Natural	No or negligible modification of instream and riparian habitats and biota.	Natural	Human activity has caused no or minimal changes to the historically natural structure and functioning of biological communities, hydrological characteristics, chemical concentrations and the bed, banks and channel of the resource.
Good	Ecosystem essentially in good state; biodiversity largely intact.	Moderately used or impacted	Resource conditions are slightly to moderately altered from the Natural class due to the impact of human activity and water use.
Fair	Sensitive species may be lost, with tolerant or opportunistic species dominating.	Heavily used or impacted	Resource conditions are significantly changed from the Natural class due to human activity and water use, but are nonetheless ecologically sustainable.
Poor	Mainly tolerant species present or alien species invasion; disrupted population dynamics; species are often diseased.	Unacceptably degraded resources	Due to over-exploitation, these rivers are already in a state that is ecologically unsustainable.

Overview of the Crocodile (West) Marico Water Management Area

Overview

The Crocodile (West) Marico Water Management Area (WMA) lies primarily within the North West Province with parts of it in the northern region of Gauteng and the south-western corner of the Limpopo Province. Along the north-western side, the WMA borders on Botswana.

The Crocodile and Marico rivers are the two main rivers in this WMA, which at their confluence forms the Limpopo River that flows eastwards to the Indian Ocean. The Limpopo River is an international river that is shared by Botswana, Zimbabwe and Mozambique. The head-waters of the west flowing Molopo River, a tributary of the Orange River, also forms part of the WMA.



Important features in this WMA include the Bafokeng Tribal Area, the Pilanesberg Nature Reserve, the Cradle of Humankind Heritage site, the dolomitic wetland or "eye" system found at the source of the Marico and Molopo rivers and large dams such as Hartbeespoort, Rooikopjes, Vaalkop, Roodeplaat, Klipvoor and Molatedi.

The natural mean annual runoff (MAR) of the Crocodile (West) Marico WMA is 855 million m^3/annum. Approximately 75 % of the total surface runoff from the WMA flows down the Crocodile River, while the Marico catchment contributes 20 % and the Upper Molopo catchment 5 %.

More than half of the total water use in the WMA comprises urban, industrial and mining use, approximately a third is used by irrigation and the remainder of the water requirements are for rural water supplies and power generation. These water requirements are far more than what can be provided by the current water resources. In order to meet the current demand, much of the water in the WMA is being imported mainly from the Vaal River system for domestic and industrial use purposes. Rand Water, which is the largest water board in South Africa, together with Magalies Water and Botshelo Water (the North West Water Supply Authority), are the three water boards that supply water in this WMA.

Physical Characteristics

Climate and rainfall

Climatic conditions in the Crocodile (West) Marico WMA vary significantly from east to west. The climate across the Water Management Area is temperate, and semi-arid in the east to dry in the west. Rainfall is strongly seasonal, with most rainfall occurring as thunderstorms during the summer period of October to April. Mean annual rainfall ranges from 400 to 800 mm and decreases from the eastern to the western side of the WMA. The mean annual temperature ranges between 18 and 20 °C. Maximum and minimum temperatures are experienced during January and July respectively.

Topography

The Crocodile (West) Marico WMA has a fairly uniform terrain with an altitude ranging from approximately 1700 m.a.s.l. on the Witwatersrand to about 900 m.a.s.l. at the confluence of the Crocodile and Limpopo rivers.

The topography of the southern parts of the WMA varies from plains which have a moderate to low relief to more complex lowlands, hills and mountains to closed hills and mountains with relief varying from moderate to high. The central parts consist predominantly of plains with a low relief and towards the north the WMA is recognised by plains and lowlands with a low to moderate relief.

Main topographic features of the WMA include the Witwatersrand, Magaliesberg, Waterberg and Pilanesberg.

Geology

The diverse geology in the WMA has some of the richest mineral deposits in the world.

North of the Magaliesberg the geology is largely dominated by the Bushveld Igneous Complex. Formations in this complex are extremely rich in minerals and a number of mines have been developed in the area as a result. Platinum, chrome and vanadium mining in particular, are taking place at a large scale.

In the Upper Crocodile sub-catchment, dolomitic rock is found in the Rietvlei Dam catchment, towards Krugersdorp, the Marico and Molopo catchments and north of Randfontein and Krugersdorp. Pretoria abstracts a significant quantity of its water supply from these water rich dolomitic compartments. Dolomitic rock is also found at the confluence of the Tolwane and Pienaars rivers as well as the confluence of the Pienaars and Crocodile rivers in the Apies/Pienaars sub-catchment.

The rest of the catchment consists of sedimentary rock, with the quartsitic Magaliesberg being the prominent feature. These mountains are regarded among the oldest in the world at 2.5 billion years old.

Soils

Soil types of the Crocodile (West) Marico WMA are broadly classified as:

- Moderate to deep sandy loam - southern and far eastern regions
- Moderate to deep clayey loam - the rest of the catchment.

Most of the clayey loam soils in particular are highly suitable for commercial agriculture when sufficient water is provided.

Natural Vegetation Types

According to Low and Rebelo's (1998)* vegetation map of South Africa, the Crocodile (West) Marico WMA is dominated by the Mixed Bushveld vegetation type. The vegetation found here varies from dense short bushveld to a more open tree savanna. This vegetation type is found in areas where the rainfall varies between 350 and 650 mm/a and the altitude comprises low relief plains at an altitude range of 700 to 1000 m.a.s.l.

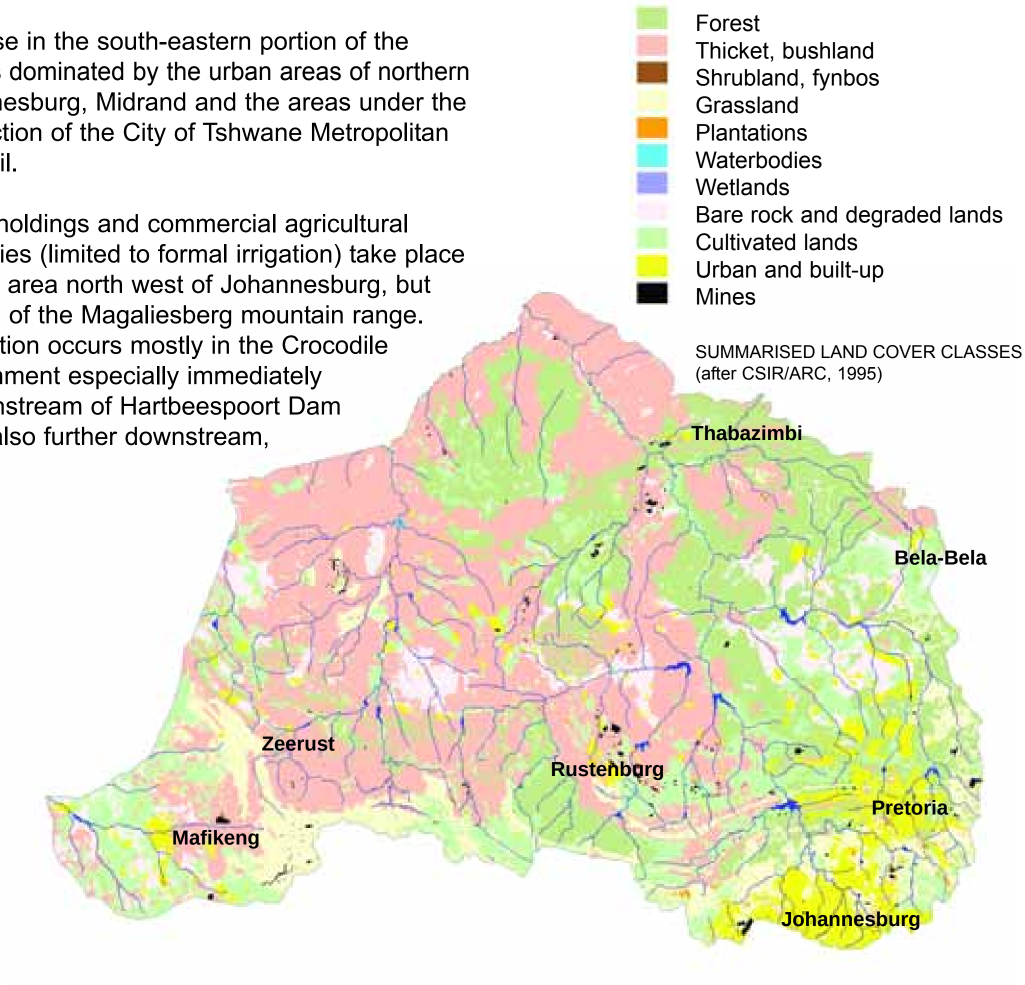
The northern parts of the WMA is dominated by Mixed Bushveld, Sweet Bushveld and Mopane Bushveld vegetation types. The central and western parts are dominated by Mixed Bushveld, while North-eastern Mountain Grassland and Mixed Bushveld vegetation types are found in the eastern parts. Dry Sandy Highveld Grassland and Moist Cool Highveld Grassland vegetation types are largely found in the southernmost region of the WMA.

* Low and Rebelo’s (1998) description of vegetation types is referred to since the vegetation component of the Ecoregion approach is based on this version of the classification of vegetaton types. Ecoregions were key in the delineation of the Ecological Study Units.

LAND-USE

Land-use in the south-eastern portion of the WMA is dominated by the urban areas of northern Johannesburg, Midrand and the areas under the jurisdiction of the City of Tshwane Metropolitan Council.

Smallholdings and commercial agricultural activities (limited to formal irrigation) take place in the area north west of Johannesburg, but south of the Magaliesberg mountain range. Irrigation occurs mostly in the Crocodile catchment especially immediately downstream of Hartbeespoort Dam but also further downstream,



south of Thabazimbi as well as along the mainstem of the Crocodile River. A very wide variety of crops are produced, ranging from intensive vegetable production to tobacco, maize, cotton, citrus and sub-tropical fruits, sorghum, sunflowers and soya bean. A significant amount of irrigation also takes place near Mafikeng, situated in the Molopo catchment with water sourced from the Grootfontein dolomitic compartments. Dry land crops (usually maize) are grown in the south and south-eastern parts of the WMA where the rainfall is higher, while in the drier northern and western regions, land-use consists mostly of stock and game farming. Further away from the main river channels, most of the land-use is small-scale irrigation from farm dams as well as the raising of small and large livestock and game animals.

Extensive mining activities occur north and east of Rustenburg - mainly in a circular belt around the perimeter of the Bushveld Igneous Complex. These mines are mainly focused on the platina group of metals which are in great demand on the world market at the moment, as well as granite mining. Rustenburg is considered one of the fastest growing cities in Africa because of the platinum mining operations. In the Upper Crocodile River sub-catchment, small open-cast stone and sand quarries are common as well as a number of large platinum and chrome mines. Limited mining occurs in the rest of the WMA.

Social and Economic Characteristics

Population

The Crocodile (West) Marico WMA is the second most populous water management area in the country.

In 2001, the population of this WMA has been estimated to be 6.7 million people. Approximately 85 % of the population in the WMA live in the urban metropolitan area of Johannesburg and Tshwane, situated in the Upper Crocodile and Apies / Pienaars sub-catchments where they are attracted by the economic activity and employment opportunities in the region. Extensive informal settlements have as a result, sprung up around the periphery of the major urban centres.

The number and density of population declines with increasing distance from these upper reaches and the rural population is more evenly distributed than the urban population.

Economic Profile

Economic activity in the WMA is dominated by the urban and industrial complexes of northern Johannesburg and Pretoria and platinum mining north-east of Rustenburg.

About 25 % of the Gross Domestic Product (GDP) of South Africa originates from the Crocodile (West) Marico WMA. This constitutes the largest, single contribution to the national wealth from any of the water management areas. The WMA's gross geographic product (GGP), which is the total value of all final goods and services produced within the economy in a geographic area for a given period, was R130.1 billion in 1997. The major sectors contributing to the GGP are manufacturing (22.7 %), government (18.7 %), transport (15.7 %), finance (17.7 %) and the significant mining activities in the Rustenburg, Bafokeng and Madibeng (Brits) areas. Mining is an important and stable sector of the regional economy that provides strong employment opportunities.

Other Water Resources of the WMA

Groundwater

An important feature with regards to the water resourses in the Crocodile (West) Marico WMA, are the large dolomitic aquifers which occur along most of the southern part of the Water Management Area from Pretoria to Mafikeng. Large quantities of water are abstracted from these aquifers, mainly for urban and irrigation use, while a significant portion of the base flow of several rivers originates as springs from these aquifers. Along the lower Crocodile River, sandy aquifers are found from which large quantities of water are abstracted for irrigation purposes. Sandy aquifers also occur in the catchments of the Molopo River. The remainder of the WMA is mostly underlain by fractured rock aquifers, which are well utilised for rural water supplies.

Wetlands

The wetlands of the Crocodile (West) Marico WMA occur in a variety of biomes contributing to an amazingly rich diversity of wetlands in terms of setting, type, biodiversity and extent. Some of these are:

- The extensive Moretele floodplain wetlands and the Dolomitic Eyes of Marico and Molemane in the wetter Mixed Lowveld Bushveld biome;
- The pristine Waterval valley bottom mire in the mountains of Kgaswane Nature Reserve within the Clay Thorn Bushveld biome;
- The Dry Sandy Highveld Grassland hosts extensive karst related wetland systems such as the mire at Gerhard Minnebron or the one at Schoonspruit; and
- The arid Kalahari Plains Thorn Bushveld hosts unique wetlands such as the endorheic Heuningvlei with its seep zones or the Molopo wetland complex on the border with Botswana.

Two unique wetland groups needs further mentioning. One group is the eyes, mires and peatlands associated with the karst landscape which dominates large parts of the North West Province and that underlay a variety of the biomes. The second group are the endorheic pans. These pans are as diverse in character as they are in setting. They vary from small permanently inundated pans to temporary playa-like pans from the wetter east to the more arid western parts of the province.

Sub-Management Areas

The Crocodile (West) Marico water management area is divided into six sub-areas by the Department of Water Affairs and Forestry for water resources planning purposes. The delineation was largely based on practical considerations such as size and location of sub-catchments, homogeneity of natural characteristics, location of dams, and economic development. The six sub-management areas are described below:

Apies / Pienaars sub-management area

The Apies / Pienaars sub-management area comprises the Apies River catchment, the Pienaars River catchment and the catchment of the Moretele and Tlholwe rivers down to its confluence with the Crocodile River. The Apies River joins the Pienaars River to the north of Hammanskraal. The Apies River drains the Pretoria CBD, parts of the central-eastern suburbs and most of the western Pretoria industrial and urban areas. Increased high surface water runoff is channelled into the Apies River from these areas. The Pienaars River joins the Crocodile River just below the confluence of the Crocodile and Elands rivers. Roodeplaat Dam and Klipvoor Dam are the major dams in the sub-catchment while Pretoria in the southern part and Bela-Bela, situated in the northern part of the sub-catchment, are the major towns. The upper and middle reaches of this sub-management area in particular are densely settled.

The Pienaars River drains the area from Pretoria northwards to the Waterberg Mountains near the town of Bela-Bela. All the main rivers are perennial and their flows are supplemented by substantial discharges of treated domestic and industrial effluent. Flows in these rivers are also enhanced by water imported from the Vaal River system to the south of Johannesburg, which is used principally for domestic and industrial water supplies prior to treatment and discharge.

Upper Crocodile sub-management area

This area corresponds to the catchment of the Crocodile River upstream of the confluence of the Elands River which includes the major tributaries of the Sterkstroom, Magalies, Bloubankspruit, Jukskei and Hennops rivers. The Crocodile River has its source in the Witwatersrand mountain range at a height of 1 700 m.a.s.l. The northern suburbs of Johannesburg, as well as parts of adjacent cities such as Kempton Park and Krugersdorp are situated in this sub-catchment. There are two large dams in this sub-catchment, namely Hartbeespoort and Roodekopjes. The upper reaches of the catchment are densely settled.

Elands sub-management area

The Elands sub-management area consists of the Elands River catchment which includes the tributaries of the Koster, Selons and Hex rivers. The Elands River is a tributary of the Crocodile River and the confluence is situated below Roodekopjes Dam. Large portions of this catchment are tribal areas. Rustenburg is the only major city in this sub-catchment and the major dams are Bospoort Dam on the Hex River and Vaalkop Dam on the Elands River. Mining of platinum and its associated platina group of minerals are the dominant land-use in the catchment and is rapidly expanding.

Lower Crocodile sub-management area
This sub-management area represents the remainder of the Crocodile River catchment, downstream of the confluence of the Elands River. The river flows in a north/north-westerly direction until the confluence with the Marico River. After the confluence the river is known as the Limpopo River. The Lower Crocodile River has two large tributaries, namely the Sand River and the Bierspruit which join the Crocodile River west of the town of Thabazimbi. Irrigation is the dominant water demand in this sub-area.

Marico sub-management area
The Marico sub-management area corresponds to the catchment of the Marico River. Main tribuitaries of the Marico River include the Klein and Groot Marico rivers. This sub-area forms the western part of the WMA. Major dams in this sub-catchment are the Marico-Bosveld Dam in the upper catchment and the Molatedi Dam further downstream. The town of Zeerust is found in this sub-management area with smaller settlements scattered throughout. The Groot Marico River is fed by a number of springs within the Groot Marico dolomitic aquifer compartment. These dolomitic eyes include the Molemane Eye and the Marico Eye. The upper reaches of this catchment are not densely populated.

Upper Molopo sub-management area
The Upper Molopo sub-management area comprises the upper part of the Molopo River catchment. The Molopo River rises from the Molopo Eye near Mafikeng and flows westwards to form the northern border of the North West Province with Botswana. The Molopo River is a tributary of the Orange River. It ceases as a surface flow and discharges into pans in Botswana before turning south and emerging as surface flow just before it reaches the Orange River. The source of the Molopo River is the main supplier of water to the town of Mafikeng. Irrigation is also a dominant water demand in this sub-management area.

Management and conservation challenges for the burning Bodibe peatland in the North West Province

The Bodibe is a karst related peatland located between the towns of Mafikeng and Lichtenburg in the North West Province. The peat in this wetland has been on fire since early 2003. A current partnership between the community, local/district government, provincial departments and Working for Wetlands is trying to arrest the spreading of the fire.

The onset of organised agriculture and the formation of large townships have lead to pressures on the water resources of this area. Surface runoff, dammed streams and groundwater were used to irrigate maize fields and to supply water to the human settlements. The increase in population has lead to more demands on water resources. It was especially the groundwater resources that were targeted for exploitation. This resulted in a drop in the regional water table to such an extent that the eyes and springs have dried up and could not sustain flow to the peatland. The peatland started to dry out, desiccation fissures developed all over the surface. Severe drought desiccated the peatland even further and the upper portion started to burn when the veld was burnt to improve grazing for the local community's stock.

Working for Wetlands has put in place a cut-off wall (about 120 m long, 5 m deep and 0.6 m wide). This wall has arrested the spread of the fire and it is hoped that it would trap water entering the system thereby facilitating the restoration of this system.

It is expected that with global climatic change peatlands in semi-arid regions will come under more pressure. These marginal peatlands such as Bodibe would eventually become a victim of struggle for water between man and the environment.

Burning peatland at Bodibe, near Mafikeng due to over utilisation of groundwater by local municipalities and farmers.

Status of the Crocodile (West) Marico Water Management Area

Summary

The overall EcoStatus of the Crocodile (West) Marico WMA is poor, with 13 of the 23 units surveyed classified as poor. Only 10 were classified as fair or better (see table below). This WMA is highly developed: about 25 % of the Gross Domestic Product (GDP) of South Africa originates from the Crocodile (West) Marico. The industrial, mining and agricultural sectors within this WMA play a vital role in contributing to this economic achievement and are highly dependent on water resources within the WMA.

Some parts of the WMA are still in good to natural condition. These are found primarily in the headwaters of catchments with very little development and human impact. Examples of river reaches in near pristine condition include the headwaters of the Groot Marico and Skeerpoort rivers.

EcoStatus Category*	Number of Ecological Study Units
POOR	13
FAIR	7
GOOD	2
NATURAL	1

* Note: In those instances where the EcoStatus was intermediate, e.g. POOR/FAIR, the first stated category is the predominant status and is classified as such in the above table. Using the POOR / FAIR example - this would fall under the POOR category.

There are a number of management responses that have been identified throughout the course of the survey. Some of these need to focus directly on the riparian zone and instream habitat, some need to be addressed at the catchment level and others are directly related to water use and quality.

Riparian Zone
The riparian zone is an important ecological link between the river and the terrestrial component of a catchment. In addition it provides a necessary buffer between the river itself and any potential impacts that might originate from within the catchment. The protection of the riparian zone should be a management priority, where management responses should include:

- The minimisation of future development within the riparian zone, and control and management of existing activities that occur within the riparian zone, such as grazing, sand winning and slasto mining. All these activities change the structure and functioning of the riparian zone - sometimes irreversibly (the responsibility of landowners; farmers; developers; rural communities; Department of Water Affairs and Forestry (DWAF); National Department of Agriculture (NDA); Department of Mineral and Energy Affairs (DME); Department of Environmental Affairs and Tourism (DEAT); Gauteng Department of Agriculture, Conservation and Environment; North-West Department of Agriculture, Conservation, Environment & Tourism; as well as District and Local Municipalities).
- The clearing of alien vegetation within the riparian zone (the responsibility of Working for Water, DWAF and provincial departments responsible for environmental quality).

In order to successfully restore and rehabilitate the riparian zone, rivers need to be prioritised in terms of their desired conservation status. This will provide guidance as to which rivers require urgent attention within the WMA (the future CMA will play a role in this regard).

Instream Habitat
The integrity of the instream habitat is vital for maintaining biota and a healthy river system. Aquatic flora and fauna are often highly specific in terms of their habitat preferences, for example the depth of the water, type of bottom substrate and velocity of flow. Instream flow patterns are often affected by impoundments which alter the variability and quantity of flows. Within the Crocodile (West)

Marico WMA there are many impoundments - it is recommended that DWAF investigate these impoundments for opportunities to manage releases that simulate natural flow patterns. This will ensure that aquatic flora and fauna that are dependent on seasonal flows, for example to trigger reproductive responses, will return or flourish within specific river reaches downstream of impoundments. Environmental flow requirements are deter-mined as part of an ecological reserve determination. The implementation of instream flow objectives is subject to classifying a river in terms of the level of protection that it should receive. Both of these actions are the responsibility of DWAF.

More directed management responses related to instream habitat integrity include:

- The control of instream alien flora and fauna - alien species not only alter instream habitat, for example through their feeding behaviour, but may contaminate the natural gene pool through cross-breeding (the responsibility of provincial environmental affairs), and
- The installation of fish ladders and eelways in suitable flow regulating structures - this will allow natural migration patterns and will improve the functional connectivity down the length of a river system (the responsibility of provincial environmental affairs).

Catchment and Land-use

It is important to realise that what happens within the broader catchment, can have a direct impact on the ecological integrity of the river within it. Within the Crocodile (West) Marico WMA, two issues linked to broader catchment management were identified:

- Wetlands form an integral part of the water resources within a catchment and are often degraded by activities that occur in the surrounding catchment. There are a number of seeps, springs and palustrine wetlands within the WMA. These are largely unknown and require urgent characterisation, delineation and classification in terms of their desired protection status (the responsibility of DWAF, DEAT and provincial environmental affairs).
- Urban development within the catchment is often accompanied by impervious surfaces (roads, paving, roofs etc). Flows that would normally percolate into the ground now travel across the land surface. Surface runoff flows more rapidly and at greater volumes than groundwater flows. There is a need to manage surface stormwater runoff at the source in order to sustain groundwater flows and attenuate stormwater damage (the responsibility of Local Municipalities, DWAF and landowners).

Water use

The water budget is the balance between supply and demand within a system (e.g. a WMA). It is important that the demand for water resources in the Crocodile (West) Marico WMA is within the systems sustainable capability. A number of economic activities, most notably agriculture, industry and mining, within the WMA use vast quantities of water. (In the Gauteng portion of the WMA, domestic use is a major contributor to overall water use, although most of this water is imported from the Upper Vaal WMA.) In a water scarce country it is essential that water use is efficient and not wasteful. There is a specific need to monitor and control mining activities through the issuing of water use and discharge licences, with the recognition that in some cases a license application can and should be declined (the responsibility of DWAF & DME).

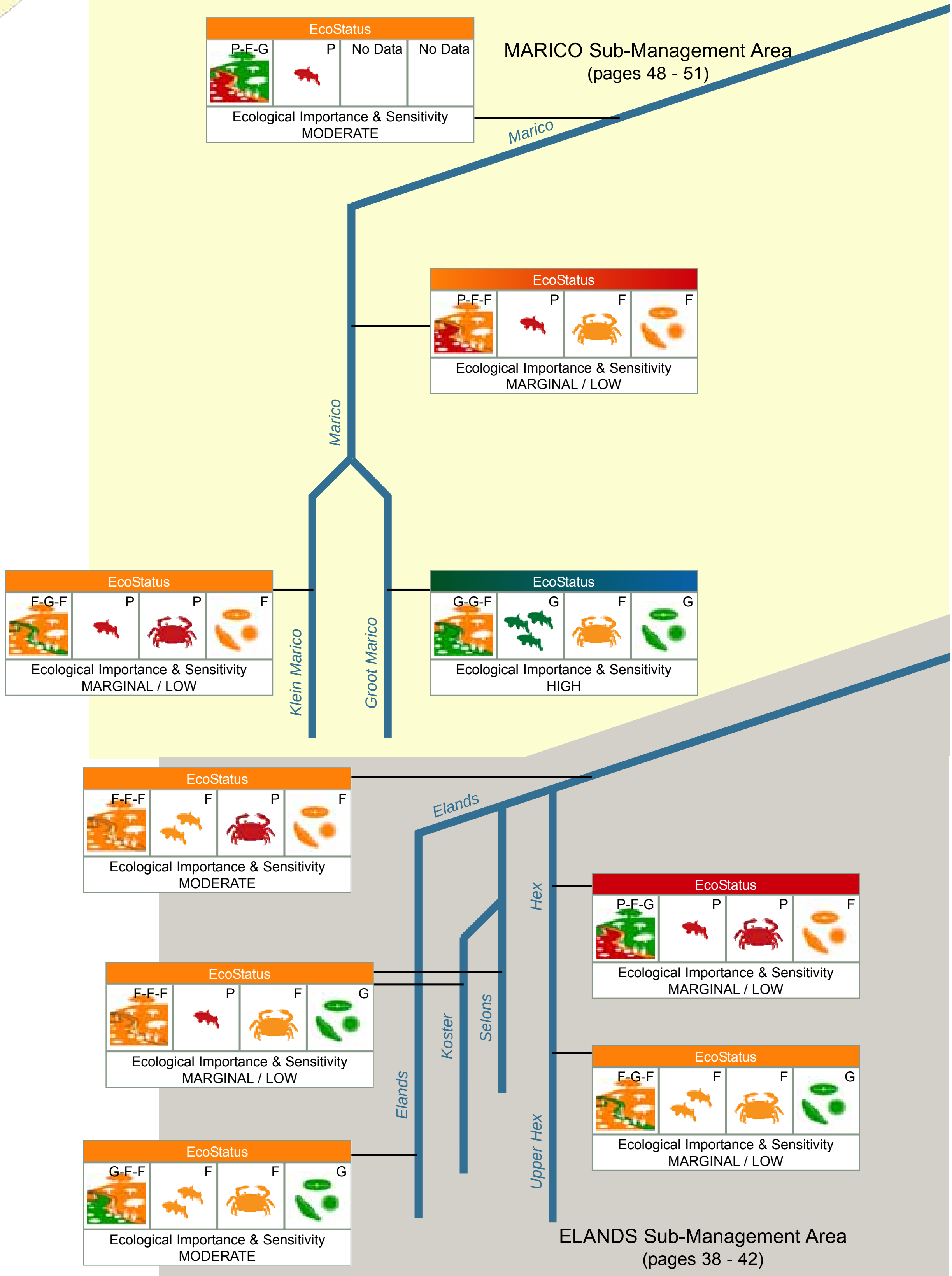
Water quality

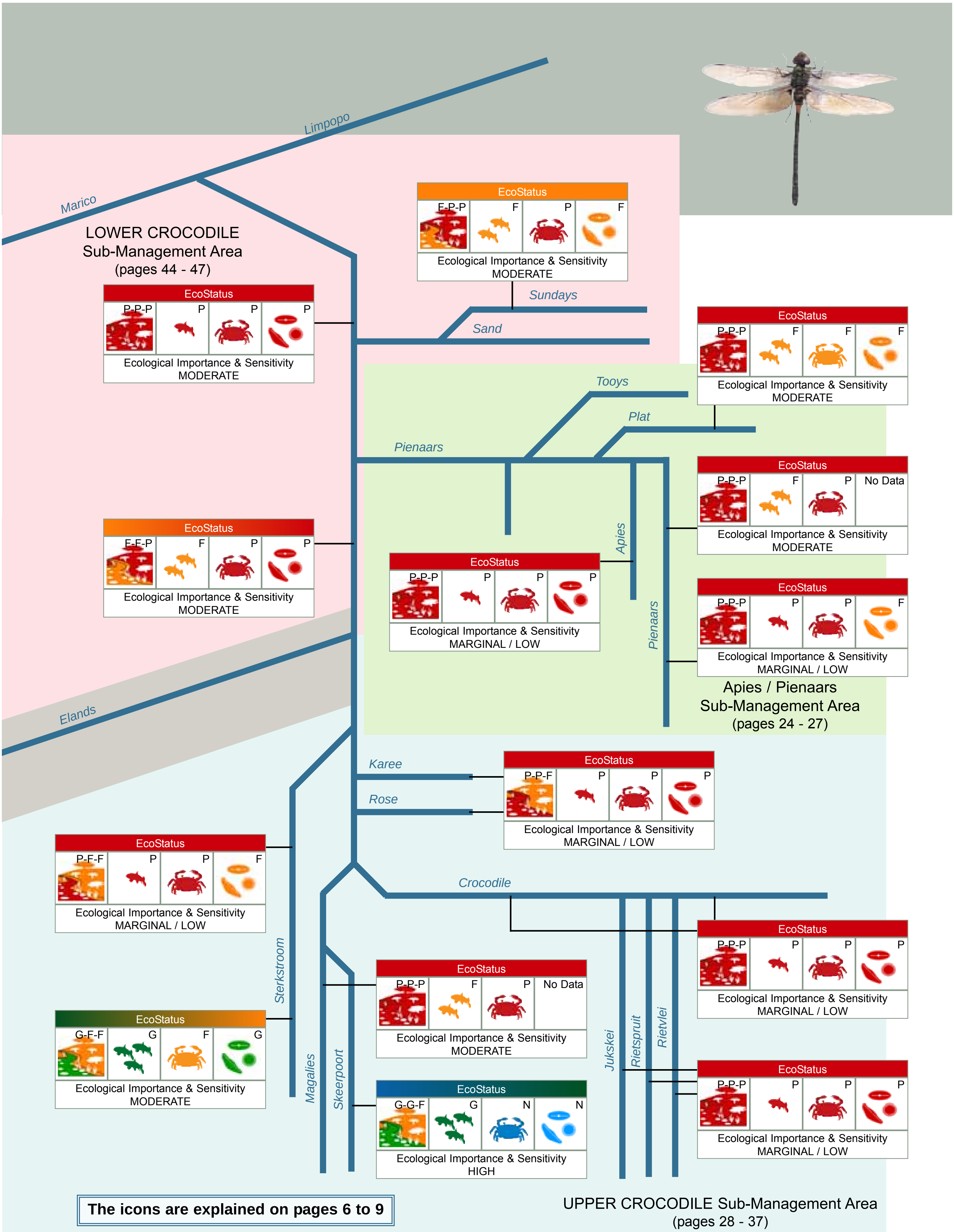
In highly developed WMAs, such as the Crocodile (West) Marico, water quality issues are always on the list requiring management responses. The first step in managing water quality problems would be to set the water quality objectives for the rivers within the WMA. Once this has been undertaken it is important to monitor water quality to ensure that the objectives are being adhered to. Within the WMA there are a number of sources of pollution that are contributing to the reduced levels of water quality. These include:

- Agricultural return flows (the responsibility of DWAF, NDA, organised agriculture and farmers to improve their practices),
- Industrial discharges (the responsibility of DWAF and industry to adhere to licence conditions and take responsibility for the health of the water resources they use), and
- Sewage spills and discharges (the responsibility of DWAF and municipalities to upgrade the sewerage systems and improve their management).

The Crocodile (West) Marico Water Management Area

Summary




Limpopo
Marico
LOWER CROCODILE
Sub-Management Area
(pages 44 - 47)
EcoStatus
F-P-P F P F
Ecological Importance & Sensitivity
MODERATE
Sundays
Sand
EcoStatus
P-P-P P P P
Ecological Importance & Sensitivity
MODERATE
EcoStatus
P-P-P F F F
Ecological Importance & Sensitivity
MODERATE
Tooys
Plat
Pienaars
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P-P-P F P No Data
Ecological Importance & Sensitivity
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Apies
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Ecological Importance & Sensitivity
MARGINAL / LOW
Pienaars
EcoStatus
P-P-P P P F
Ecological Importance & Sensitivity
MARGINAL / LOW
Apies / Pienaars
Sub-Management Area
(pages 24 - 27)
Elands
Karee
Rose
EcoStatus
P-P-F P P P
Ecological Importance & Sensitivity
MARGINAL / LOW
EcoStatus
P-F-F P P F
Ecological Importance & Sensitivity
MARGINAL / LOW
Crocodile
EcoStatus
P-P-P P P P
Ecological Importance & Sensitivity
MARGINAL / LOW
Sterkstroom
EcoStatus
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Ecological Importance & Sensitivity
MODERATE
EcoStatus
G-F-F G F G
Ecological Importance & Sensitivity
MODERATE
Magalies
Skeerpoort
Jukskei
Rietspruit
Rietvlei
EcoStatus
P-P-P P P P
Ecological Importance & Sensitivity
MARGINAL / LOW
EcoStatus
G-G-F G N N
Ecological Importance & Sensitivity
HIGH
The icons are explained on pages 6 to 9
UPPER CROCODILE Sub-Management Area
(pages 28 - 37)

Longterm Monitoring and Management Programme for River Health

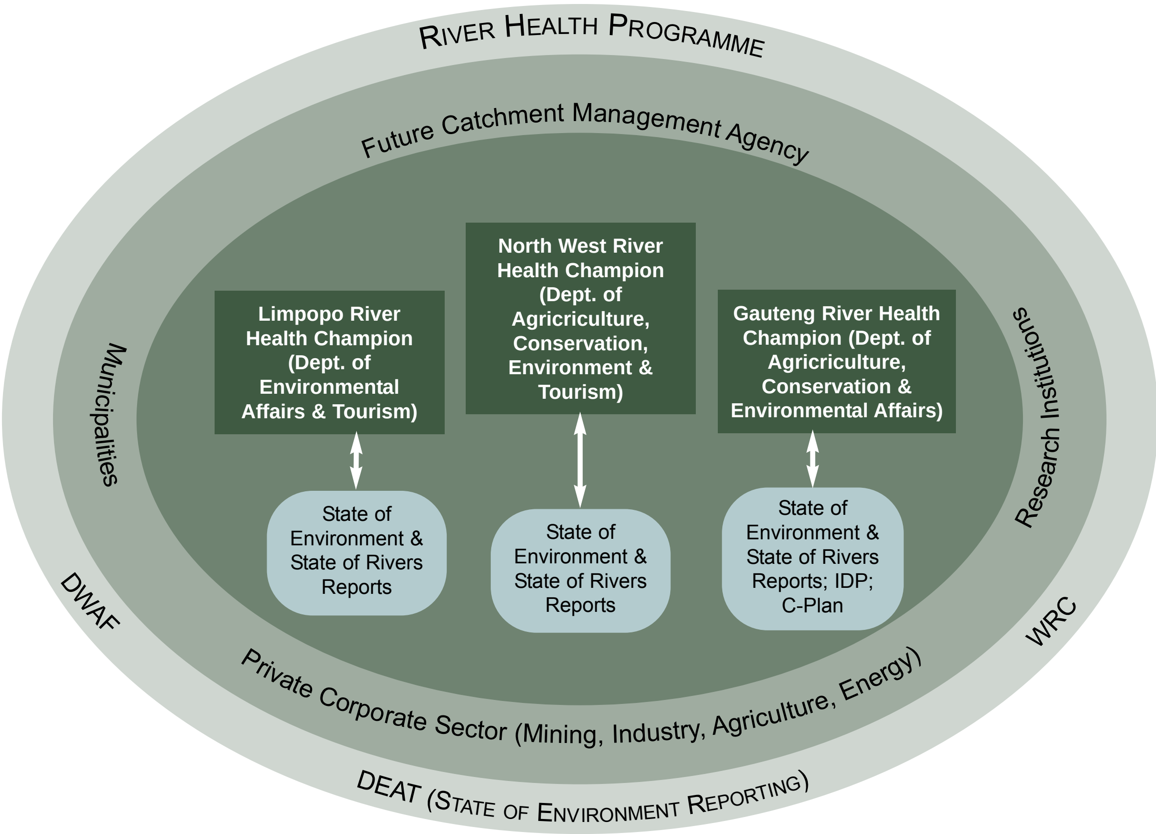
Institutional Arrangements

The Crocodile (West) Marico Water Management Area falls mainly within the North West Province, but it also incorporates sections of the Limpopo and Gauteng provinces. A cross-provincial team of river ecologists is therefore required to ensure the successful implementation of the River Health Programme (RHP) in this Water Management Area (WMA).

The institutional arrangements that currently exist for the RHP in this WMA are depicted in the figure below. Three River Health Provincial Champions are responsible for ensuring implementation of the programme in their respective provinces and to coordinate monitoring programmes with provincial neighbours. These champions are also responsible for reporting back to the National Coordinating Team. In each province, relevant stakeholders are assisting the champions with the biomonitoring programmes, for example research institutions, mining companies and municipalities.

A Catchment Management Agency (CMA) will be established in terms of Chapter 7 of the National Water Act (Act No. 36 of 1998) for the Crocodile (West) Marico WMA within the next two years (by end of 2006). It is anticipated that the CMA will have an important role to play in the River Health Monitoring Programme for this WMA, especially as a coordinating body on a catchment level between provinces and the relevant national departments.

In the three provinces, application of the RHP occurs mainly in the context of the State of Environment reporting obligation of the provinces and also to inform the aquatic biomonitoring programme of the province in Gauteng. Although links have been established between the provincial departments responsible for environmental monitoring and reporting and the regional offices of the Department of Water Affairs and Forestry, true partnerships between these provincial and national agencies are still lacking.



Monitoring Programmes

Monitoring protocols have been implemented by each province in the 2004 survey with recommended timeframes for repeat surveys. Repeat surveys will revisit existing sites as well as aim to expand the number of monitoring sites in areas of low coverage, for example the Upper Molopo sub-management area.

In Limpopo Province, the RHP started in earnest in 1997. However, the Crocodile (West) Marico WMA was only surveyed in 2004, the survey on which this report is based. Due to capacity constraints, the future surveys of this WMA in the Limpopo Province, will only be done in a 3-4 year return period.

Gauteng Province started to implement the RHP in 1999. The upper Crocodile (West) Marico WMA was surveyed for the first time in 2001 and is monitored on a 4-year cycle. The first cycle ended in 2005 and the next sampling period for this WMA in Gauteng, will start in 2007 and the results will be published in a SoR report in 2009.

In North West Province the RHP will be implemented in earnest from 2005. Previously, in 1999, a few sites in the WMA were surveyed, but due to capacity constraints, monitoring ceased. In 2004 a renewed effort by the Department of Agriculture, Conservation, Environment & Tourism to revive the programme in the North West Province was initiated and the RHP was revitalised with the assistance of the Department of Water Affairs & Forestry. The 2004 survey was conducted for the Crocodile (West) Marico WMA and it will be monitored in future on a 2-3 year cycle.

Management Actions

The biomonitoring indices used in the River Health Programme are good indicators of the ecological state of rivers and can therefore flag problem areas where corrective measures are required. However, river management can be quite complex and therefore different institutions have to plan and work together to reach appropriate decisions. Different stakeholders might have different interests in a specific river, but in the end each one needs to understand and respect the others' interests in order to manage their collective interest in river health.

In the Crocodile (West) Marico WMA, management decisions are not yet being co-ordinated on a catchment level, but it is anticipated that the coming Catchment Management Agency will play a key role in this regard. However, in the Limpopo and Gauteng provinces (where the RHP has been running for a few years), the respective RHP teams have identified problem issues and have proposed and implemented appropriate management actions (see pages 16 and 17). In the North West Province, the RHP has only recently been initiated therefore management actions have yet to be addressed. In the next State of the River report for the Crocodile (West) Marico WMA, the North West Province will be able to contribute in this regard.

Ecological Study Units

For the purpose of this study, the rivers in the Water Management Area were clustered based on ecological similarity - these clusters are referred to as ecological study units. A total of twenty two ecological study units were assessed. These ecological study units, the sub-management areas in which they fall, the ecoregions that they comprise, as well as the main rivers and tributaries that form part of each ecological study unit (and hence the ecological assessment), are summarised on the following page.

Ecological Study Units

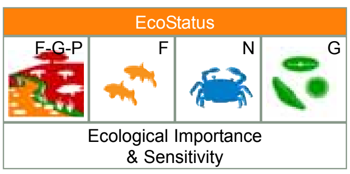
The ecological study units provided the boundaries within which ecological assessments were done. The delineation of these ecological study units was largely based on ecoregions and rivers that were similar, available information relating to riverine ecology, as well as the importance of the river from an ecological and land-use point of view.

Ecoregions

Ecoregion delineation is an approach that is followed to classify rivers into areas of broad ecological similarity (DWAF, 2003). Factors such as terrain morphology, natural vegetation, geology, soil characteristics, altitude, rainfall and runoff variability are considered when ecoregions are delineated. Rivers in the same ecoregion are hence ecologically more similar than rivers in different ecoregions.

Results of this survey

The overall ecostatus and individual integrity of the ecological indicator groups (i.e. fish, macroinvertebrates, instream and riparian habitat and diatoms), and the ecological importance and sensitivity of each ecological study unit, are presented per sub-management area and are summarised by the following icon which is colour-coded according to the results of each index i.e. the appropriate river health category.



Sub-management Area:
LOWER CROCODILE

Ecological Study Units:
- Middle Crocodile
- Sundays
- Lower Crocodile

Ecoregions:
- Western Bakenveld
- Bushveld Basin
- Limpopo Plain

Main Rivers and Tributaries:
- Crocodile downstream of Roodekopjes
- Sundays

Sub-management Area:
MARICO

Ecological Study Units:
- Klein Marico
- Groot Marico
- Middle Marico
- Lower Marico

Ecoregions:
- Highveld
- Western Bakenveld
- Bushveld Basin
- Limpopo Plain

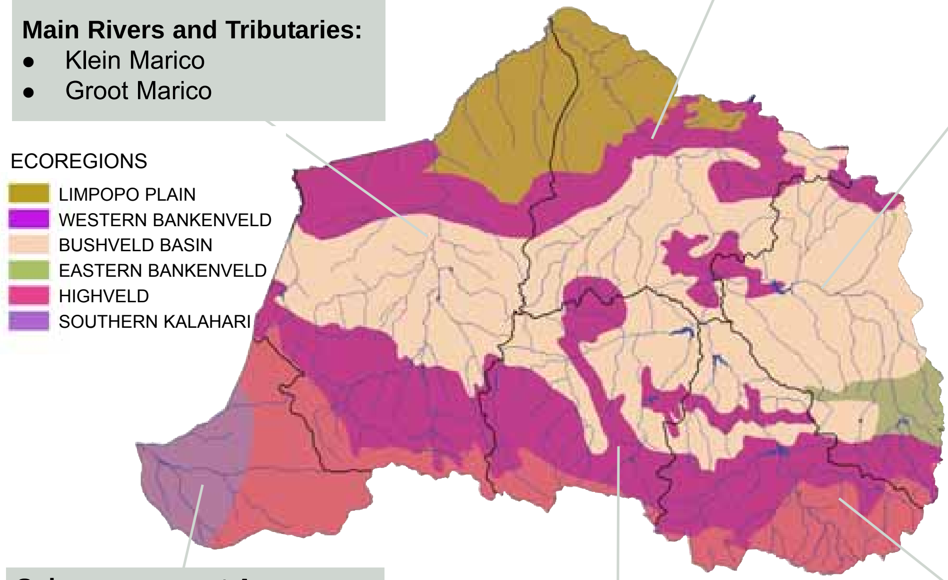
Main Rivers and Tributaries:
- Klein Marico
- Groot Marico

Sub-management Area:
Apies / Pienaars

Ecological Study Units:
- Plat
- Lower Pienaars
- Upper Pienaars
- Apies

Ecoregions:
- Western Bakenveld
- Bushveld Basin
- Eastern Bakenveld

Main Rivers and Tributaries:
- Pienaars
- Tshwane
- Kutswane
- Apies
- Moretele / Plat
- Tooyspruit



Sub-management Area:
UPPER CROCODILE

Ecological Study Units:
- Crocodile Highveld
- Magalies
- Skeerpoort
- Crocodile Western Bakenveld
- Upper Sterkstroom
- Lower Sterkstroom
- Middle Crocodile
- Rose / Kareespruit

Ecoregions:
- Highveld
- Western Bakenveld
- Bushveld Basin

Main Rivers and Tributaries:
- Sterkstroom
- Magalies
- Skeerpoort
- Rosespruit
- Kareespruit
- Crocodile
- Jukskei
- Klein Jukskei
- Hennops

Sub-management Area:
UPPER MOLOPO

Ecological Study Units:
Not surveyed

Ecoregions:
- Highveld
- Southern Kalahari

Main Rivers and Tributaries:
- Molopo
- Ramatlabama
- Polfonteinspruit
- Madebe

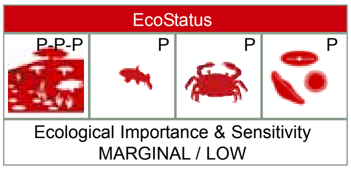
Sub-management Area:
ELANDS

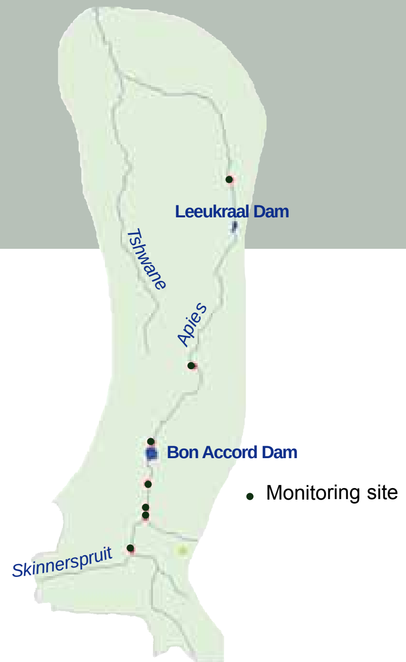
Ecological Study Units:
- Upper Elands
- Selons/Koster
- Upper Hex
- Lower Hex
- Lower Elands

Ecoregions:
- Highveld
- Western Bakenveld
- Bushveld Basin

Main Rivers and Tributaries:
- Elands
- Koster
- Selons
- Hex
- Klein Hex

APIES





EcoStatus

The overall EcoStatus for this study unit is POOR and comprises the following indices:

The ***Instream Habitat Integrity*** is POOR - this reach of river has been canalised and straightened in the urban areas. This has resulted in higher flows which in turn have also altered channel and bed shape. Urban runoff, sewage spills and litter from settlements impact heavily on water quality and the functional integrity of the river. ***Riparian Zone Habitat Integrity*** is POOR - channel modification plays the largest role in altering the habitat integrity of the riparian zone by changing the natural flow and flood patterns of the river.
Riparian Vegetation Integrity is POOR - most riparian vegetation has been cleared due to high levels of development. Alien vegetation encroachment is high in some areas; mulberries, jacaranda and sesbania are the most common species.

Fish Assemblage Integrity is POOR - sensitive species such as *Chiloglanis sp.* (rock catlet or suckermouth), *Amphilius sp.* (stargazer mountain catfish) and *Aplocheilichthys sp.* (topminnow) are lost, even hardy species have lowered frequencies of occurrence.
Macro-invertebrate Integrity is POOR - diversity and abundance is heavily impacted by urban runoff (increased volumes and reduced lag times) as well as reduced water quality.

Water Quality is POOR, flows have intermediate levels of nutrients and are free from significant organic pollution. Sources of pollution are primarily from urban activities. Pretoria Central, Iscor and large parts of Attridgeville contribute to reduced quality.

Ecological Importance and Sensitivity (EI&S)

EI&S is MARGINAL / LOW - species and habitat diversity is low because of canalised system, however some riffle and wetland habitats are present and sections of the river near Bon Accord Dam have been earmarked for rehabilitation. The Wonderboom Nature Reserve conserves some natural area.

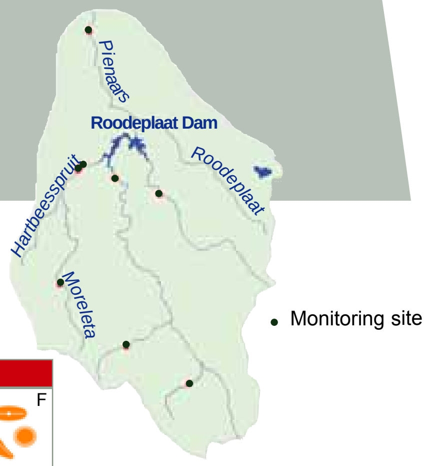
Drivers of Change

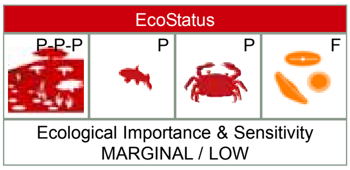
- High levels of development and urbanisation
- Canalisation and alteration of flow patterns

Management Responses

- Restore and rehabilitate channel morphology and riparian vegetation
- Control of urban runoff which is impacting on water quality
- Reduction and clean-up of litter from human settlements

Upper Pienaars





EcoStatus

The overall EcoStatus for this study unit is POOR and comprises the following indices:

The ***Instream Habitat Integrity*** is POOR primarily because of flow and bed modifications upstream from the Roodeplaat Dam caused by high levels of urbanisation and land-use activities such as small holdings, and chicken and dairy farming. Urban return flows contribute to higher than normal flows in the summer months and illegal dumping of garden refuse and building rubble on unoccupied land is problematic. The ***Riparian Zone Habitat Integrity*** is POOR - urban flood waters cause severe bank erosion. Below Roodeplaat Dam there is quite a lot of sedimentation. Channel modifications are mainly due to berms used for storm water management purposes. ***Riparian Vegetation Integrity*** is POOR - riparian vegetation in many areas has been cleared for development, from Lynwood Road to Magaliesberg there is no vegetation in the riparian zone and below the Roodeplaat Dam many developments are impacting on the riparian fringe. There is also serious alien vegetation infestation mainly of blue gum and wattle species.

The ***Fish Assemblage Integrity*** is also POOR - sensitive species such as *Chiloglanis sp.* (rock catlet or suckermouth) and *Amphilius sp.* (stargazer mountain catfish) are lost because of urbanisation and flows from sewage works. Even hardy species are under stress. ***Macro-invertebrate Integrity*** is POOR - reduced water quality has the largest impact on invertebrates.

Water Quality is FAIR, flows have intermediate levels of nutrients but are free from significant organic pollution. Main sources of pollution include urban return flows, sewage spills, and chicken and dairy farming activities.

Ecological Importance and Sensitivity (EI&S)

EI&S is MARGINAL / LOW - riparian and instream biotic diversity is low. Habitat types although not varied provide some unique examples: the Colbyn wetland in Hartebeespruit is a peat wetland, with more wetland types found upstream of the Silverlakes Golf Estate. Conservation areas include Bronberg, Fairie Glen and Moreletaspruit.

Drivers of Change

- Roodeplaat Dam altering natural flow regimes - wall construction does not allow flexible releases
- An increase in impervious surfaces due to increasing urbanisation has resulted in higher than normal peak flows, especially in the summer months
- Lack of riparian vegetation zone in many areas because of high levels of development and poor management
- Reduced water quality impacting on aquatic fauna and flora

Management Responses

- Control impingement of development into the riparian zone
- Improve solid waste disposal facilities and educate people of the impacts of littering
- Stabilise bank erosion
- Identify and control sources of pollution that are reducing water quality
- Map wetlands that require protection and investigate ways to conserve them
- Remove alien trees, especially wattle and blue gum

The Wonderboom Nature Reserve

The Wonderboom Nature Reserve is situated in the northern part of Tshwane straddling the Magaliesberg Mountains. This reserve is famous for its magnificent specimen of the Wonderboom. The Wonder tree is a wild fig (*Ficus salicifolia*) that is more than 1 000 years old, and legend has it that it grew this big because the chief of an indigenous tribe lies buried beneath its roots. Branches of this trunk first spread out radially but gradually drooped towards the ground, where they sent out roots from which sprang a circle of new trunks. In time, two of the offspring produced a third generation. Today the Wonderboom has 13 distinct trunks that cover an area of 1,5 ha. It is recorded that the tree was once big enough to shade 1 000 people at a time, or 22 ox-wagons with 20 oxen in front of each! Today, it is much smaller - probably because of the devastating fire in 1870 started by a hunting party or because of infestation by a parasite, which put it in quarantine for 20 years.

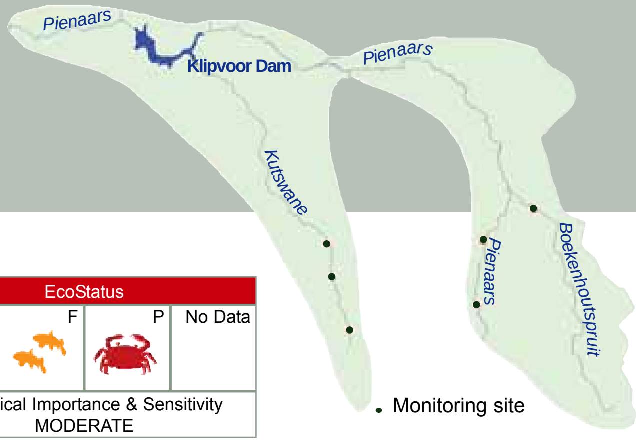
Sources:http://www.places.co.za and http://www.tshwane.gov.za

25

Apies / Pienaars

SUB-MANAGEMENT AREA

LOWER PIENAARS



EcoStatus			
P-P-P	F	P	No Data
Ecological Importance & Sensitivity MODERATE			

EcoStatus

The overall EcoStatus for this study unit is POOR and comprises the following indices:

The ***Instream Habitat Integrity*** is POOR because of flow modifications caused by the Klipvoor Dam, abstraction for irrigation purposes and weirs. Sedimentation caused by runoff from overgrazed areas in the riparian zone is impacting on channel bed morphology. Some parts of this river reach are still in good condition. The ***Riparian Zone Habitat Integrity*** is POOR - changes in the flow regime have impacted on riparian ecosystems. Sand mining activities along the Boekenhoutspruit are resulting in increased sedimentation. Two tributaries to the Lower Pienaars lie close to Bela-Bela and both are highly disturbed by alien plant species. The main alien species found here are blue-gum and lantana. ***Riparian Vegetation Integrity*** is POOR - vegetation decrease in the riparian zone is due to overgrazing and results in many open areas along the river up to the confluence with the Apies River. Alien vegetation encroachment is high in the upper sections of this reach and less in the lower sections.

Fish Assemblage Integrity is FAIR to POOR - stress conditions created by urbanisation and flows from sewage works have resulted in the loss of sensitive species. Even hardy species have lowered frequencies of occurrence. Eels are lost due to obstructions. ***Macro-invertebrate Integrity*** is POOR - diversity and abundance is heavily impacted by return flows from urban and industrial areas.

Water Quality - although no diatom data is available, water quality is very poor and requires urgent intervention to reduce pollution levels.

Ecological Importance and Sensitivity (EI&S)

The EI&S is MODERATE - a diversity of species types is still present in this river reach (e.g. otters and yellowfish). The floodplain landscape offers a variety of habitat types and refugia. The Wallmannsthal Military Base offers some degree of protection of natural areas.

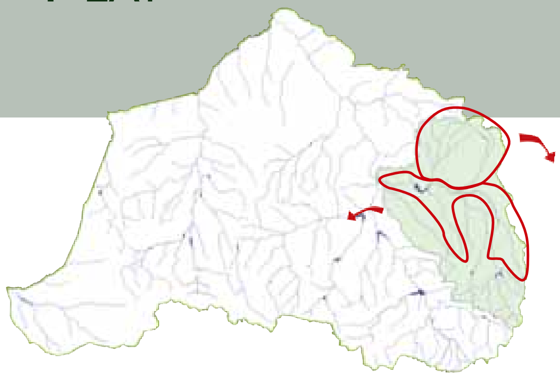
Drivers of Change

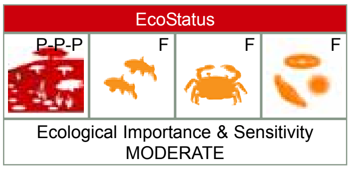
- Impacts of impoundments on natural hydrological regime of river
- High levels of urbanisation and industrial discharges impacting on water quality

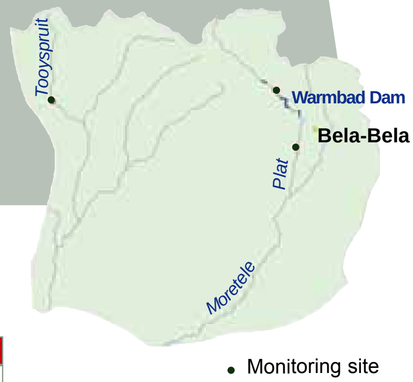
Management Responses

- Clear alien vegetation from riparian zone and catchment
- Manage and control overgrazing in riparian zone
- Manage and enforce compliance of sand winning activities in the riparian zone
- Identify sources of pollution and enforce water quality standards

PLAT







EcoStatus

The overall EcoStatus for this study unit is POOR and comprises the following indices:

The ***Instream Habitat Integrity*** is POOR - there are a number of agricultural dams and weirs as well as the Bela-Bela Municipality that have a serious impact on the aquatic biota and connectivity of this river reach because large volumes of water are being abstracted. The flows in the middle sections of this river reach are also being choked by debris from alien vegetation, mainly Eucalyptus species. The ***Riparian Zone Habitat Integrity*** is POOR with water abstraction having a large impact on riparian habitat. The presence of alien vegetation is causing a reduction in the undergrowth, leading to bank instability - banks on river bends are badly eroded, leading to a change in terrace structure. ***Riparian Vegetation Integrity*** is POOR with indigenous vegetation largely replaced by aliens - extensive growth of blue gum, lantana, poplar, seringa, prickly pear, bramble and sesbania were observed.

The ***Fish Assemblage Integrity*** is FAIR to POOR - in the lower sections the river is naturally very dry but these conditions are exacerbated by water abstraction and as a result only hardy species are found and often are limited to isolated pools. Upper sections are in fair condition with *Clarias theodorae* (snake catfish) still present. ***Macro-invertebrate Integrity*** is FAIR to POOR - water abstraction and subsequent riparian habitat alteration has the largest impact on invertebrates. The upper sections are still in reasonably fair condition.

Water Quality is FAIR, flows have between low and intermediate levels of nutrients and are free from significant organic pollution. Some localised urban runoff and sewage outflow contribute to moderate water quality scores.

Ecological Importance and Sensitivity (EI&S)

The EI&S is MODERATE - riparian vegetation diversity is moderate because the river is in a transition zone between mountain and bushveld. Instream habitat is high, comprising wetlands, riffles, pools, runs and cascades in high flow conditions. There are a number of private nature reserves offering some degree of protection for natural areas.

Drivers of Change

- Agriculture - demand for irrigation water has resulted in reduced flows in the river altering natural habitat
- Alien vegetation encroaching in riparian zone and blocking flows in some areas

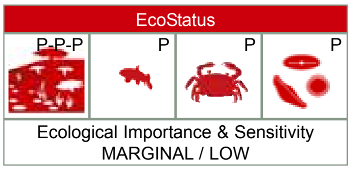
Management Responses

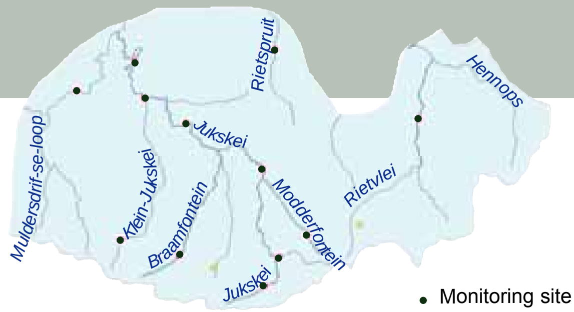
- Control and manage water use especially for irrigation purposes
- Determine environmental flow requirements and implement ecological reserve
- Eradicate invasive alien plants and rehabilitate degraded riparian habitats
- Identify and control sources of urban pollution especially sewage spills

Upper Crocodile

SUB-MANAGEMENT AREA

CROCODILE HIGHVELD





EcoStatus

The overall EcoStatus for this study unit is POOR and comprises the following indices:

The ***Instream Habitat Integrity*** is POOR because of urban development - the majority of the river is canalised, urban runoff is high because of paved areas and sewage spills and industrial discharges are common because infrastructure can not cope with the high levels of utilisation. It must be mentioned that some of the tributaries feeding the Crocodile River are not as severely impacted. The ***Riparian Zone Habitat Integrity*** is also POOR primarily because the river has been engineered and the flow patterns completely altered. ***Riparian Vegetation Integrity*** is POOR - natural vegetation has been completely altered because of urbanisation, and encroachment by poplar species is severe.

The ***Fish Assemblage Integrity*** is POOR - increased flow volumes and increased peak flows after heavy rains because impervious surfaces have altered natural flow regimes. There is complete loss of sensitive species and even hardy species have lowered frequencies of occurrence. ***Macro-invertebrate Integrity*** is POOR - diversity and abundances are severely impacted by urban runoff including sedimentation, sewage flows and industrial discharges.

Water Quality is POOR with high levels of nutrients and an increased frequency of water quality problems. The percentage of species tolerant to organic pollution indicates that the water is free from significant organic pollution. Water quality in the urban areas is severe - mostly because of sewage spillages and industries discharging into the sewer network. The sewerage system is not able to cope with the increase in housing density.

Ecological Importance and Sensitivity (EI&S)

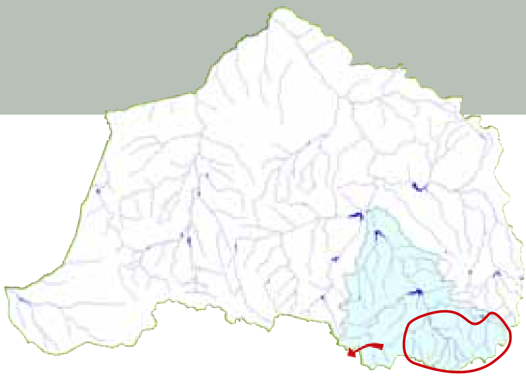
The EI&S is MARGINAL / LOW, the number of functional habitat types and species diversity is low because of the complete alteration of channel morphology and the natural flow regime. The African bullfrog is one unique species that manages to survive in this reach of river but is under constant threat as a result of changing land-use.

Drivers of Change

- Urbanisation - impervious surfaces, lack of sufficient capacity of sewer system, channel and flow modification
- Increased change of land-use from natural to urban and industrial

Management Responses

- Upgrade sewerage system and improve management
- Reduce pollution from sewers, illegal discharges and reduction of instream solid waste (litter)
- Manage surface stormwater runoff at source
- Clear alien invasives from riparian zone
- Encourage and support the Giant Bullfrog Project to ensure the survival of this 'near threatened' species and its habitat
- Encourage infiltration by reducing impervious surfaces to aid flood attenuation



Magaliesberg Protected Natural Environment (MPNE)

On 12 August 1977, the Magaliesberg which comprises approximately 37 000 ha, was declared a "Nature Area" in terms of the Physical Planning Act 88 of 1967. This Act introduced the concept of "Nature Areas" or Protected Natural Environments as they are now called in South African law.

The Magaliesberg Mountain range, stretching 125 km between Tshwane Metropolitan area, Johannesburg and Rustenburg, is of great geological importance. It has a rich concentration of valuable minerals and contains an archeological history representing the origins of humankind with a rich collection of hominid and pre-hominid fossils.

Evidence of occupation by early San communities is found in the rock paintings in the mountain. Bakgatla, Bakwena and Bafokeng can all trace their history in this area. A few monuments exist that commemorate the wars between Nguni and Sotho and the Boer and the English.

Presently the mountain is under severe pressure from developments such as mining, agriculture and recreation.

History of Johannesburg Streams

Johannesburg may not be built on a river or harbour, but its streams are the source of two of southern Africa's mightiest rivers.

A number of streams meander through the suburbs of Johannesburg, and form the source of two of southern Africa's primary rivers - the Limpopo and the Orange. Most of the springs from which many of these streams emanate are now covered in concrete and canalised, accounting for the fact that the names of early farms in the area often end with "fontein", meaning "spring" in Afrikaans. Braamfontein, Rietfontein, Zevenfontein, Doornfontein, Zandfontein and Randjesfontein are some examples.

When the first white settlers reached the area that is now Johannesburg, they noticed the glistening rocks on the ridges, running with trickles of water, fed by the streams - giving the area its name, the Witwatersrand, "the ridge of white waters". Another explanation is that the whiteness comes from the quartzite rock, which has a particular sheen to it after rain.

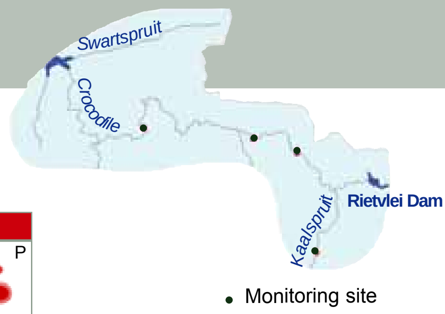
Adapted from Lucille Davie's article 'Water, water….everyway' - December 24, 2004
Source: Johannesburg News Agency; www.joburg.org.za

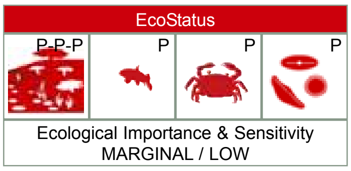
Johannesburg
Canalised stream running through Johannesburg

Upper Crocodile

SUB-MANAGEMENT AREA

CROCODILE WESTERN BANKENVELD





EcoStatus

The overall EcoStatus for this study unit is POOR and comprises the following indices:

Instream Habitat Integrity is POOR - this can be attributed to the severe modifications to the channel morphology and flow patterns. Patterns have changed because of development, an increase in return flows resulting in higher peak flows, water being imported into the system and sewer discharges into the river. Solid waste in the form of general litter is problematic in the riparian zone and instream. The ***Riparian Zone Habitat Integrity*** is POOR - the modifications of channel morphology and flow has had a serious impact on the riparian habitats; bank erosion and inundation of the riparian zone have all contributed to low scores. ***Riparian Vegetation Integrity*** is POOR with alien vegetation encroachment and vegetation clearing both impacting on riparian vegetation integrity. At Ben Albert's Nature reserve, however, there are relatively fewer alien species, greater cover and recruitment of indigenous riparian species and the riparian zone is well covered with vegetation. The Sweethome site has a considerable forest of monkey thorn which is in need of conservation. It has a reasonably high percentage of indigenous riparian species and recruitment, but with highly invasive alien species like the castor-oil plant and herbaceous alien species present.

The ***Fish Assemblage Integrity*** is POOR - there is a complete loss of sensitive species (*Amphilius sp.* (Stargazer mountain catfish) and *Opsaridium sp.* (barred minnow)). Even hardy species are under stress with lowered frequencies of occurrence. ***Macro-invertebrate Integrity*** is POOR - reduced water quality and flow modifications due to urban and industrial runoff have a severe impact on invertebrates.

Water Quality is POOR - flows have high levels of nutrients and water quality problems but are free from significant organic pollution. This is primarily the result of urban runoff and industrial discharges.

Ecological Importance and Sensitivity (EI&S)

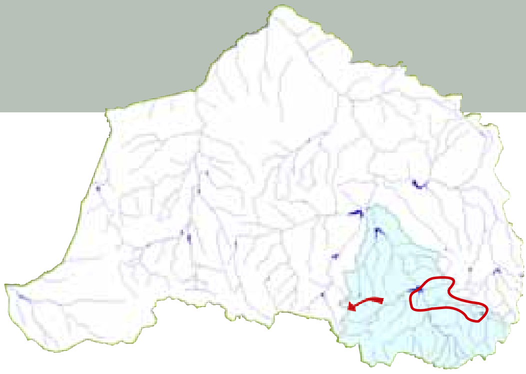
The EI&S is MARGINAL / LOW although there is some diversity of habitat due to the influence of the Brakenveld ecoregion. Overall however species and habitat diversity is low with little natural area left for protection or conservation.

Drivers of Change

- High levels of urbanisation - sewerage system unable to cope resulting in sewage discharges
- Discharges from industries into the sewer system
- Canalisation and alteration of flow patterns
- Invasive alien plants in riparian zone and in catchment

Management Responses

- Reduce and clean-up litter pollution
- Control of discharges into river - both sewage and industrial - to improve water quality
- Clear invasive aliens in riparian zone



Hartbeespoort Dam

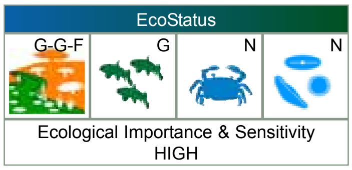
The Hartbeespoort Dam, located in the Crocodile River Catchment in the North West Province, is a landmark for many people, attracting tourists from all over South Africa. However, Hartbeespoort Dam has, for decades, received large loads of wastewater effluent from Johannesburg, Midrand and Krugersdorp and will continue to do so well into the foreseeable future. The subsequent level of pollution is such that the dam regularly experiences dense blooms of cyanobacterial algae, with associated levels of algal toxins that pose a significant threat to human and animal health. Increasing development around the dam also results in additional pressure on the water quality of the dam.

Some interventions to address this problem include:

- Enhanced wastewater treatment in the watershed draining to Hartbeespoort Dam;
- Maximizing wastewater re-use for irrigation and other purposes at or close to the point of generation;
- Sound land-use planning in the shoreland area and in the catchment draining to Hartbeespoort Dam;
- Development and operation of an instream ferric sulphate dosing facility to reduce phosphorus loads from both point and nonpoint sources within the Crocodile River catchment;
- Development of a commercial fishery on the dam to manage and control coarse fish populations and promote sustainable game fishery;
- Restoration of shoreland wetland and floodland ecosystems;
- Development and delivery of a programme of public information to the Hartbeespoort Dam communities inclusive of non-resident users;
- Ongoing monitoring of the response of the reservoir to the aforementioned interventions, and the conduct of further pilot scale studies as may be required; and
- Liaison with the relevant government entities, such as the local municipality, DWAF and NW-DACET, on matters relevant to the reservoir ecosystem and continuing development of the catchment, shoreline and recreational uses.

Hartbeespoort Dam

SKEERPOORT



EcoStatus

The overall EcoStatus for this study unit is NATURAL / GOOD and comprises the following indices:

The **Instream Habitat Integrity** is GOOD - there are several dolomitic eyes at the source of the Skeerpoort River which are still in pristine condition. Some farming activities have impacted on flows lower down in the system. The **Riparian Zone Habitat Integrity** is GOOD - there is very minimal impact on the riparian zone with some localised bank erosion. The **Riparian Vegetation Integrity** is FAIR with alien vegetation encroachment having an impact at a small number of localities and some vegetation clearing for agriculture.

Fish Assemblage Integrity is GOOD to NATURAL with some impacts due to farming activities influencing fish diversity. Eels are lost due to obstructions, especially Hartbeespoort and Roodekopjes dams. **Macro-invertebrate Integrity** is NATURAL - macro-invertebrate diversity and abundance is high and close to natural conditions with species present that require permanent flows and high water quality conditions.

Water Quality is NATURAL, flows have low to intermediate levels of nutrients and free from significant organic pollution.

Ecological Importance and Sensitivity (EI&S)

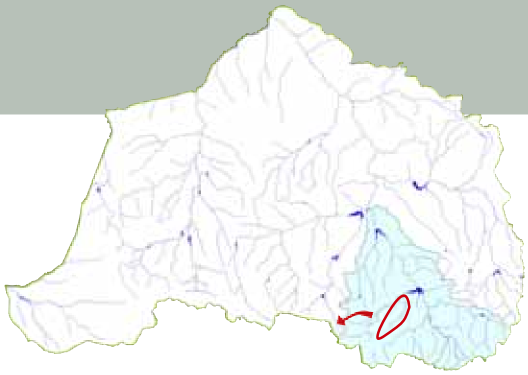
The EI&S is HIGH with high scores for unique and diverse biota as well as for providing habitat as refugia for biota during periods of environmental stress. The Cradle of Humankind, a World Heritage Site, is a significant conservation achievement for the area.

Drivers of Change

- Farming activities - although currently have minimal impact

Management Responses

- Restrict development to a minimum, as the greater part of the Skeerpoort catchment is situated within a proclaimed nature reserve
- Monitor farming activities - ensure impacts are minimal into the future
- Eradicate alien invasive plant species

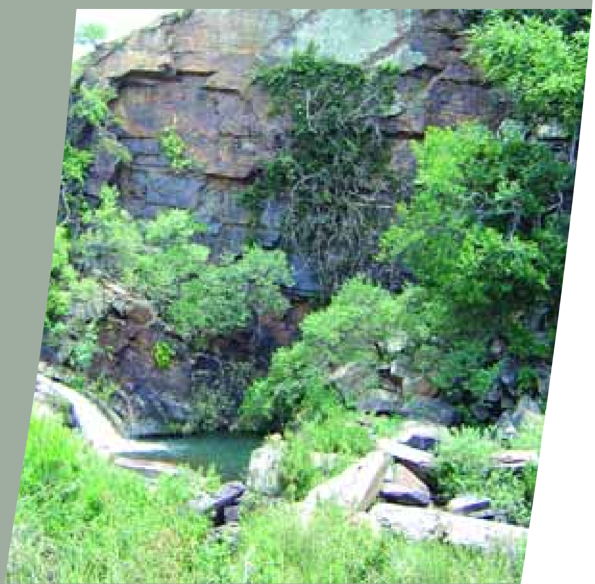


Cradle of Humankind

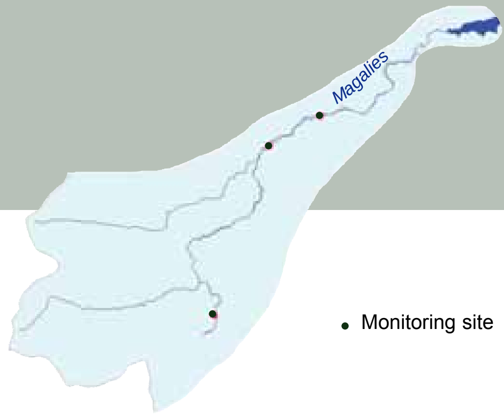
In 1997 the South African government signed the 1972 UNESCO Convention on the protection, preservation and promotion of the world's natural and cultural heritage making South Africa eligible to nominate sites of unique international significance. In 1999 the National Department of Environmental Affairs and Tourism, the office of the Premier of Gauteng and the Gauteng Department of Agriculture, Conservation, Environment and Land Affairs (DACEL, now known as GDACE) nominated the fossil hominid sites of Sterkfontein, Swartkrans, Kromdraai and environs known as the Cradle of Humankind. The Cradle of Humankind was inscribed on the World Heritage List on 2 December 1999. The Cradle of Humankind World Heritage Site comprises a strip of thirteen dolomitic caves containing the fossilised remains of plants, animals and, most importantly, hominids (members of the human family and our near relatives). These fossils are a superbly preserved record of the stages in the evolution of humankind within the past 4 million years.


Taung Child

Source: http://www.cradleofhumankind.co.za/index_.html
Accessed 15 Nov 2004


Skeerpoort

MAGALIES



EcoStatus			
P-P-P	F	P	No Data
Ecological Importance & Sensitivity MODERATE			

EcoStatus

The overall EcoStatus for this study unit is POOR and comprises the following indices:

The ***Instream Habitat Integrity*** is POOR - this is attributed to high levels of water abstraction primarily for bottling. Water abstraction by farmers is also high with 25 furrows on the Magalies River alone. Many people rely on the furrow water for domestic use. The Magalies River has it's main source at Malony's eye upstream from the town of Magaliesburg. A constant flow of water surfacing at Malony's eye from the Steenkoppies dolomitic compartment feeds the river throughout the year.
The ***Riparian Zone Habitat Integrity*** is POOR - furrows have resulted in inundation of the riparian vegetation and flow modification has altered natural riparian habitats.
The ***Riparian Vegetation Integrity*** is POOR with riparian vegetation being cleared for agricultural and housing purposes. Alien vegetation encroachment is serious; poplars, wattles and blue gums are the most common.

Fish Assemblage Integrity is FAIR - the upper reaches still sustain some sensitive species, while the lower sections are impacted by water abstraction and flow modifications.
Macro-invertebrate Integrity is POOR overall, primarily because of water abstraction and therefore habitat alteration and some localised impacts on water quality from the town of Magaliesberg. Although in the upper reaches integrity can be classified as fair.

Water Quality - although no diatom data is available water quality is good with localised impacts from lodge developments along the river and return flow from pig farms, chicken farms and flower farms in the area.

Ecological Importance and Sensitivity (EI&S)

The EI&S is MODERATE - in the upper reaches species intolerant to changes in flows are present as well as some unique species of stoneflies, Perlidae, and fish (*Amphilius uranoscopus* (stargazer mountain catfish)). In the lower reaches habitat and species diversity are reduced due to flow modifications.

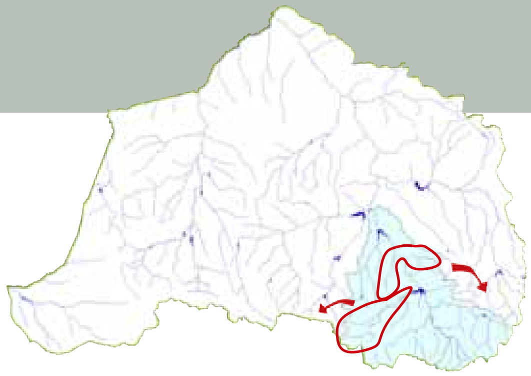
Drivers of Change

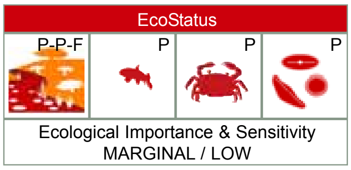
- Serious encroachment of alien vegetation in the riparian zone
- High levels of water abstraction resulting in changes in the natural flow regime of the river
- Large volumes of water extracted from the Steenkoppies/Holfontein compartment for agricultural use
- Flow regulating structures - large number of weirs for irrigation altering flow patterns

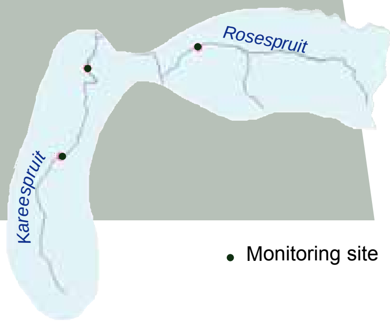
Management Responses

- Monitor and control water use and abstraction - ensure that the ecological reserve is determined and maintained
- Clear alien vegetation in the riparian zone
- Consider installing fish ladders in suitable flow regulating structures

Rosespruit / Kareespruit







EcoStatus

The overall EcoStatus for this study unit is POOR and comprises the following indices:

The ***Instream Habitat Integrity*** is POOR - the Kareespruit and the Rosespruit are impacted on by agricultural return flows, industry, and sewage inflows respectively. The ***Riparian Zone Habitat Integrity*** is POOR primarily because of channel modification and bank erosion caused by industrial and agricultural activities. The ***Riparian Vegetation Integrity*** is FAIR to POOR with some removal of vegetation from the riparian zone and the occurrence of alien vegetation encroachment.

The ***Fish Assemblage Integrity*** is POOR - only hardy species are present in the Kareespruit while no fish were caught in the Rosespruit. ***Macro-invertebrate Integrity*** is POOR due to severe water quality problems. Diversity of macro-invertebrates is very low.

Water Quality is POOR with flows measuring high levels of nutrients and the sites sampled were classified as heavily contaminated with organic pollution. The high vanadium levels in the area around Rosespruit are impacting so severely on the water quality that farmers have stopped using borehole water in the vicinity because of contaminated groundwater.

Ecological Importance and Sensitivity (EI&S)

EI&S is MARGINAL / LOW - instream flow modifications and water quality problems have impacted on the diversity of species and habitat types found in this river reach. This is typical of a river being heavily utilised for agricultural purposes where flows are regulated by impoundments and water quality modified by irrigation return flows and the use of agricultural chemicals. This results in stress conditions for the biota with a resultant reduction in sensitive species.

Drivers of Change

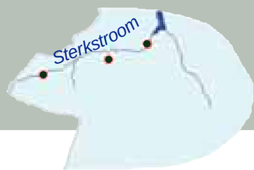
- Industrial activities impacting on flow and channel morphology
- Surface and groundwater contamination by industries
- Flow modifications due to agricultural return flows and bank erosion

Management Responses

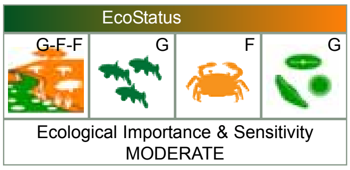
- Manage and minimise industrial and agricultural water pollution
- Restore and rehabilitate channel morphology and riparian vegetation
- Ensure compliance to water quality objectives especially with regards to groundwater contamination

Upper Crocodile
Sub-Management Area

Upper Sterkstroom



• Monitoring site



EcoStatus

The overall EcoStatus for this study unit is GOOD / FAIR and comprises the following indices:

The ***Instream Habitat Integrity*** is GOOD with some water abstraction upstream of Buffelspoort Dam for farming. Some small weirs are present but have minimal impact on flows and channel morphology.
The ***Riparian Zone Habitat Integrity*** is FAIR - there are a few weirs which when full inundate the riparian zone which cause some localised bank erosion.
The ***Riparian Vegetation Integrity*** is FAIR - this is primarily attributable to the widespread invasion of alien vegetation, mostly poplars and blue gums.

The ***Fish Assemblage Integrity*** is GOOD to NATURAL - sensitive species with permanent flow and high water quality requirements are present. Frequency of occurrence is close to natural. Eels are lost due to flow regulating structures obstructing their migration routes.
The ***Macro-invertebrate Integrity*** is FAIR with flow and habitat modifications contributing to localised impacts on invertebrates.

The ***Water Quality*** is GOOD - flows have between low and intermediate levels of nutrients and are free from significant organic pollution.

Ecological Importance and Sensitivity (EI&S)

The EI&S is MODERATE with a high proportion of species dependent on permanently flowing water and some protection of natural areas offered by the Magalies Protected Natural Environment.

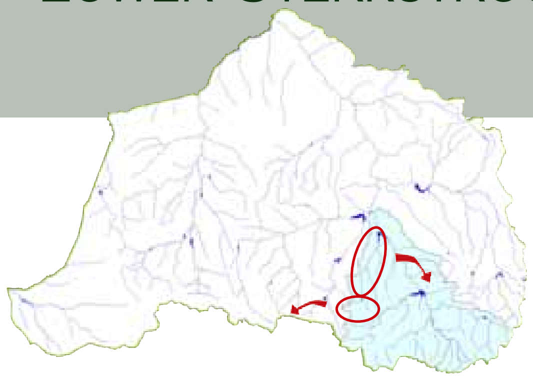
Drivers of Change

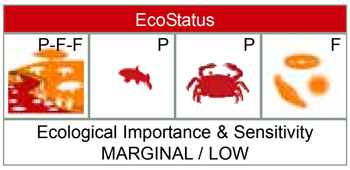
- Water abstraction for irrigation
- Widespread infestation by alien vegetation
- Resort development

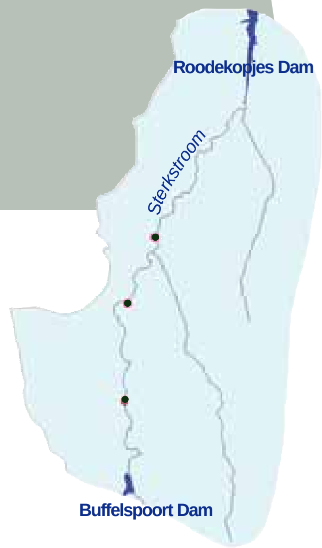
Management Responses

- Clear alien vegetation in riparian zone and in catchment
- Construct fish ladders where appropriate
- Monitor water use for irrigation
- Determine environmental flow requirements and implement the ecological reserve
- Identify and monitor wetlands to ensure ecological functions are maintained

Lower Sterkstroom







EcoStatus

The overall EcoStatus for this study unit is POOR and comprises the following indices:

The ***Instream Habitat Integrity*** is POOR primarily because of mining activity in the area. Water abstraction and mine de-watering has altered the natural flow regime of the river to such an extent that the upper reaches of the river are drier than they should be and the lower reaches are wetter than they should be. In some cases water abstraction points have become mining process dams severely impacting on flows and channel morphology. The ***Riparian Zone Habitat Integrity*** is also FAIR because of flow and channel modifications due to mining activities. The ***Riparian Vegetation Integrity*** is FAIR with moderate abundances of alien vegetation in the riparian zone. Increasing levels of development has resulted in vegetation removal.

The ***Fish Assemblage Integrity*** is POOR - water quality problems originating from mines create stress conditions for most fish species, sensitive species are lost due to the cumulative impacts of reduced water quality and flow modifications and obstructions. The ***Macro-invertebrate Integrity*** is POOR with water quality having the largest impact on invertebrate diversity and abundances and flow and habitat modifications contributing to low scores.

Water Quality is FAIR - flows have intermediate levels of nutrients and emerging signs of water quality problems with organic pollution likely to contribute to eutrophication of the sites sampled. Impacts on water quality originate primarily from mining activities with mines acting as a salt sink, increasing salinity levels in both surface and groundwater resources.

Ecological Importance and Sensitivity (EI&S)

The EI&S is MARGINAL / LOW - low scores can be attributed to the low diversity of species and habitat types within this reach of river, primarily the result of severe modifications of flows and of channel morphology.

Drivers of Change

- Mining operations - flow and channel alterations, and reduced water quality (high salinity levels)
- Informal settlements related to mining activities - impacts on water quality and natural resource use
- Groundwater usage for citrus farming along the northern foot of the Magaliesberg range

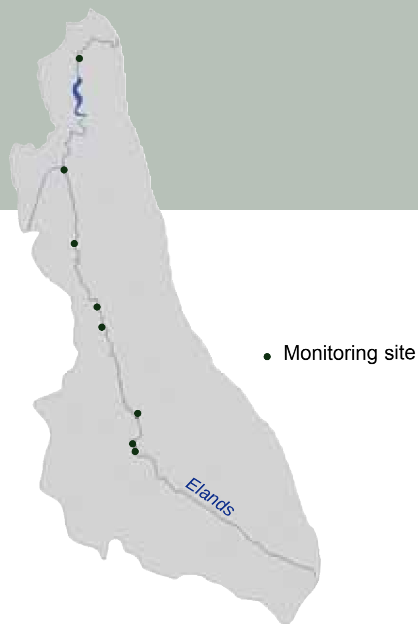
Management Responses

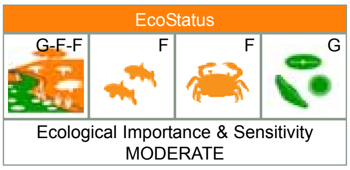
- Zone, licence and monitor mining activities
- Monitor water quality to ensure resource directed water quality objectives are being adhered to
- Rehabilitate riparian vegetation
- Monitor and manage sewage and solid waste disposal from informal settlements

Elands

SUB-MANAGEMENT AREA

UPPER ELANDS





EcoStatus

The overall EcoStatus for this study unit is FAIR and comprises the following indices:

Instream Habitat Integrity is GOOD - water abstraction and flow modification is low due to the presence of small dams and weirs which have little impact. However there is some turbidity which may be sedimentation from the shale and slasto mining and farming activities adjacent to the river. The ***Riparian Zone Habitat Integrit***y is FAIR - alien vegetation encroachment has resulted in some bank erosion and mining activities have resulted in some channel modification. The ***Riparian Vegetation Integrity*** is FAIR - there is a large infestation of wattle species along this reach and some vegetation clearing for dryland agriculture along the river banks.

The ***Fish Assemblage Integrity*** is FAIR to GOOD with some sensitive species possibly lost due to turbidity and sedimentation from slate quarries. The ***Macro-invertebrate Integrity*** is FAIR, primarily because of the sensitivity of invertebrates to turbidity and sedimentation.

Water Quality is GOOD - flows have between low and intermediate levels of nutrients and are free from significant organic pollution.

Ecological Importance and Sensitivity (EI&S)

EI&S is MODERATE - there is a range of diverse instream habitats (waterfalls, rapids and pools) as well as many wetlands in the highveld area. In the lower reaches there is cattle and game farming with some overgrazing.

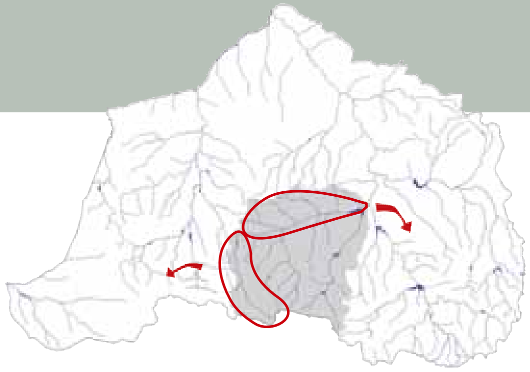
Drivers of Change

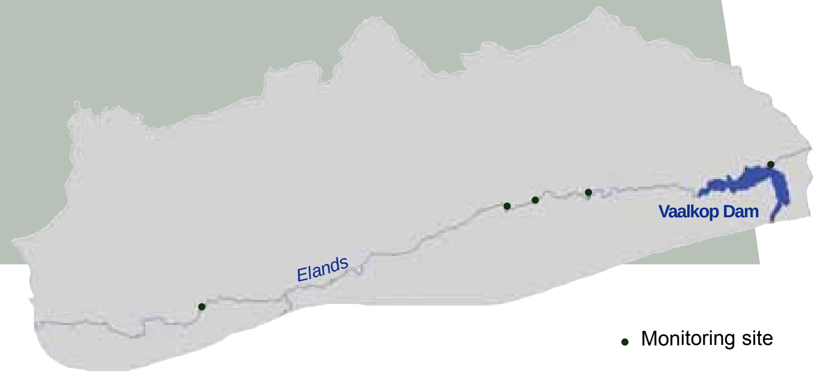
- Sedimentation resulting from the slate quarries and agriculture
- High infestation of alien plant species
- Inadequate management of some sewage treatment facilities

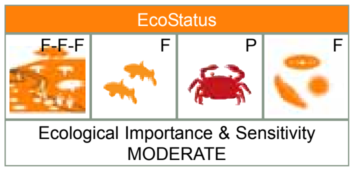
Management Responses

- Manage mining activities to reduce sedimentation
- Clear alien vegetation in riparian zone
- Map and monitor highveld wetlands to ensure protection and continued functioning as sediment traps

Lower Elands







EcoStatus

The overall EcoStatus for this study unit is FAIR and comprises the following indices:

The ***Instream Habitat Integrity*** is FAIR to POOR - this is primarily due to the mines and development in the area. The majority of these negative impacts are however confined to localised areas; the upper reaches of the river are in satisfactory condition. The ***Riparian Zone Habitat Integrity*** is FAIR with some return flows from the mines, although very localised, and some potential bank erosion problems in the future due to large toppling bushwillow trees. The ***Riparian Vegetation Integrity*** is FAIR due to some degraded areas in the lower reaches.

The ***Fish Assemblage Integrity*** is FAIR - sensitive species are lost due to flow modification and obstruction of movement. Water quality problems originating from mines create stress conditions for fish species along some sections. ***Macro-invertebrate Integrity*** is POOR mostly because of reduced water quality and habitat alteration.

Water Quality is FAIR mainly because of large settlements and mines in the area. Flows have intermediate levels of nutrients and there is emerging evidence of organic pollution - this might be nutrients from the surrounding platinum mines.

Ecological Importance and Sensitivity (EI&S)

The EI&S is MODERATE - diversity of habitats is low with some deep kloofs and pools in the Pilanesberg area. The Vaalkop Dam and the Pilanesberg Nature Reserve provide some protection of the indigenous vegetation in the area as well as a number of game farms along the river.

Drivers of Change

- Informal settlements contributing to organic pollution
- Platinum mining operations - nutrient rich return flows
- Urban settlements - spillages and discharges reducing water quality

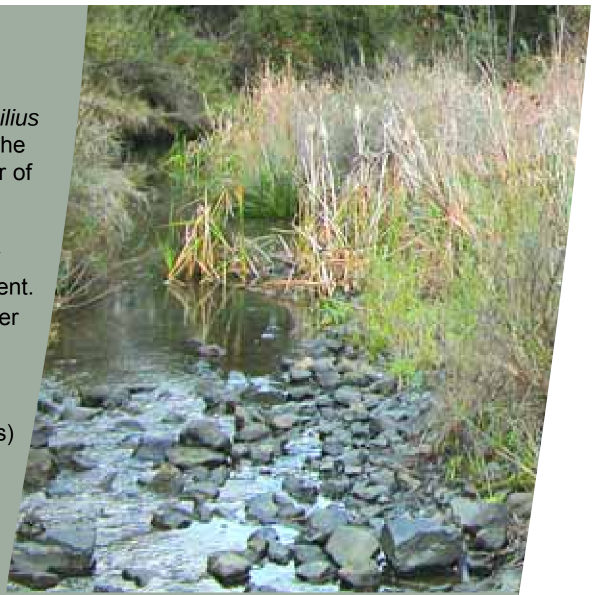
Management Responses

- Manage mining activities to reduce water quality problems
- Plan settlements not to impinge on riparian zone
- Stabilise bank erosion
- Improve on management of water quality impactors, e.g. tannery and sewage treatment works

The Elands River

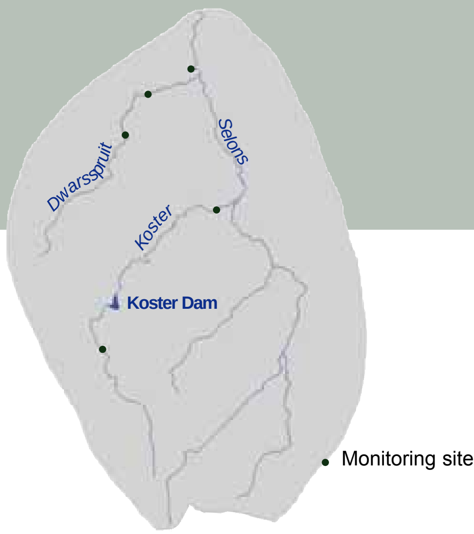
Two fish species, *Chiloglanis pretoriae* (shortspine suckermouth) and *Amphilius uranoscopus* (stargazer mountain catfish) were conspicuously absent from the Elands River. Both species are highly dependent on clear, fast flowing water of good quality. Possible scenarios that may explain their absence include:

- The Elands River was naturally perennial but these species were never able to invade this tributary due to a natural obstruction to their movement.
- The Elands was perennial and colonized by both species. When the river stopped flowing in the dry season (became seasonal) due to water abstraction for agricultural use together with use by alien trees next to the river, both species disappeared.
- Extensive slate quarries along the upper Elands River (up to Swartruggens) had a detrimental influence on the water quality, especially in terms of increased turbidity and fine sediment, influencing their spawning success and food source (riffle-dwelling aquatic invertebrates). This, together with generally decreasing flows, has led to the disappearance of both species from the Elands River.



Koster River, tributary to the Elands River

Selons / Koster



EcoStatus			
F-F-F	P	F	G
Ecological Importance & Sensitivity MARGINAL / LOW			

Elands

Sub-Management Area

EcoStatus

The overall EcoStatus for this study unit is FAIR and comprises the following indices:

Instream Habitat Integrity is FAIR - downstream of Koster Dam flow is regulated for irrigation purposes. Releases are fairly constant but not all the water is used for irrigation therefore some water flows downstream of the dam. In the upper reaches of the catchment tributaries are seasonal and are fed by natural springs. The ***Riparian Zone Habitat Integrity*** is FAIR with some flow modification between the Koster Dam and the confluence of the Koster and Selons rivers. Water abstraction for irrigation is evident. The ***Riparian Vegetation Integrity*** is FAIR because of severe infestation by alien vegetation, primarily seringa and poplars in the upper reaches.

The ***Fish Assemblage Integrity*** is POOR with considerably reduced frequency of occurrence of species due to flow obstructions and water abstraction - mostly hardy species are present. These are *Barbus paludinosus* (straightfin barb), *Pseudocrenilabrus philander* (Southern mouthbrooder) and *Tilapia sparrmanii* (vleikurper or banded tilapia). The ***Macro-invertebrate Integrity*** is FAIR mainly due to flow modifications.

Water Quality is GOOD, flows have between low and intermediate levels of nutrients and the water is free from significant organic pollution.

Ecological Importance and Sensitivity (EI&S)

EI&S is MARGINAL / LOW with very low scores for species and habitat diversity. Most refugia and migration corridors are localised and very little land is formally protected.

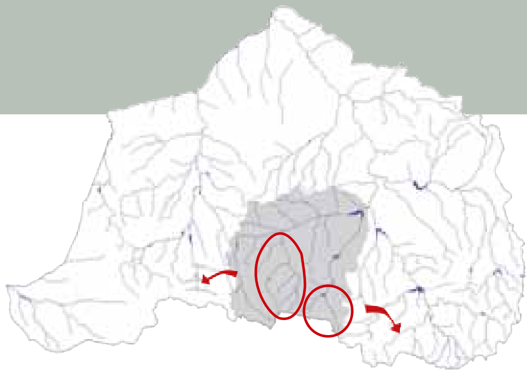
Drivers of Change

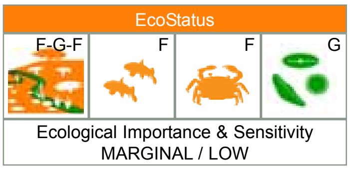
- Impoundment of river altering natural flow regimes
- Water abstraction for irrigation not efficient

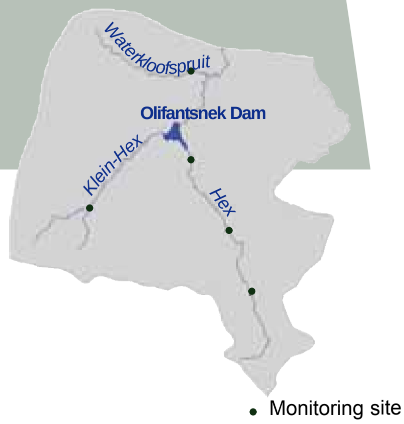
Management Responses

- Clear alien vegetation from riparian zone and catchment
- Manage water use and investigate abstraction for irrigation to ensure scheme is efficient

Upper Hex







EcoStatus

The overall EcoStatus for this study unit is FAIR and comprises the following indices:

Instream Habitat Integrity is FAIR - Olifantsnek Dam is situated at the confluence of the Hex and Klein Hex rivers, there is some water abstraction from the river for irrigation purposes and some sedimentation as a result of bank erosion. Low abundances of *Myriophyllum aquaticum* (parrot's feather) were observed. The ***Riparian Zone Habitat Integrity*** is GOOD with only localised areas of erosion adjacent to river bridges. Downstream of the Olifantsnek Dam there is some localised impacts, upstream of the dam natural vegetation predominates. The ***Riparian Vegetation Integrity*** is FAIR - there is some infestation by poplar species, although not very abundant. Wattle infestation in parts of the catchment is severe.

The ***Fish Assemblage Integrity*** is FAIR - this is attributable to water abstraction in some sections which lowers the frequency of occurrence of some species, although some sensitive species such as minnows and yellowfish are still present. ***Macro-invertebrate Integrity*** is FAIR - impacts are mostly due to localised habitat alteration.

Water Quality is GOOD - flows have between low and intermediate levels of nutrients and are free from significant organic pollution. Waterkloofspruit is well known for its exceptionally good water quality.

Ecological Importance and Sensitivity (EI&S)

EI&S is MARGINAL / LOW primarily because of the low diversity of habitat in this reach of the river, although the wetlands at Waterkloofspruit are significant. The Kgashwane Mountain Reserve and the Magaliesberg Protected Natural Environment (MPNE) conserve the natural landscape and restrict development.

Drivers of Change

- Water abstraction for irrigation
- Downstream impacts of dam - alteration of natural flow regimes

Management Responses

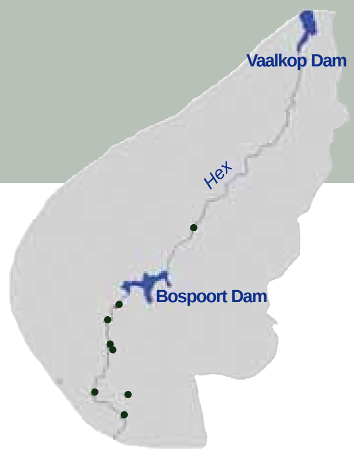
- Clear alien species from riparian zone and catchment
- Stabilise localised erosion points
- Control solid waste dumping and burial
- Regulate water use of irrigation schemes
- Determine environmental flow requirements and implement the ecological reserve
- Identify and monitor wetlands to ensure ecological functions are maintained

Lower Hex

Elands

Sub-Management Area

EcoStatus			
P-F-G	P	P	F
Ecological Importance & Sensitivity MARGINAL / LOW			



EcoStatus

The overall EcoStatus for this study unit is POOR and comprises the following indices:

The ***Instream Habitat Integrity*** is POOR, primarily because of high levels of development especially in terms of mining activities as well as water abstraction for irrigation purposes. There are a number of weirs that comprise the irrigation scheme but their use is limited. Stretches of the river have been diverted for the mines but more recently for the upgrade of the N4 Platinum Toll Highway. The ***Riparian Zone Habitat Integrity*** is FAIR - channel modifications caused by diversions for mining have impacted on riparian zone habitats. The ***Riparian Vegetation Integrity*** is GOOD - there is some vegetation clearing for sand winning activities and some pockets of sesbania and blue gums, both of which are very localised.

The ***Fish Assemblage Integrity*** is POOR - sensitive species are lost due to flow modifications and obstructions. Water quality problems originating from the mines and from agriculture have created stress conditions for fish species. The ***Macro-invertebrate Integrity*** is POOR, the cumulative impacts of reduced water quality and, flow and habitat modifications have had a large effect on invertebrate diversity and abundance.

Water Quality is FAIR - flows have between low and intermediate levels of nutrients but are largely free of significant organic pollution. High conductivity readings were recorded - high salinity levels are possibly due to mines.

Ecological Importance and Sensitivity (EI&S)

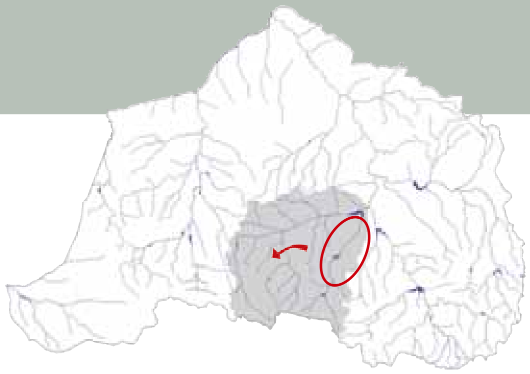
The EI&S is MARGINAL / LOW - diversity of habitat and species is low with some localised refugia for slightly sensitive species and protected natural area in the form of a conservancy around Bospoort Dam.

Drivers of Change

- Mining operations - river diversions and polluted discharges and seepages
- Road construction - river diversions for the N4 Platinum Toll Highway and mining activities

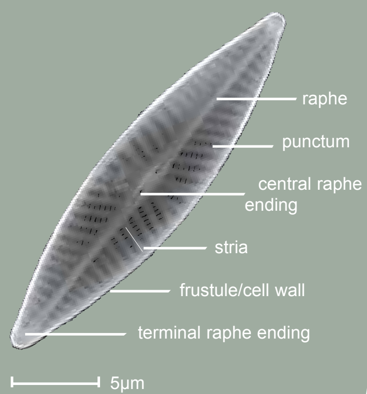
Management Responses

- Manage mining activities through the issuing of water use and discharge licences
- Monitor water use for irrigation purposes - investigate more efficient use of irrigation water
- Consider installing fish ladders in suitable weirs



What are the characteristics of a Diatom?

- A unique cell wall composed of silica known as a frustule
- A raphe fissure or slit (found in many but not all diatoms) which allows the diatom to move when mucilage is excreted
- A golden-brown chloroplast containing the pigment fucoxanthin
- Food reserves are stored as oil droplets, which add buoyancy to counteract the heavy silica frustule and chrysolaminarin for starch storage
- Diatom communities are composed of a myriad of species, each of which has a specific preference or tolerance towards water quality variables
- For many diatom species these specific pollution tolerances are known
- If the water quality tolerances for the majority of species within a community (the dominant species) are known then conclusions may be drawn regarding the water quality of the site from which the diatoms were taken



Diatom

Lower Crocodile

SUB-MANAGEMENT AREA

SUNDAYS

EcoStatus			
F-P-P	F	P	F

Ecological Importance & Sensitivity
MODERATE

EcoStatus

The overall EcoStatus for this study unit is FAIR and comprises the following indices:

The ***Instream Habitat Integrity*** is FAIR to GOOD - the river flows through cattle and game farming land. Water abstraction is limited to a few irrigation areas. Numerous weirs were present in the river prior to the 2000 floods - most of these were destroyed, those remaining have limited take-off. Some overgrazing combined with Sandveld has led to considerable deposition of sand in pools and on bends. Flow variability is still good. The ***Riparian Zone Habitat Integrity*** is POOR mainly due to bank erosion that was caused by damaged weirs, while some erosion is due to overgrazing. There is some encroachment of terrestrial species into the riparian zone which indicates some flow modification - although vegetation is largely natural and bank structure is generally intact. ***Riparian Vegetation Integrity*** is POOR - Agriculture, resorts and stock have the greatest impact on the riparian vegetation. The Sundays system has a high number of riparian species and in the upper sections the vegetation is good (e.g. the Vingerkraal-se-loop tributary to the Sundays). There is some alien vegetation encroachment by seringa, prickly pear and herbaceous aliens.

The ***Fish Assemblage Integrity*** is FAIR - moderately sensitive species still occur; the lower sections are impacted by water abstraction during low flow periods. ***Macro-invertebrate Integrity*** is POOR - the upper sections of this river reach are generally in good condition, it is the lower reaches where reduced water quality and habitat alteration impact on invertebrate diversity.

Water Quality is FAIR, flows have between low and intermediate levels of nutrients and are free from significant organic pollution - impacts are primarily due to agricultural return flows.

Ecological Importance and Sensitivity (EI&S)

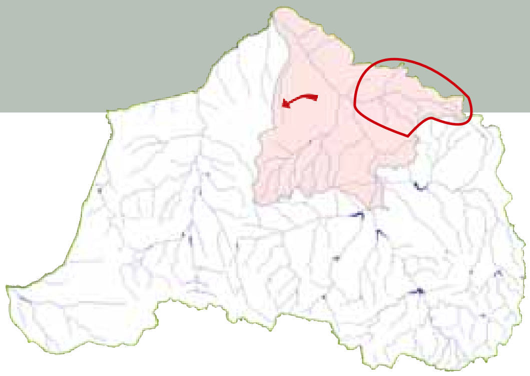
The EI&S is MODERATE - diversity of species is moderately high, influenced by the location of the river in a transition zone between Waterberg and Bushveld. There are many interconnecting tributaries which provide a diverse range of habitat and refugia for many species. The Marakele Reserve and numerous game farms offer protection of natural areas.

Drivers of Change

- Alien vegetation encroachment in the riparian zone
- Flow modifying structures - damaged weirs

Management Responses

- Clear alien vegetation from riparian zone
- Ensure that an EIA is undertaken if the repair of any of the damaged weirs is being considered
- Monitor agricultural return flows
- Determine natural flow regime of river and the ecological reserve
- Minimise overgrazing to reduce sediment input into the river



The Importance of the Sundays River to the Lower Crocodile River

Cumulative impacts arising from the upper and middle Crocodile River catchments have seriously affected the flow regime of the lower Crocodile River. In most years, the river stops flowing and the diversity of aquatic fauna is declining. The river is also seriously fragmented by the placement of dams and weirs and most of these structures are barriers to the free movement and migration of fish species.

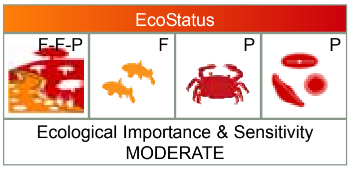
The Sundays River and its Sand River tributary, rise in the Waterberg Mountains and join the Crocodile River a short distance upstream from Thabazimbi and the Ben Alberts Nature Reserve. For most of its length, the river flows through game and cattle country. The foothill zone of the Sundays River also boasts a number of wetlands. There are very few weirs along the river and there is good connectivity between the Crocodile River and the Waterberg. These factors result in the Sundays River to be in a markedly better ecological condition than the remainder of the lower Crocodile River catchment.

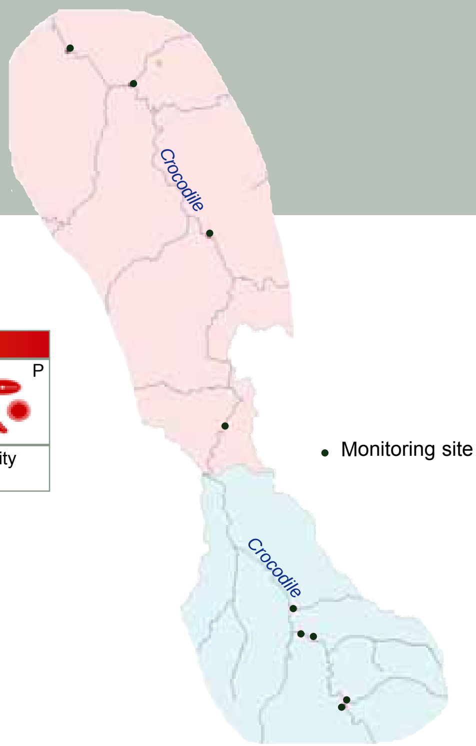
It is therefore apparent, that the Sundays River is both acting as a refuge for the more sensitive biota of the lower Crocodile River, while also providing a near permanent supply of relatively clean water to the lower river. This rejuvenation of flow is viewed as an important factor in maintaining the status of the Crocodile River in the Thabazimbi area and for maintaining the river in the Ben Alberts Reserve.

It is strongly recommended that no developments should be considered in this catchment without a detailed study of the environmental implications.

Sundays River, tributary to the lower Crocodile River

MIDDLE CROCODILE





EcoStatus

The overall EcoStatus for this study unit is FAIR / POOR and comprises the following indices:

Instream Habitat Integrity is FAIR - the Hartbeespoort Dam releases water into canals and the river for irrigation purposes. Releases from the Roodekopjes Dam are also fairly constant therefore the river flows throughout the year. Excess water seeps into sand aquifers from which it is abstracted. This excess base flow impacts on the natural flow and flood regimes. The ***Riparian Zone Habitat Integrity*** is FAIR with some localised areas of bank erosion in the Atlanta area. Higher than normal base flows stimulate the growth of riparian vegetation. The ***Riparian Vegetation Integrity*** is POOR to FAIR with some land cleared for irrigation of agricultural fields. Alien vegetation is widespread - seringa, sesbania, weeping willow and Spanish reed are the most common species.

The ***Fish Assemblage Integrity*** is FAIR to POOR with sensitive species such as *Chiloglanis spp.* (rock catlet or suckermouth) still present due to increased base flows and presence of rapids and riffles where dissolved oxygen concentrations are high. Reduced water quality due to urban return flows, impoundments and industrial discharges is impacting on fish diversity. Eels are lost from the system due to migration obstructions. ***Macro-invertebrate Integrity*** is POOR - eutrophication causes algal growth on hard surfaces in rapids and riffles leading to loss of suitable habitat.

Water Quality is POOR - flows have between low and intermediate levels of nutrients with some evidence of organic pollution. Sources of pollution are primarily urban and industrial diffuse source return flows.

Ecological Importance and Sensitivity (EI&S)

EI&S is MODERATE - habitat diversity is low primarily because the landscape has lost the various floodplain geomorphic features. Roodekopjes Dam has some surrounding wetland habitat which attracts water birds, however very little natural area is formally protected. Some sections of the river are still fine - the value of the natural environment as a legitimate land-use still needs to be recognised.

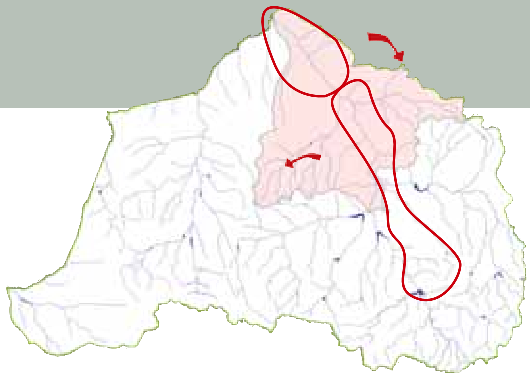
Drivers of Change

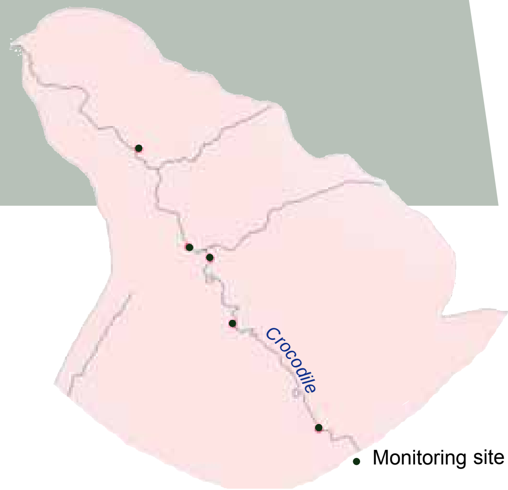
- Impoundments altering natural flow regime
- Reduced water quality - eutrophication of water
- Invasive aliens encroaching on riparian zone

Management Responses

- Control snowball effect of development - monitor land-use changes
- Enforce compliance of water quality standards
- Clear alien vegetation in riparian zone and in catchment
- Rehabilitate riparian habitat to restore the river's ability to provide ecosystem services such as flood attenuation and sediment trapping

Lower Crocodile





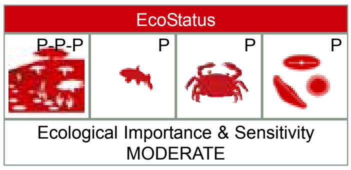
EcoStatus

The overall EcoStatus for this study unit is POOR and comprises the following indices:

The ***Instream Habitat Integrity*** is POOR - there is extensive irrigation and multiple abstraction points along this reach of river which has a severe impact on river functioning. Flows are regulated through a series of weirs and dams resulting in unseasonal releases (to maintain irrigation) which leads to undercutting of river banks and increased sedimentation.
Riparian Zone Habitat Integrity is POOR - the large number of dams in this region and upstream are causing a loss in flow variability. Low flows are depositing fine sediments in pools and on bends. A lack of high flow events is resulting in reed encroachment and the encroachment of terrestrial vegetation on flood benches.
Riparian Vegetation Integrity is POOR - riparian vegetation has been cleared in many areas for agriculture and the setting up of pumps using the water from the river. A number of game farms along the river protect certain sections of the riparian vegetation. Syringa and castor-oil plants are the main alien species threatening this section of the river as they are found in large numbers. The area of the Crocodile River near the Kilpspruit confluence has high levels of agriculture which has degraded the riparian zone in many areas.

Fish Assemblage Integrity is POOR - only hardy species are present, loss of habitat and connectivity of the river has resulted in stress conditions for most fish species.
Macro-invertebrate Integrity is POOR - reduced water quality and diminished flows are leading to dry sections and isolated pools. This reduction in suitable habitat has a severe impact on invertebrate diversity.

Water Quality is POOR - flows have between low and intermediate levels of nutrients and the sites sampled are heavily contaminated with organic pollution. Low scores can be attributed to high agricultural return flows.



Ecological Importance and Sensitivity (EI&S)

EI&S is MODERATE - this is a low gradient river therefore instream habitat diversity is naturally low, however a large number of pools and weir backwaters provide refuge for a variety of species including crocodiles, hippos and otters. This river reach provides a corridor from the Limpopo to the Bushveld which is very important for the migration of birds and animals. Private game farms offer some degree of protection of the surrounding natural landscape.

Drivers of Change

- Extensive water use for agricultural purposes - abstraction for irrigation impacts on natural flow regime of the river
- Dams and weirs act as barriers to flow and the migration of fauna
- Reduced water quality due to agricultural return flows

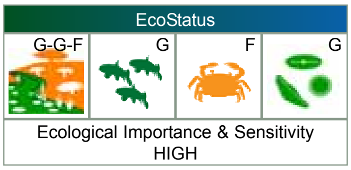
Management Responses

- Control water use and manage water abstraction for irrigation purposes
- Determine environmental flow requirements and implement the ecological reserve
- Monitor and control agricultural return flows
- Stabilise bank erosion
- Clear alien invasives from the riparian zone

GROOT MARICO

Marico

SUB-MANAGEMENT AREA



EcoStatus

The overall EcoStatus for this study unit is GOOD / NATURAL and comprises the following indices:

Instream Habitat Integrity is GOOD but is affected by agricultural return flows and water abstraction. There are a number of farm dams and old furrows adjacent to and within the river channel as well as some development upstream of the Marico-Bosveld Dam. Overall the ***Riparian Zone Habitat Integrity*** is GOOD. There is minimal impact on the riparian zone due to existing flow regulating structures and no increasing trend of land clearing for agriculture in the riparian zone.
The ***Riparian Vegetation Integrity*** is FAIR. This is primarily because of the presence of alien vegetation such as various species of wattle, blue gum, seringa and Spanish reed.

Fish Assemblage Integrity is GOOD to NATURAL. The tributaries feeding into the Groot Marico are fairly clear of sediment and several sensitive species were found, these include *Amphilius sp.* (stargazer mountain catfish), *Chiloglanis sp.* (rock catlet or sucker-mouth), *Labeobarbus marequensis* (large-scale yellowfish) and *Labeobarbus polylepis* (small-scale yellowfish).
The ***Macro-invertebrate Integrity*** is FAIR, this is mainly due to localised poor water quality and habitat alteration.

Water Quality is GOOD. The nitrogen and phosphate levels were classified as between low and intermediate and according to the percentage of species tolerant to organic pollution the water was considered free from significant organic pollution.

Ecological Importance and Sensitivity (EI&S)

EI&S is HIGH, development in this region is low therefore natural vegetation predominates. This reach of the river is perennial therefore it provides refugia for a number of species, however the Marico-Bosveld Dam and other weirs prevent the migration of certain fish species and eels upstream. There are a number of wetlands above the dam along the tributaries feeding into the Marico. The Marico-Bosveld Nature Reserve conserves the area surrounding the dam. The *Tilapia sparmanii* (banded tilapia) in the Marico Eye are genetically unique and unique invertebrate species also occur here.

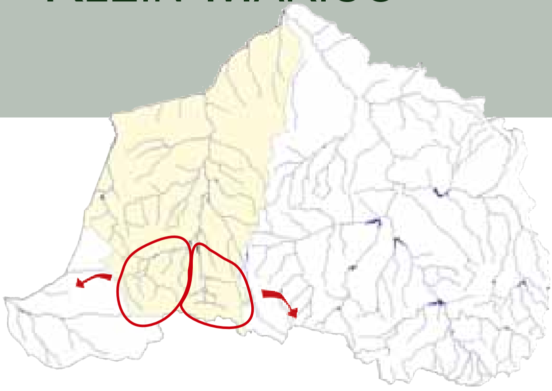
Drivers of Change

- Flow regulating structures - dams and weirs - especially the Marico-Bosveld Dam
- Water abstraction for irrigation
- Shale and slasto mining on the Highveld along some tributaries
- Recreational activities (diving and camping) in the source area
- Alien fish (Bass) in the upper reaches of the Kaaloog-se-loop

Management Responses

- Clear alien vegetation in riparian zones
- Consider installing fish ladders and eelways in suitable flow regulating structures
- Identify and map wetlands that occur for potential future protection
- Stabilise areas of local erosion
- Monitor agricultural return flows to ensure reduced non-point source pollution
- Monitor recreational activities and implement control measures
- Control alien fish
- Ensure that the ecological reserve is determined and maintained.

Klein Marico



EcoStatus			
F-G-F	P	P	F
Ecological Importance & Sensitivity MARGINAL / LOW			

EcoStatus

The overall EcoStatus for this study unit is FAIR and comprises the following indices:

Instream Habitat Integrity is FAIR, this is primarily due to the presence of the Klein-Maricopoort and Kromellenboog dams. Both dams impact on the levels of water in the river and natural sedimentation patterns. Above the Klein-Maricopoort Dam habitat integrity is less impacted. The ***Riparian Zone Habitat Integrity*** is GOOD primarily because of the low levels of development in the area. At Oopgenoeg and Nahoek water abstraction has resulted in some wetlands drying up.
The ***Riparian Vegetation Integrity*** is FAIR due to the presence of alien vegetation and the removal of some vegetation for agriculture.

Fish Assemblage Integrity is POOR, only the most hardy of species are present due to reduced flows and localised poor water quality.
The ***Macro-invertebrate Integrity*** is POOR due to the impact of the dams on water flow but primarily due to the impacts of reduced water quality especially near the town of Zeerust.

The ***Water Quality*** in general is FAIR - flows have inter-mediate levels of nutrients and there is some evidence of organic pollution.

Ecological Importance and Sensitivity (EI&S)

EI&S is MARGINAL / LOW, overall diversity of habitat types is low. There are however some locally unique areas with note-worthy features such as abundant and often large, Wild Olive trees at Ottoshoop and Molemane Eye Game Reserve. The Molemane dolomitic eye and associated wetland represents a unique, relatively undisturbed wetland ecosystem and is rich in invertebrate species with some unique and isolated fish populations.

Drivers of Change

- Water abstraction
- Return flows from urban runoff at Zeerust
- Impoundment of river altering natural flow regimes
- Sedimentation of Kromellenboog Dam
- Alien fish (Bass) in the upper reaches of the Molemaneloop

Management Responses

- Identify sources of urban runoff that impacts on water quality
- Clear alien vegetation from riparian zone
- Ensure that the ecological reserve is determined and maintained
- Map and monitor wetlands to ensure future ecological functioning
- Control alien fish

Herman Charles Bosman

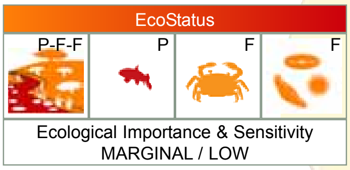
Herman Charles Bosman is regarded as one of South Africa's pre-eminent writers of short stories. His work has left a treasury of stories, essays, chronicles and poems, mostly woven around the follies, idiosyncrasies, humaneness and nobility of the human spirit. Bosman was born in 1905 at Kuilsrivier, near Cape Town. Shortly afterwards his family moved to Johannesburg where he was educated. On receiving his degree, Bosman was appointed to a teaching post in the Groot Marico district. A most fruitful year, for the place and the people enthralled him. They provided him with the background for his best-known works, the Oom Schalk Lourens and Voorkamer sketches, in which he managed to capture the Great Marico of the 1930s and 1940s in a timeless air of nostalgia.

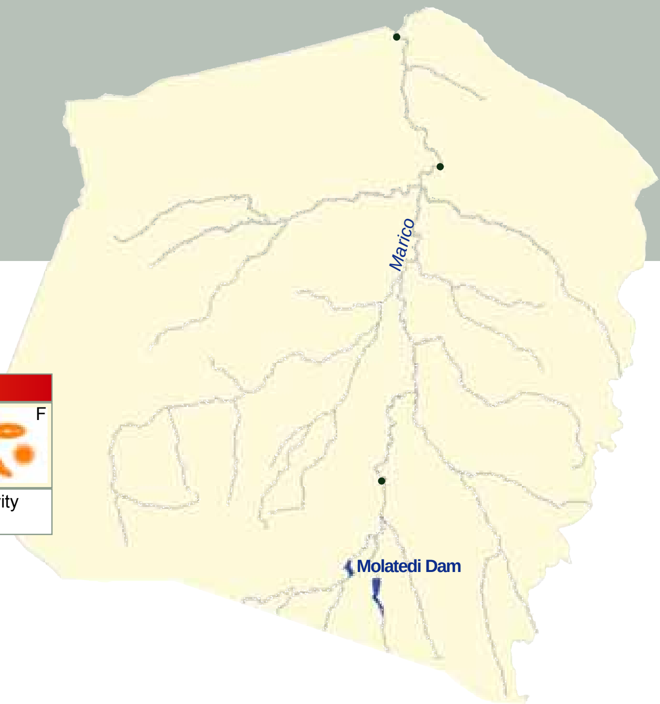
(Source: The Herman Charles Bosman Literary Society; http://www.marico.co.za/ accessed 4/1/2005)

Middle Marico

Marico

Sub-Management Area





EcoStatus

The overall EcoStatus for this study unit is FAIR / POOR and comprises the following indices:

Instream Habitat Integrity is POOR - the demand for water exceeds supply. Water abstraction for irrigation is high resulting in serious flow modification, resulting in some cases in tributaries becoming dry. The impoundments Kromellenboog, Marico Bosveld and Molatedi dams impact on the natural flow regime of the river. The ***Riparian Zone Habitat Integrity*** is FAIR - water abstraction has resulted in vegetation in the riparian zone tending towards a terrestrial nature because of the drying up of the river. This has also resulted in some areas experiencing heavy erosion which is exacerbated by overgrazing. The ***Riparian Vegetation Integrity*** is FAIR to GOOD with some clearing of riparian vegetation for maize fields.

Fish Assemblage Integrity is POOR - considerably lowered frequency of occurrence of species was encountered, primarily due to water obstructions and abstractions. Only hardy species were naturally present. ***Macro-invertebrate Integrity*** is FAIR to POOR primarily because of flow modification and reduced water quality from agricultural return flows.

Water Quality is FAIR - flows have between low and intermediate levels of nutrients and the percentage of species tolerant to organic pollution indicates flows are free from significant organic pollution.

Ecological Importance and Sensitivity (EI&S)

EI&S is MARGINAL / LOW - species and habitat diversity is low, the three dams impact on the river's flow regime and thus on the riparian ecosystems. There are some game farms below Molatedi Dam and the Madikwe Nature Reserve conserving some natural habitat in the area.

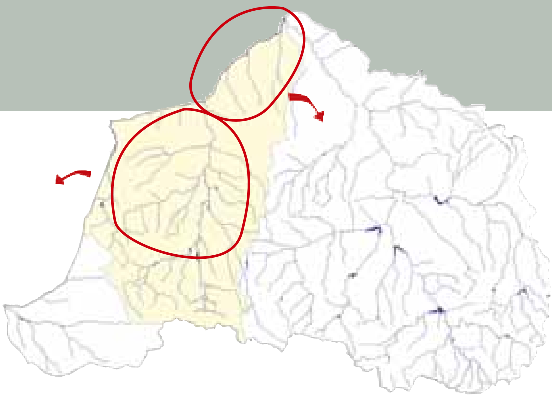
Drivers of Change

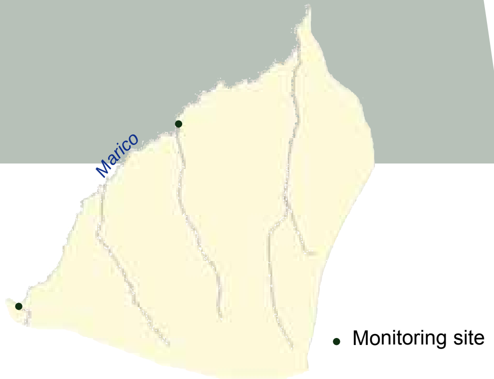
- Water demand exceeding supply
- Impoundments altering natural flow regimes
- Irrigated commercial farmland and water abstraction
- Overgrazing

Management Responses

- Establish environmental flow requirements
- Determine measures to facilitate a more favourable water balance (demand vs. supply)
- Investigate dam releases that simulate natural flow patterns
- Establish erosion rehabilitation programmes
- Control overgrazing and infestation by sickle bush

Lower Marico





EcoStatus			
P-F-G	P	No Data	No Data
Ecological Importance & Sensitivity MODERATE			

EcoStatus

The overall EcoStatus for this study unit is FAIR and comprises the following indices:

The **Instream Habitat Integrity** is POOR, this is primarily because of flow releases from the Molatedi Dam. Water is released every 4 to 6 weeks into some weirs for irrigation and supply to Botswana. The surrounding area is quite flat and this results in the inundation of large areas covering shallow instream habitats. Therefore although the releases are beneficial to riparian vegetation, they are having a detrimental effect on aquatic biota. The ***Riparian Zone Habitat Integrity*** is FAIR - At Molatedi Dam there is a large variety of vegetation, which is abundant due to the mixed bedrock streambed. The vegetation is highly impacted by the lack of water downstream of Molatedi. Although there are many old established trees, the extent of riparian vegetation cover is low, ground cover and indigenous riparian tree species recruitment is low. The flow regulation in this area can potentially impact on the extent of the riparian zone and the riparian species present. The large thorn apple, Kariba weed and the large cock-lebur are the alien plant species found in this area. The ***Riparian Vegetation Integrity*** is GOOD - the vegetation is in good condition because on the South African side of the border fence there is a foot and mouth fence which protects the riparian zone. Cultivation adjacent to the river is limited due to steep riverbanks.

The ***Fish Assemblage Integrity*** is POOR - frequency of occurrence of species is low due to reduced flows. Mostly hardy species are present. ***Macro-invertebrate Integrity*** - there are no SASS scores for this reach of river.

Water Quality - there was no diatom data sampled in this reach of river therefore no score is available; however water quality is reduced because of irrigation return flows.

Ecological Importance and Sensitivity (EI&S)

EI&S is MODERATE - the surrounding landscape is dry therefore the riparian zone offers refugia for many species of amphibians and birds including the white-backed vulture. The area comprises mostly game farms, while on the Botswana side communal farming and hunting game farms are common.

Drivers of Change

- Irrigated commercial agriculture - farmers request water from dams when levels in weirs are low

Management Responses

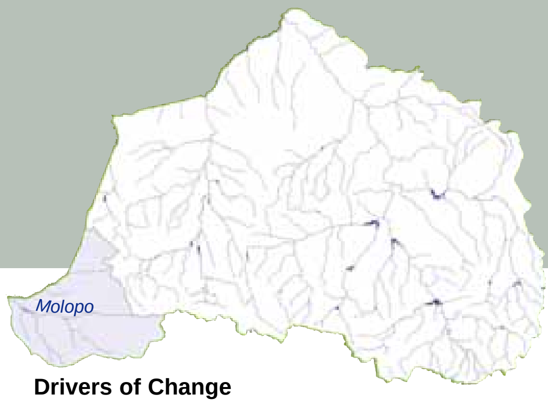
- The riverbed downstream of Molatedi Dam is dry and the rapid release of water would lead to erosion. Natural flow should be simulated by regulated releases

The Lower Crocodile and Marico fish and aquatic insect life

In both the Lower Crocodile and Marico rivers, flows are largely managed on demand for irrigation purposes. This results in unseasonally high pulses of flow in the river and extended periods of low flow. The managed flow regime, when combined with the large numbers of dams and weirs, has resulted in river habitats becoming severely fragmented with what were largely perennial rivers now being distinctly seasonal in nature. For extended periods, weirs and deep pools are the only refuge for any aquatic life. The fish and invertebrates that still occur in the river are very tolerant species that can survive the impacts of water regulation and pollution.

While it is recognised that these rivers have been in a largely modified condition for many years, it is suspected that the status of the lower catchment could still be declining. Through improved management this downward trend can be mitigated.

Upper Molopo



Upper Molopo
Sub-Management Area

No bio-monitoring took place in the Upper Molopo sub-management area in this 2004 survey. It is the intention of subsequent surveys that the scope of biomonitoring be broadened to include this area. The information presented in this section is based on previous, unrelated surveys and provides some interesting background information on the area.

Drivers of Change

- Dams in the river
- Poor sanitation and sewage return flows
- Mining/cement industries in the catchment
- Insufficient storm water systems
- Erosion due to over grazing
- Lack of solid waste management

Unique biota	Major threats	Surrounding land-use
Fish The cichlid fish (*Tilapia sparmanii*) is genetically and morphologically distinct from other known conspecific populations and reclassification as a new species is currently under investigation. Insecta Three new mayfly (Ephemeroptera) distribution records One new dragonfly (Odonata) species Two new caddisfly (Tricoptera) species Crustacea Four new seed shrimp (Ostracods) distribution records and one new species	Habitat loss due to weir construction and water abstraction. The ecological reserve still has to be determined. Wetlands do not receive enough water flow due to abstraction for domestic use in Mafikeng. This is a direct threat to the shortfin barb population. Pesticide and herbicide use for red-billed quelea *(Quelea quelea)* control threatens the water quality and reed habitat. Alien bass (*Micropterus salmoides*) predate on indigenous fish and invertebrate species. The bass have already restricted the distribution of the shortfin barb to the reed areas downstream of the eye. Construction of roads and presence of weirs leads to genetic isolation of biota. Water quality threats include leaching from septic tanks and agricultural chemicals.	Area around the eye is used as holiday accommodation (100 houses, used since 1900's) and is part of a conservancy. Recreational use is limited to non-motorized activities. Major land-use in the area is agriculture, mostly cattle.

The unique dolomitic eyes of the Crocodile (West) Marico Water Management Area

Dolomitic eyes are water bodies fed by groundwater originating from fractures in the underlying dolomite. The fractures and intrusions of geological formations impenetrable to water in the dolomite form aquifers, dolomite compartments and dolomitic eyes. Aquifers are subterranean waterways/ tunnels and reservoirs from which water is forced above ground through openings (fractures), which are called dolomitic eyes or springs.

The dolomitic area covers approximately 4022 km^2 of the North West Province and forms the main catchment of the east-flowing Limpopo River system and the west-flowing Molopo River. The interdependence of ground and surface water is apparent in the ecology of the dolomitic eyes. These eyes are influenced by the water quality and quantity of both the surface water and the groundwater.

The sources of the Molopo, Molemane and Marico rivers are unique dolomitic eyes (springs) and associated wetland systems. These dolomitic eyes are of great conservation significance as they are biologically unique. One of the main contributing factors to the unique resident ecological communities is the geographical isolation at surface water level. Eyes have been isolated for millennia, allowing for speciation through genetic mutations and adaptation to localized environmental conditions. The groundwater linkages in the aquifer systems contribute to the sensitivity of the dolomitic eyes as groundwater abstraction kilometers away could reduce the water levels at the dolomitic eyes.

The J.L.B Smith Institute of Ichthyology was contracted by the Department of Nature and Environmental Conservation to study the dolomitic ecosystems in the Western Transvaal. The table above highlights some of the results of this study that emphasized the biological diversity and major threats to the dolomitic eyes (Skelton et al. 1994).

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CONTRIBUTORS

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Appendix B

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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PRIMARY RESEARCH PAPER

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

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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.

Keywords Diatoms · Indices · Water quality · Biomonitoring

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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 receiving water bodies by the addition of chemical compounds, colloidal material and suspended solids, often resulting in changes in salinity, nutrient status and turbidity. 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 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 well-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; 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 biomonitoring 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 (98%) of benthic diatoms present in the heavily impacted Vaal River 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 topic 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* (*Achnanthidium*), *A. standeri* was described by Cholnoky, and is found in abundance in certain mountain streams (Cholnoky, 1957). The occurrence of these endemic species 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

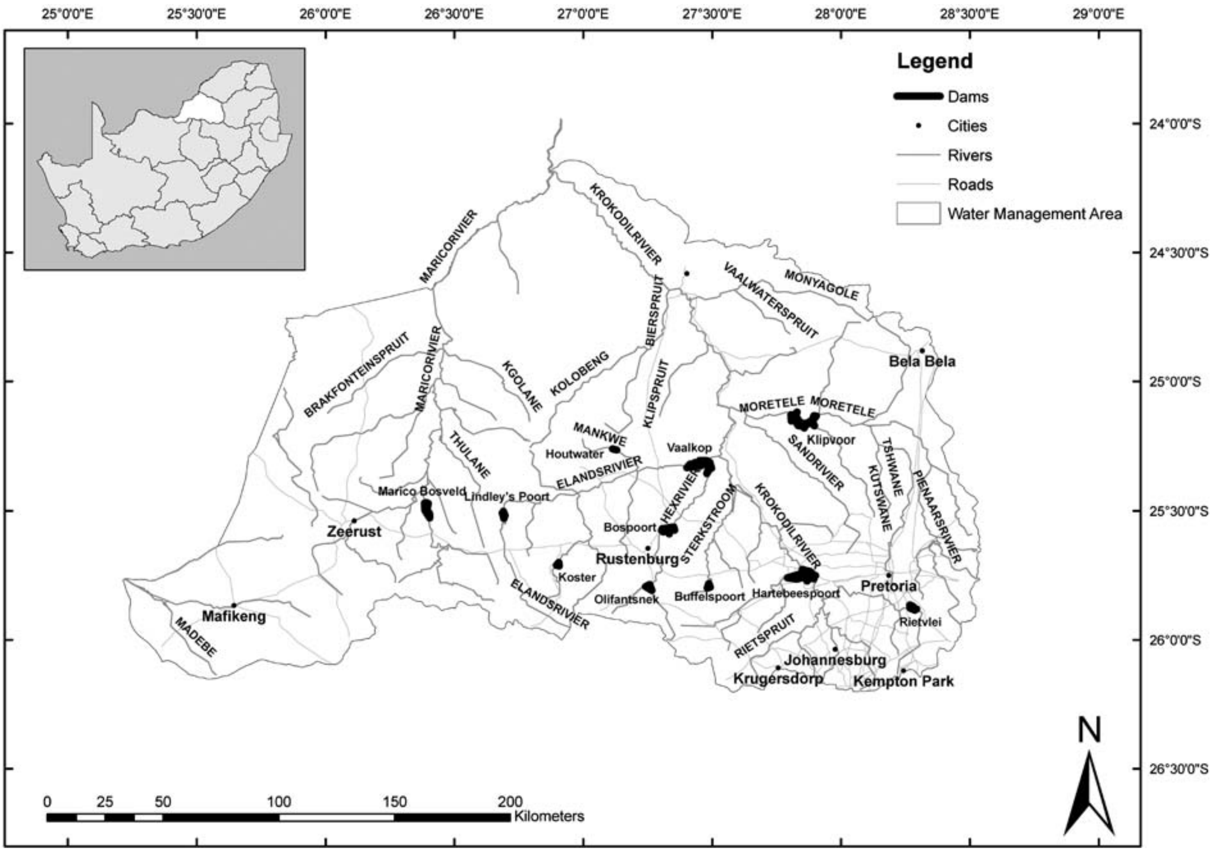

Fig. 1 Map showing location of some of the larger rivers in the Crocodile West and Marico water management area

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 (1986–1991) was used for identification. Other taxonomic guides consulted include Schoeman & Archibald (1976–1980), 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 & 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 1.

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 Fig. 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 3). Measured values (mean, median, minimum and maximum) for the physical and chemical variables (n = 50) in the Crocodile West and Marico WMA are presented in Table 4. 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 Fig. 2 may be found in Table 1.

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

Table 1 Acronyms used in the CCA biplot (Fig. 2)

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

Table 2 Pearson correlation coefficients between measured environmental variables and diatom index scores generated for sites in the Crocodile West and Marico WMA

	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[a]	0.45	0.43	0.59	0.50	0.50	· · ·	0.45	0.61	· · ·	0.39	· · ·	0.57

Numerical values indicate significant correlations at $P < 0.05$, $n = 50$ (casewise deletion of missing data), while (· · ·) indicates no significant correlation. 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

[a] $n = 40$

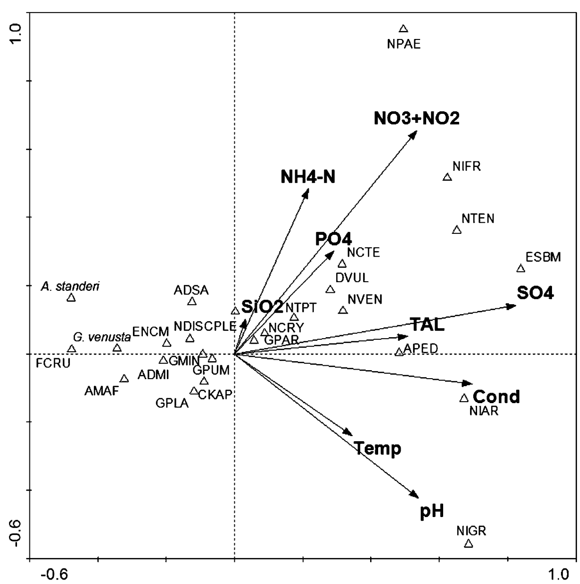

Fig. 2 CCA biplot showing the relationship between measured environmental variables and some diatom species in the Crocodile West and Marico water management area. Species with a weight range of 1–100% are shown. Acronyms are presented in Table 1

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 (Fig. 2) was used to demonstrate that certain widely distributed taxa have similar ecological characteristics in widely separated geographic areas. It is clear from Fig. 2 that those species commonly associated with poor water quality in Europe e.g. *Eolimna subminuscula* Lange-Bertalot, *Nitzschia frustulum* (Kützing) Grunow ordinate on the right hand side of the diagram together with elevated levels of water quality variables. Another typical example is *Diatoma vulgaris* Bory, which commonly occurs worldwide in freshwaters with elevated levels of

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

phosphate-phosphorus and is closely associated with the vector for this variable in Fig. 2. Taxa typical of cleaner, less impacted waters ordinate out on the left hand side of the diagram e.g. *Achananthidium minutissimum*, *Achnanthidium affine* (Grunow) Czarnecki, *Encyonopsis microcephala* (Grunow) Krammer and *Fragilaria capucina* var. *rumpens* (Kützing) 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 Fig. 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

Table 4 Summary table of measured chemical and physical environmental variables in the Crocodile West and Marico WMA during the study period (n = 50)

	Unit	Mean	SE	Median	SD	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

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* (Kützing) 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. pyrenaicum* 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.

(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 lead then 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 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 unique South African diatom index.

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Appendix C

On the use of diatom-based biological monitoring.
Part 1: A comparison of the response of diversity and autecological diatom indices to water quality variables in the Marico-Molopo River catchment

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On the use of diatom-based biological monitoring Part 1: A comparison of the response of diversity and aut-ecological diatom indices to water quality variables in the Marico-Molopo River catchment

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Abstract

Two main approaches have been followed in using diatoms as bio-indicators in the past few decades namely species diversity indices and aut-ecological indices. This study, based on 102 water quality and epilithic diatom samples from the Crocodile Groot-Marico catchment in South Africa, evaluated both types of indices by establishing how well they reflect changes in water quality. It was found that less of the variation in diversity indices could be attributed to changes in water quality variables than was the case for the aut-ecological indices. Furthermore it was found that species diversity indices tend to be higher at intermediate levels of pollution, rather than at low levels of pollution.

Keywords: diatoms; Bacillariophyceae; bioindicators; species diversity indices; water quality; aut-ecological indices

Introduction

Southern Africa is a subcontinent notorious for its unpredictable rainfall. South Africa is a semi-arid country, and the decline in the quality of available water is one of the biggest problems currently facing the country (Davies and Day, 1998). For this reason the integrated management of water resources has enjoyed high priority in the National Water Act as well as in research and management actions by both government and non-government organisations.

Walmsley et al. (2000) state that indicators are an ideal means by which progress towards integrated water resource management can be monitored, in that they provide a summary of conditions, rather like temperature and blood pressure are used to measure human health. Using the same analogy, it is important to be able to distinguish between indicators which can truly be linked to health and are not just features of the system which do not have relevance to the question posed. For the aforementioned reason it is important to be able to test indicators quantitatively in order to assess how closely they can be linked to the health of aquatic ecosystems, in the case of water resource management, and/or water quality. Diatoms have been shown to have narrow tolerance ranges for many environmental variables and respond rapidly to environmental change, making them ideal indicators (Reid et al., 1995).

Several kinds of indicators have been proposed over the years to evaluate ecosystem health. Two main groups of indicators using diatom community data will be discussed in this paper namely diversity indices and aut-ecological indices.

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Species diversity/species richness indicators

Ecosystem stability can be defined as the ability of a system to recover to an equilibrium state after disturbance, or simply the persistence of the system (May, 1976). The diversity-stability hypothesis asserts that species vary in their traits and that in a highly diverse (species rich) system, there will be some species than can compensate for the loss of others should disturbance occur in such a system (Pimm, 1984; Elton, 1958). Thus, species rich systems are more likely to be considered stable. Another common view is that this theory predicts a decrease of diversity as pollution increases. The pollution intolerant species decline in abundance and the pollution tolerant species can grow rapidly without competition for space, nutrients, or other resources. This results in community abundance patterns of heavy dominance and fewer species (Van Dam, 1982).

It is on the basis of the two abovementioned hypotheses that species diversity indices enjoy widespread use in ecology and, more specifically, aquatic ecology. Diversity indices are related to community structure and are not specific to any type of contamination. Species diversity indices consist mainly of three measures namely: species richness (related to the number of species), the evenness (how evenly the individuals are distributed between the species) and a combined measure called the diversity index such as the Shannon Diversity Index (Shannon and Weaver, 1949).

Species diversity indices based on benthic diatom assemblages are regularly used in the study of water resources. In such instances these indices are mainly used to determine the impact of certain actions and pollutants on aquatic systems (Cunningham et al., 2003; Gómez 1999; Gracia-Criado et al., 1999). An alternative to species diversity/species richness indicators are aut-ecological indices.

Aut-ecological Indices

Aut-ecological indices use the relative abundance of species in assemblages, their ecological preferences, sensitivities, or tolerances to infer environmental conditions in an ecosystem (Stoermer and Smol, 1999). Put in another way, aut-ecological indices make use of the niche requirements and habitat preferences of the individual species or higher taxonomic groupings. In such indices long-term data gathered about the tolerances of a species are used to compile an index which can, in turn, be used to deduce environmental conditions from the species composition by taking into account the specific tolerances of the species in the community surveyed. These indices can be constructed to measure specific pollutants or general environmental conditions. A number of diatom-based aut-ecological indices are based on the weighted average equation of Zelinka and Marvan (1961) and have the basic form:

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

where:

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

The method mainly utilises the distribution of species along a water quality gradient in terms of sensitivity to pollution as well as broadness of the species distribution along the water quality gradient. This equation is used in many diatom-based indices including the 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 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 and Maquet, 1987), etc. Such indices mainly vary in terms of species included in the calculation and the tolerances assigned to such species.

Diatom indices constructed by this approach have been tested with success in South Africa (Taylor et al., 2007; De la Rey et al., 2004) and in many countries in Europe and the rest of the world (Sabater, 2000; Kelly, 1998; Reavie et al., 1995; Zeeb et al., 1994; Hall and Smol, 1992).

Rationale

This research paper consists of two parts. This paper (Part 1) tests the performance of two approaches to the use of diatoms as bio-indicators namely diversity indices and aut-ecological indices. The second part of this paper (Part 2) (De la Rey, 2008) compares the performance of diatom-based indices with that of a macro-invertebrate index (SASS 5) in terms their ability to indicate changes in water quality variables.

The purpose of Part 1 is to compare diversity indices and aut-ecological indices as measures of aquatic ecosystem health by comparing their response to water quality variables. This will enable an informed decision as to which of the two approaches will be most appropriate when using diatoms as bio-indicators in river and stream ecosystems in South Africa.

Since water quality is one of the main environmental factors affecting the ecology in rivers and streams, it can be used as a measure of the applicability of indicators for integrated water resource management. Diatoms are appropriate for the purpose of this study as they provide interpretable indications of specific changes in water quality (McCormack and Cairns, 1994).

Materials and methods

Sampling localities

Thirty three sites were identified in the Groot Marico and Molopo River systems for the study (Fig. 1). The identified sites form part of the River Health Program of the North West Province, South Africa. Samples were collected in April, June, September and November 2005 at the sites, as water levels permitted (at times certain samples could not be collected due to low water levels), resulting in a total of 102 separate data points with diatom data and same-day water quality data. These sites were chosen to represent a wide range of water quality (see water quality summary in Table 1) as well as the wide range of diatom index scores recorded (SPI: 1.3-19.7 out of a possible 20).

The Marico River originates south of Groot Marico town and feeds into the Limpopo River in the north. The origin of the Marico River falls within the Groot Marico dolomitic aquifer compartment. The two primary sources of this river are the Grootfontein dolomitic eye, that gives rise to Kaaloog-se-loop and an eye on the farm Renosterhoek 343JP that feeds the Rietspruit. Secondary sources include Draaifontein tributary that is of a mixed dolomitic nature and the Sterkstroom tributary that is fed by springs of a non-dolomitic nature. Other tributaries of the Marico River include the Klein Marico River. This tributary is seasonal and is thus not a major flow contributor to the Marico River. The 'Molemane-se-Loop' is of a dolomitic nature originating in the Molemane Nature Reserve. Major dams in this sub-catchment include the Marico-Bosveld Dam in the upper catchment and the Molatedi Dam further downstream. The upper reaches of this sub-catchment are not densely populated (RHP, 2005).

The Molopo River originates east of Mafikeng and feeds into the Vaal River in the south west. The origin of the Molopo River falls within the Groot Marico dolomitic aquifer compartment. The primary source of this river is the Molopo dolomitic eye. Major dams in this sub-catchment include Cooke's Lake, Montshioa Dam, Lotlamoreng, Modimolo Dam (Setumo) and the main dam in the system is the Disaneng Dam close to the Botswana Border.

TABLE 1
Descriptive statistics of water quality variables

	Mean	Median	Min	Max	Standard deviation
Ca (mg/ℓ)	32.18	30.06	1.49	82.25	23.78
Cl (mg/ℓ)	14.98	4.99	2.00	145.57	24.12
F (mg/ℓ)	0.18	0.15	0.05	0.61	0.14
K (mg/ℓ)	2.17	0.73	0.15	11.28	2.91
Mg (mg/ℓ)	24.27	18.52	1.02	65.79	18.52
NH_4 (mg/ℓ)	0.68	0.02	0.02	16.78	2.43
NO_3+NO_2	0.38	0.09	0.04	7.52	1.02
Na (mg/ℓ)	11.91	3.45	1.00	109.31	18.05
PO_4 (mg/ℓ)	0.21	0.02	0.01	4.90	0.70
SO_4 (mg/ℓ)	15.18	5.70	2.00	113.27	20.22
Si (mg/ℓ)	6.26	5.94	1.27	11.20	2.16
pH	8.16	8.23	7.31	8.66	0.30
DO (mg/ℓ)	6.55	6.94	1.81	11.48	2.49
Temperature (°C)	17.98	18.60	7.70	29.52	4.18
Turbidity (NTU)	12.49	6.77	0.00	75.10	14.34
EC (mS/m)	39.21	31.10	2.66	113.70	28.57
TAL (mg/ℓ)	168.73	146.83	4.00	497.00	121.00

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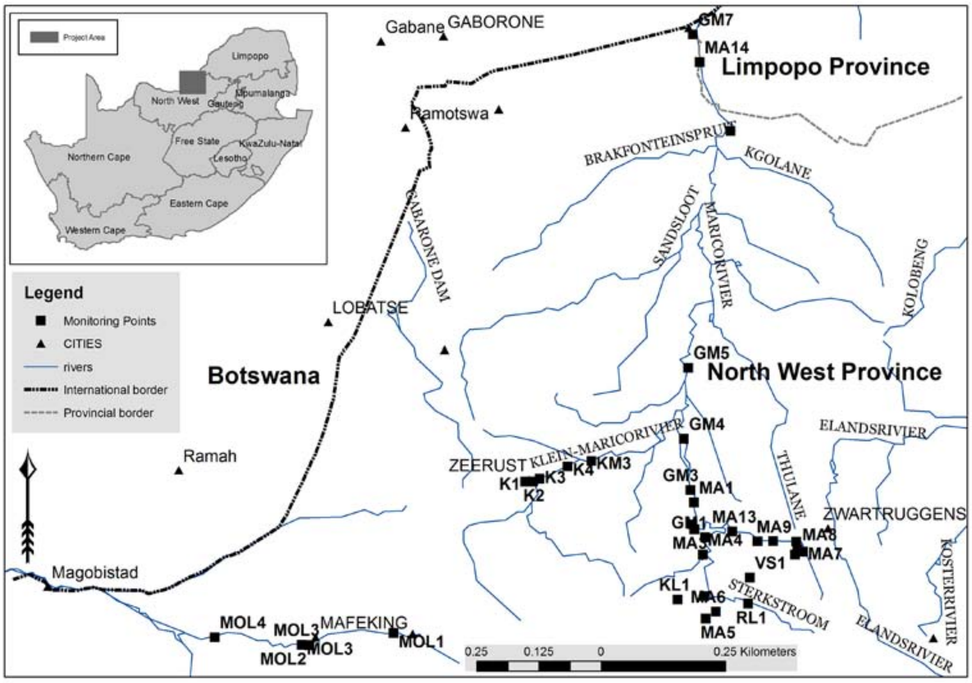

Figure 1
The Groot Marico and Molopo River systems (North West Province, South Africa) showing the location of the study area

From the results in the study, it is clear that the water quality deteriorates downstream, especially as it flows through the towns of Groot Marico, Zeerust and Mafikeng. Downstream water quality impacts may also occur from impoundments (dams and weirs) as well as from agricultural activities.

Indices

Diatoms were collected, prepared and enumerated according to the protocol as set out in Taylor et al. (2005). Only epilithic diatoms were sampled for the study due to the fact that this is the community of preference for the most diatom indices as well as being the community that yields the best coefficients when multiple regressions are performed with water quality variables (Hodgkiss and Law, 1985). Diatom identification was carried out according to the nomenclature of Krammer and Lange Bertalot (1986-1991) and the SPI, BDI, SPI was chosen as it has the broadest species base; BDI showed the best overall correlation to water quality variables in studies performed recently on the Vaal River (Taylor et al., 2007).

Species diversity index, species evenness and number of species were calculated using the OMNIDIA software package (Lecointe et al., 1993). For the biodiversity indicator (species diversity, species evenness and number of species) calculations, the same numbers of cells counted for the aut-ecological index calculation were used (400 cells per slide). It is a well-known fact that there is a relationship between diversity indices and sample size (Seber, 1986; Lewins and Joanes, 1984). Since the same numbers of cells were counted on each slide, the data are comparable between samples as well as between indices.

Water quality

Instream water quality measurements for pH, dissolved oxygen (mg/ℓ), electrical conductivity (mS/m) as well as temperature (°C) were done at each locality by means of an YSI 556 MPS Multimeter. Water samples were taken concomitantly with biological samples at every site.

The water sample was preserved by means of an $HgCl_2$ ampoule broken into each sample and delivered to Resource Quality Services (Department of Water Affairs and Forestry, Roodeplaat) for analysis. The following water quality variables formed part of the analysis:

Calcium (mg/ℓ Ca), chloride (mg/ℓ Cl), fluoride (mg/ℓ F), potassium (mg/ℓ K), magnesium (mg/ℓ Mg), ammonia (mg/ℓ NH_4-N), nitrate and nitrite (mg/ℓ NO_3+NO_2), sodium (mg/ℓ Na), ortho-phosphate (mg/ℓ PO_4-P), sulphate (mg/ℓ SO_4), silica (mg/ℓ Si), total alkalinity (mg/ℓ TAL) and turbidity (NTU, nephelometric turbidity units).

Statistical analysis

Multiple regressions and correlation analysis were performed using the STATISTICA software package (Release 7, Stat Soft. Inc., United States of America), while principle component analysis (PCA) was performed using CANOCO for Windows (Version 4.51, Biometris-Plant Research International, The Netherlands). Before analysis, all the data were standardised by subtracting the sample mean and dividing the result by the sample standard deviation (Hair et al., 1998). This was done to ensure that all the variables could contribute equally to the regressions and correlations by removing the scale factor. For the regressions the forward stepwise method was chosen to eliminate variables that do not contribute to the regression. For the purpose of the multiple regressions the Electrical Conductivity and Total Alkalinity were left out of the analysis as these variables contributed to multi-colinearity in the data, due to high correlation with other water quality variables (e.g. Ca, Cl, F, K, Mg, Na, SO_4). The R^2 value was used, instead of the R value, as it is a stricter measure of the predictability of multiple regression.

Results

Diversity indices

Table 2 represents the results for the multiple regression analysis for water quality and diatom species diversity. The R^2 for the regression was 0.215. This shows that approximately 21.5% of the variation in the diatom species diversity could be attributed to the measured water quality variables. The beta values in the table are the regression coefficients while the p-values indicate whether a specific variable contributed statistically significant to the regression. Water quality variables that contributed significantly ($p<0.05$) to the regression were fluoride and the pH of the water. The fact that changes in fluoride and pH concentrations influence diatom community structures have been noted by several authors (Joy and Balakrishnan, 1990; Ares et al., 1983; Lewin, 1962).

TABLE 2
Regression summary for Shannon species diversity with water quality (italicised values significant at p<0.05)

N=102	R^2= 0.215		
	Beta	Std. err. of Beta	p-level
Intercept			*<0.001*
F (mg/ℓ)	***0.385***	***0.131***	***0.004***
pH	***-0.347***	***0.109***	***0.002***
SO_4 (mg/ℓ)	0.175	0.125	0.164
DO (mg/ℓ)	0.101	0.100	0.313

The results for the regression performed to explain species evenness with water quality are provided in Table 3. The R^2 value for prediction significance of water quality for this variable was only slightly lower than for the species diversity at 20%. Once again the significant contributors to the regression were pH and fluoride.

TABLE 3
Regression summary for Pielou species evenness with water quality (italicised values significant at p<0.05)

N=102	R^2= 0.201		
	Beta	Std. err. of Beta	p-level
Intercept			*<0.001*
F(mg/ℓ)	***0.430***	***0.104***	***<0.001***
pH	***-0.211***	***0.105***	***0.048***
PO_4 (mg/ℓ)	0.183	0.093	0.051

The regression performed for the number of diatom species and water quality (Table 4) indicates that considerably more of the variation in the values of this index can be explained by the measured water quality variables. Just over 32% of the variation in the number of species encountered in samples could be attributed to water quality variables and Ca, Mg, Si, and K were the significant contributors in the regression model.

Archibald (1971) stated, in his studies on South African diatom species diversity, that clean water samples displayed diatom diversity scores between that of moderately enriched and polluted samples. This result was supported by findings of Van Dam (1982) by demonstrating that the highest number of diatom species occurred in moorland pools which were moderately affected by acidification as opposed to the low diversity found in very acidic pools. That diversity tends to be high at 'medium water quality' and lower at either 'good' or 'poor' water quality suggests a quadratic relationship of species diversity to disturbance may be more appropriate than a linear one. To test whether this tendency is also followed in the current data set, a quadratic or 2nd degree polynomial regression was fitted on the data. This

TABLE 4
Regression summary for the number of diatom species with water quality (italicised values significant at p<0.05)

N=102	R^2= 0.321		
	Beta	Std. err. of Beta	p-level
Intercept			*<0.001*
pH	-0.239	0.123	0.056
F (mg/ℓ)	0.151	0.219	0.494
Na (mg/ℓ)	-1.462	0.845	0.087
Temperature (°C)	-0.090	0.102	0.379
Ca (mg/ℓ)	***-1.072***	***0.270***	***<0.001***
Mg (mg/ℓ)	***0.802***	***0.310***	***0.011***
Si (mg/ℓ)	***0.319***	***0.149***	***0.036***
SO_4 (mg/ℓ)	0.251	0.174	0.153
K (mg/ℓ)	***0.592***	***0.287***	***0.042***
Turbidity (NTU)	-0.198	0.115	0.089
Cl (mg/ℓ)	0.740	0.735	0.317

was done by the use of, for example Ca as well as $(Ca)^2$ (a quadratic term), as terms in the regression with species diversity indices (see e.g. Hair et al., 1998 or Neter et al., 1985). If the results are better than those for the linear regression it would show that the relationship is therefore rather quadratic than linear. This observation would indicate that the use of diatom species diversity in a strictly linear fashion as tool to evaluate water quality is not optimal.

Tables 5, 6 and 7 present the 2nd degree polynomial regressions that were performed, for species diversity, species evenness and number of species, respectively, with water quality.

As can be seen from the R^2 values for the regression, which includes 2nd degree polynomial terms of water quality variables, much more of the variation in the diversity data was explained than with the linear regressions. The R^2 for species diversity increased from 21.5% to 54.5% and the R^2 for the species evenness increased from 20% to 40% when including the 2nd degree polynomial terms. The number of species showed the least amount of improvement in the R^2 that increased from 32% to 43%.

Aut-ecological/biotic indices

The regression summary for the biotic indices BDI and SPI is presented in Table 8 and Table 9. For both these indices water quality contributed to about 80% of the variation in the data. For the SPI the significant contributors were Na, Si, pH, PO_4, Ca, Cl and SO_4. The significant water quality contributors to the BDI were much the same as for the SPI with the addition of NO_3+NO_2, Mg and F. When 2nd degree polynomial regressions were fitted to the BDI and SPI, the R^2 value for the regressions changed marginally from near 80% to 84% (not shown). The increase in the R^2 value is probably due to a minor degree of "overfitting" due to the addition of variables to the model in the case of the 2nd degree polynomial regression (Hair et al., 1998). This change in R^2 value is small when compared to the changes encountered in the diversity related regressions (see above).

As indices are usually applied in a linear fashion (high value indicating good environmental condition, low values indicating poor environmental conditions), a linear response of index scores to water quality variables is a desirable attribute

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TABLE 5
Polynominal regression summary for Shannon species diversity with water quality (italicised values significant at p<0.05)

N=102	R^2= 0.546		
	Beta	Std. err. of Beta	p-level
Intercept			*<0.001*
F*F (mg/ℓ)	*0.658*	*0.167*	*<0.001*
Ca*Ca (mg/ℓ)	0.375	0.255	0.146
Temperature (°C)	*0.223*	*0.101*	*0.030*
Ca (mg/ℓ)	*-0.947*	*0.377*	*0.014*
F (mg/ℓ)	-0.253	0.229	0.273
Si (mg/ℓ)	0.103	0.166	0.537
DO*DO (mg/ℓ)	0.007	0.097	0.945
PO_4 (mg/ℓ)	0.720	0.552	0.196
K*K (mg/ℓ)	*-1.773*	*0.447*	*<0.001*
Mg (mg/ℓ)	0.255	0.532	0.633
Mg*Mg (mg/ℓ)	-0.378	0.265	0.158
SO_4*SO_4 (mg/ℓ)	-1.075	0.290	*<0.001*
SO_4 (mg/ℓ)	*1.648*	*0.499*	*0.001*
Turbidity*Turbidity (NTU)	*0.670*	*0.219*	*0.003*
Turbidity (NTU)	*-0.601*	*0.193*	*0.003*
DO (mg/ℓ)	0.210	0.119	0.080
K (mg/ℓ)	0.916	0.388	*0.021*
PO_4*PO_4 (mg/ℓ)	-0.141	0.524	0.789
NH_4*NH_4 (mg/ℓ)	*0.878*	*0.371*	*0.020*
NH_4 (mg/ℓ)	-1.014	0.547	0.068
Cl*Cl (mg/ℓ)	0.391	0.217	0.075

TABLE 6
Polynomial regression summary for Pielou species evenness with water quality (italicised values significant at p<0.05)

N=102	R^2= 0.404		
	Beta	Std. err. of Beta	p-level
Intercept			*<0.001*
Si*Si (mg/ℓ)	*0.416*	*0.143*	*0.005*
Ca*Ca (mg/ℓ)	*0.212*	*0.098*	*0.034*
F*F (mg/ℓ)	*0.532*	*0.138*	*<0.001*
Temperature (°C)	*0.185*	*0.087*	*0.036*
DO* DO (mg/ℓ)	-0.086	0.085	0.312
PO_4 (mg/ℓ)	*0.771*	*0.225*	*0.001*
K*K (mg/ℓ)	*-0.737*	*0.223*	*0.001*
Turbidity*Turbidity (NTU)	*0.257*	*0.118*	*0.032*
NH_4 (mg/ℓ)	-0.604	0.382	0.117
NH_4*NH_4 (mg/ℓ)	0.298	0.270	0.272

of such indices. Since the linear regressions of SPI and BDI with water quality variables are high (± 80%) and little is gained from the addition of polynomial terms, it is suggested that the linear relationship of SPI and BDI with a water quality gradient is sufficient for use of the index in a linear fashion in river systems.

TABLE 7
Polynomial regression summary for the number of diatom species with water quality (italicised values significant at p<0.05)

N=102	R^2= 0.430		
	Beta	Std. err. of Beta	p-level
Intercept			*<0.001*
F*F (mg/ℓ)	*0.341*	*0.153*	*0.029*
pH	-0.193	0.161	0.233
K*K (mg/ℓ)	-0.497	0.364	0.176
Ca*Ca (mg/ℓ)	0.710	0.262	*0.008*
Ca (mg/ℓ)	*-1.778*	*0.386*	*<0.001*
Mg (mg/ℓ)	*1.946*	*0.482*	*<0.001*
Mg*Mg (mg/ℓ)	*-0.638*	*0.281*	*0.026*
Si*Si (mg/ℓ)	-0.211	0.181	0.246
Na (mg/ℓ)	-1.153	0.413	*0.006*
Si (mg/ℓ)	0.242	0.172	0.163
PO_4*PO_4 (mg/ℓ)	0.106	0.209	0.614
K (mg/ℓ)	*0.831*	*0.414*	*0.048*
Cl*Cl (mg/ℓ)	0.492	0.308	0.115
Temperature (°C)	-0.148	0.100	0.142
pH*pH	-0.135	0.109	0.220
DO (mg/ℓ)	-0.113	0.107	0.295

TABLE 8
Regression summary for the SPI with water quality (italicised values significant at p<0.05)

N=102	R^2= 0.796		
	Beta	Std. err. of Beta	p-level
Intercept			1.000
Na (mg/ℓ)	*-1.978*	*0.524*	*<0.001*
Si (mg/ℓ)	*-0.297*	*0.093*	*0.002*
pH	*0.305*	*0.068*	*<0.001*
NO_3+NO_2	0.249	0.150	0.101
K (mg/ℓ)	-0.231	0.175	0.191
PO_4 (mg/ℓ)	*0.368*	*0.173*	*0.036*
NH_4 (mg/ℓ)	-0.054	0.171	0.752
Ca (mg/ℓ)	*-0.344*	*0.172*	*0.049*
Cl (mg/ℓ)	*1.108*	*0.449*	*0.015*
SO_4 (mg/ℓ)	*-0.195*	*0.097*	*0.048*
F (mg/ℓ)	0.186	0.137	0.179
Mg (mg/ℓ)	0.229	0.190	0.231

Principle component analysis (PCA)

A principle component analysis was performed to visually represent the response of the two types of indicators with water quality variables. The results of this analysis are shown in Fig. 2 and Table 10. From the figure we can see that the main drivers in the water quality in the catchment are Na and Cl (correlates with the first ordination axis). The BDI and SPI indices (representing the aut-ecological indices) are strongly affected by the main drivers of the water quality in the catchment and show a strong negative response to increasing salt loadings (Na and Cl). The length of the vectors representing the various indices in Fig. 2

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TABLE 9 Regression summary for the BDI with water quality (italicised values significant at p<0.05)			
N=102	R^2= 0.810		
	Beta	Std. err. of Beta	p-level
Intercept			1.000
Na (mg/ℓ)	***-2.032***	***0.458***	***<0.001***
Si (mg/ℓ)	***-0.287***	***0.080***	***0.001***
NO_3+NO_2 (mg/ℓ)	***0.305***	***0.083***	***<0.001***
K (mg/ℓ)	-0.265	0.152	0.084
pH (mg/ℓ)	***0.178***	***0.064***	***0.006***
Ca (mg/ℓ)	***-0.571***	***0.152***	***<0.001***
Cl (mg/ℓ)	***1.159***	***0.387***	***0.004***
PO_4-P (mg/ℓ)	***0.305***	***0.096***	***0.002***
Mg (mg/ℓ)	0.360	0.178	***0.046***
F (mg/ℓ)	***0.229***	***0.113***	***0.047***
SO_4 (mg/ℓ)	-0.174	0.089	0.054

also indicates a much stronger effect of water quality variables on the BDI and SPI than on the diversity index measures (as the vectors of the former are longer than those of the latter).

Diversity and evenness as shown in Fig. 2 seem to increase in the same direction as most of the water quality variables. This might be caused by the fact that diversity seems to be higher at sites with intermediate values for the water quality parameters measured in the study.

The number of species (Fig. 2) was associated with increased oxygen in the water and negatively associated with the chemical variables that denote possible organic loading of the water system (NH_4, PO_4, NO_2+NO_3). However, the relationship of the number of species to water quality variables was low, with R^2's of 0.320 and 0.430 (Tables 4 and 7).

Discussion

From the results it is clear that both types of diatom-based indicators (diversity and aut-ecological) used were significantly influenced by water quality variables. From the three different diversity indices calculated, the number of species showed the strongest response to changes in water quality variables, although this relationship is fairly weak (Fig. 2). Of all the indicators tested in the study, the species diversity and the species evenness showed the weakest response to water quality variables (Tables 2 and 3).

From the 2nd degree polynomial multiple regressions, it can be seen that the highest species diversity and evenness as well as number of species occur in moderately impacted water as suggested by Archibald (1971) and Van Dam (1982). A high degree of dominance may therefore be expected at both clean water and polluted water sites. It would seem logical to conclude that moderately impacted water can harbor species that can be dominant in either good or polluted water, as observed by Van Dam (1982).

Due to the fact that the species evenness does not exhibit a strong linear association with water quality, it would be logical to conclude that, in the case of diatoms, a high level of dominance in the population (as would be represented by a low evenness index in this study) cannot be equated to polluted or less favorable conditions. It would be more consistent with the data to expect that diatoms have well defined niches and that taxa best suited to water quality conditions at a specific point in time will become dominant. This is borne out by Cholnoky (1960) who stated that: "…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*" (own italics).

This would also suggest that specific diatom taxa will be dominant in certain (and most) water quality conditions. Representatives of many species are always present in low numbers in the population and can become dominant when water quality is suitable.

The data are also consistent with postulations from Kelly (1998) who discussed the concept that diatoms are 'subcos-

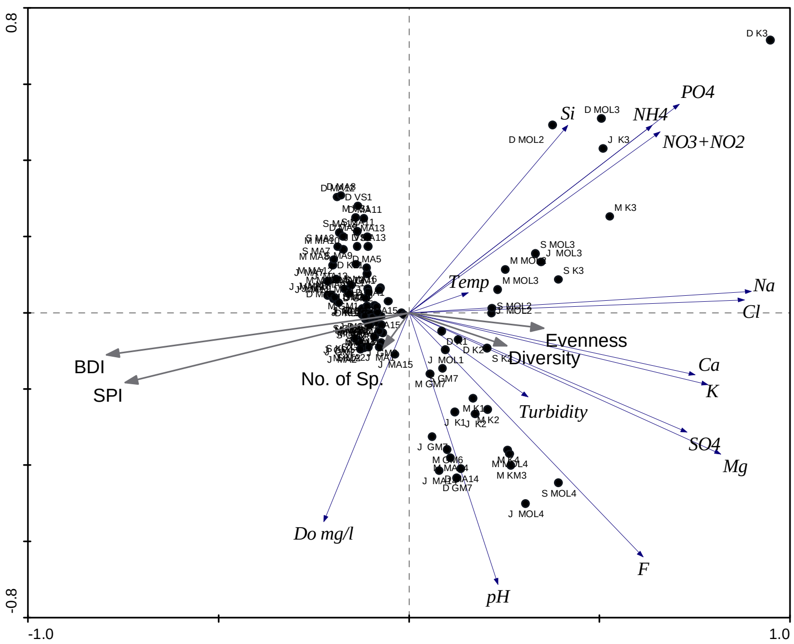

Figure 2
Principle component analysis (PCA) indicating chemical variables and diatom-based indices as vectors and sites as dots (site names are denoted by a letter denoting the month of sampling, followed by the site name as indicated in Fig. 1)

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TABLE 10 Results for PCA performed for sites in the Marico and Molopo Rivers (Biological and habitat indices were used as supplementary data in the ordination)					
Axes	**1**	**2**	**3**	**4**	**Total variance**
Species-environment correlations	0.803	0.378	0.331	0.143	
Cumulative percentage variance: of species data of species-environment relation	 40.6 81.1	 57.8 88.7	 70.5 93	 78.8 93.6	
Sum of all canonical eigenvalues					0.323

mopolitan', i.e. they occur anywhere in the world if 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. Finlay (2005) also states that it is now clear that distribution patterns of protists are quite different from those of macroscopic organisms – e.g. the recent discovery of the ubiquity-biogeography transition, where organisms smaller than about 1 mm occur worldwide wherever their required habitats are realised. However, some diatoms may be more susceptible to desiccation etc. and thus may not be so easily distributed. This would appear to be the case when (possibly) endemic diatoms such as *Achnanthes standerii* Cholnoky are found *en masse* in certain rivers and streams around South Africa but have never been reported from outside our borders. Whether diatoms such as these are in fact true endemics or if their distribution is simply governed by the factors such as local geology and climate, which may not be found elsewhere, remains a topic for further investigation.

In comparison to the diversity indices, the BDI and SPI as representatives of aut-ecological indices displayed a significantly better relationship with measured water quality variables as shown by the multiple regression results. It is also important to note that the relationship of the BDI and SPI with water quality did not increase significantly when the quadratic functions were added. The implication of this would be that the aut-ecological indices may be applied in a linear fashion in contrast to diversity indices.

The abovementioned results indicate that aut-ecological indices based on diatoms are more useful in biomonitoring programs of rivers and streams than diversity indices; a point strongly supported by the high R^2 values of the linear multiple regressions for SPI and BDI in Table 8 and 9.

Conclusions

The current study and the results presented in the different sections of the current study warrant the following conclusions:

- Diversity measures based on the abundance of diatoms appear to show a relationship to water quality variables although that relationship is not linear
- The results from the linear and 2^{nd} degree polynomial regressions show that diatom species diversity (especially as reflected by the Shannon Species Diversity Index used in this study) tends to be higher in moderately impacted water
- Due to the highly significant relationship of aut-ecological diatom indices with water quality, these indices are deemed more relevant and reliable for use in rivers and streams to inform decision making in integrated water resource management.

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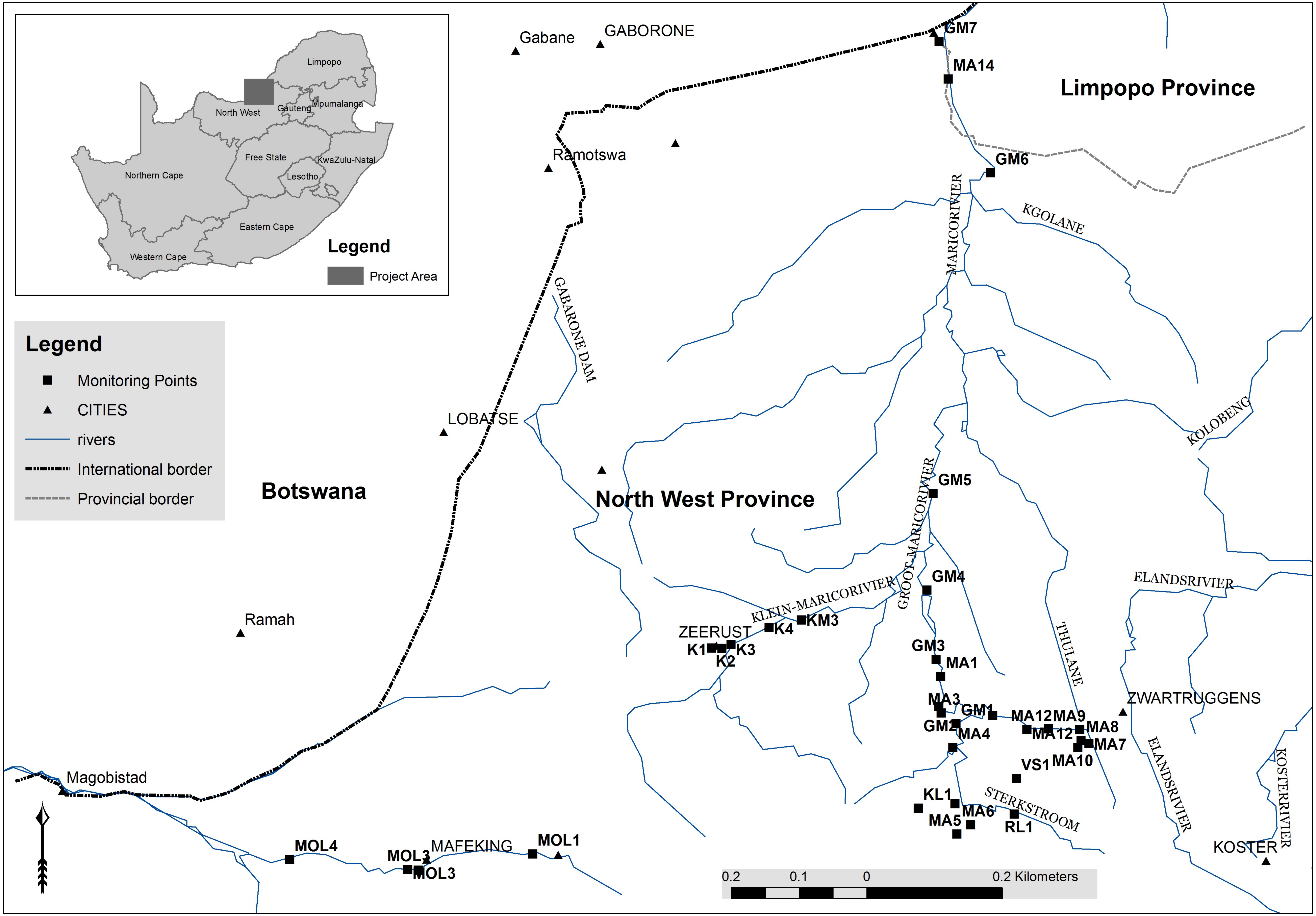
Appendix D

A3 map of Figure 1 (Manuscript IV, Chapter 5)


Limpopo
North West
Gauteng
Mpumalanga
Free State
KwaZulu-Natal
Northern Cape
Lesotho
Eastern Cape
Western Cape
Legend
Project Area
Legend
Monitoring Points
CITIES
rivers
International border
Provincial border
Botswana
North West Province
Limpopo Province
Gabane
GABORONE
Ramotswa
LOBATSE
Ramah
Magobistad
MAFEKING
ZEERUST
ZWARTRUGGENS
KOSTER
GABARONE DAM
MARICORIVIER
KGOLANE
KOLOBENG
GROOT-MARICORIVIER
KLEIN-MARICORIVIER
ELANDSRIVIER
THULANE
STERKSTROOM
KOSTERRIVIER
GM7
MA14
GM6
GM5
GM4
GM3
GM2
GM1
KM3
K4
K1
K2
K3
MA1
MA3
MA4
MA5
MA6
MA7
MA8
MA9
MA10
MA12
VS1
KL1
RL1
MOL1
MOL3
MOL4
0.2
0.1
0
0.2 Kilometers

Appendix E

On the use of diatom-based biological monitoring.
Part 2: A comparison of the response of SASS 5 and diatom indices to water quality and habitat variation

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On the use of diatom-based biological monitoring Part 2: A comparison of the response of SASS 5 and diatom indices to water quality and habitat variation

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Abstract

Due to the fact that South Africa is a water-scarce country, integrated water resource management based on sound information is essential. Bio-indicators have provided valuable information for water resource management in recent years and have enjoyed increasing popularity. Bio-indicators especially stepped to the forefront with the realisation that aquatic eco-systems are not only a source of water but also deliver several goods and services, as well as being essential for industrial growth and quality of life of many South Africans. This study aimed to quantitatively test two kinds of biomonitoring tools namely diatom-based (SPI and BDI) and macro-invertebrate based (SASS 5) in order to assess their applicability in South African River systems; and whether any additional information can be gained by using the two tools in tandem. The results showed that diatom indices are affected more by changes in water quality than SASS 5, while SASS 5 displayed a higher dependency on habitat quality, as measured by IHAS, than the diatom indices. It is therefore suggested that the two indices be utilised as complementary indicators for integrated assessment of river health.

Keywords: diatoms; Bacillariophyceae; bioindicators; SASS 5; species diversity indices; water quality

Introduction

Species of flora and fauna 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). Aquatic communities (e.g. fish, riparian vegetation, macro-invertebrates) can integrate and reflect the effects of chemical and physical disturbances that occur in river ecosystems over extended periods of time.

Walmsley et al. (2000) stated that bio-indicators are ideal means of monitoring aquatic ecosystems, leading towards integrated water resource management, and that bio-indicators provide a summary of conditions 'rather like temperature and blood pressure are used to measure human health'.

The South African Department of Water Affairs and Forestry (DWAF), as custodians of the water resources of the country, initiated the development of a National Aquatic Ecosystem Biomonitoring Programme (also called the River Health Programme or RHP) during 1995 (Roux, 1997). Examples of such indicators include the Fish Assemblage Integrity Index (Kleynhans, 1999), the Riparian Vegetation Index (Kemper, 2001) as well as the South African Scoring System, better known as SASS (Chutter, 1998). Although some methods have been available for many years, biomonitoring has only recently become a routine tool in the management of South Africa's inland waters (Davies and Day, 1998). The SASS biomonitoring system has gained a large body of support as a rapid and fairly accurate system of evaluating water quality in streams and rivers, and is currently in its 5th revised form namely SASS 5 (Dickens and Graham, 2002).

Recently diatom-based indices such as the Specific Pollution Index (SPI) and Biological Diatom Index (BDI) have come into the spotlight as potential additions to more established bio-indicators such as SASS 5. Several papers have been published in the past few years exploring the potential use of diatoms as bio-indicators such as Taylor et al. (2007b), De la Rey et al. (2004) and Harding et al. (2005). A standard protocol for assessment using diatoms has also been published (Taylor et al., 2005) to facilitate comparability of diatom index results. The value of diatoms as indicators has been recognised to the point that it has been included in the state of the rivers report for the Crocodile (West) – Marico River Water Management Area (Taylor et al., 2007c; River Health Programme, 2005).

In a recently published article, Ashton et al. (2005) called for a shift in thinking from a point where water is seen as simply a commodity to recognising that it is an integral part of a larger ecosystem, and that such an ecosystem approach demands an understanding of the various components of the hydrological cycle as well as the inter-relationships of these various components. For this reason it is believed important to understand how different bio-indicators respond to the various changes in the aquatic ecosystem.

With the above-mentioned in mind, it is also important to evaluate biological indices in terms of their relationship to habitat characteristics. In this study, the Integrated Habitat Assessment System: Version 2 (McMillan, 1998) was used as indicator of habitat condition. This assessment focuses on sampling habitat, especially habitat that can be utilised by invertebrate fauna, as well as other stream characteristics, such as water quality, which may be modified by anthropogenic or natural impacts.

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This paper represents the second part of a study that aims to evaluate the efficacy of diatom-based indices in river systems in South Africa. The paper follows from Part 1 (De la Rey et al., 2008) which concluded that aut-ecological indices should be preferentially used as they respond in a linear fashion to environmental water quality gradients.

Part 2 of this paper aims to compare the relationship of the SASS 5 invertebrate index and diatom indices to chemical water quality and habitat availability. There are several questions that the current paper strives to answer. Firstly whether there is a significant difference in the response of SASS 5 and diatom-based aut-ecological indices to changes in stream habitat and water quality. If the two indices respond similarly, and to the same extent, to water quality variables, there would be no additional benefit to be found in using both indices for monitoring changes and impacts in rivers. Secondly the present paper aims to evaluate the dependency of index response on variation in habitat and seasonal changes. The answers gained from such analysis can assist in the application and interpretation of results gained when using the various bio-indicators evaluated in this paper.

Materials and methods

Sampling localities

For information on the sampling localities please refer to Part 1 (De la Rey, 2008)

Indices calculations

Macroinvertebrates were collected using the SASS 5 methodology. The SASS score, Average Score per Taxon (ASPT) and Number of Taxa (No. of Taxa) were calculated according to standard methods as set out in Dickens & Graham (2002) and Chutter (1998).

Diatoms were collected, prepared and enumerated according to the protocol as set out in Taylor et al. (2005). The diatom identification was according to the nomenclature of Krammer and Lange Bertalot (1986-1991). For the current paper the aut-ecological method for evaluating water quality by means of diatoms were used. This choice is based on the results obtained from Part 1 of the study; a description of these indices is given in Part 1 (De la Rey, 2008). For the current study the Specific Pollution sensitivity Index (SPI) (Coste in Cemagref, 1982) as well as Biological Diatom Index (BDI) (Lenoir & Coste, 1996), indices were calculated for the various sampling localities using Omnidia v.3.1 software (Lecointe et al., 1993). The reasons for the selection of these two indices are that SPI has the broadest species base and that BDI showed the best overall correlation to water quality variables in studies performed recently on the Vaal River (Taylor et al., 2007b). More details are given in Part 1 of this paper (De la Rey et al., 2008).

The in-stream habitat was evaluated by means of the Integrated Habitat Assessment version 2 (IHAS) (McMillan, 1998). This evaluation was done for every sampling site on every sampling occasion. IHAS endeavours to numerically express the availability of in-stream habitat in terms of quantity, quality and diversity. In the system both the sampling habitat (stones, vegetation, gravel, sand and mud) and the general river/stream condition is evaluated and a total habitat score calculated.

As diatoms were only sampled from the "Stones in Current" biotope, the SASS biotopes (Stones in Current and Stones out of Current, Marginal- and Submerged Vegetation as well as Gravel Sand and Mud) were also scored individually to facilitate comparability of the responses of the indices to habitat and water quality variables. It is a well known fact that macroinvertebrates are influenced by habitat availability (Ollis et al., 2006; McMillan, 1998; Dallas, 1997; Karr and Dudley, 1981). Due to this fact, IHAS is mainly used in this study to facilitate interpretation of macroinvertebrate-based data as compared to diatom-based data, rather than as a definitive measure of stream quality. This study does therefore not directly focus on the reliability of IHAS as an indicator of habitat conditions influencing invertebrates.

Water quality

For details on the water quality analysis for the study please refer to Part 1 of the paper (De la Rey, 2008).

Statistics

Details on the methods used for the statistical analysis of the data are given in Part 1 of the study (De la Rey, 2008). However, it was felt that an abbreviated overview is justifiable due to minor alterations in statistical methods applied in Part 1.

Again, multiple regressions and correlation analysis were performed using the STATISTICA software package (Release 7, Stat Soft. Inc., United States of America), while Principle Component Analysis (PCA) was performed using CANOCO for Windows (Version 4.51, Biometris-Plant Research International, The Netherlands). Before analysis, all the data were standardised. For the purpose of the multiple regressions the Electrical Conductivity and Total Alkalinity were left out of the analysis as these variables contributed to multi-colinearity in the data. In addition to the above analysis, predicted vs. observed graphs of certain regression analysis are also shown. Such graphs were obtained from the STATISTICA software package.

Another set of multiple regressions was performed to investigate whether season has an influence on the performance of the different indicators used. Season is a categorical dependant variable and was transformed into multiple (dummy-) coded dependant variables (see Hair et al., 1998) for the analysis. In the current study, direct comparison of seasonal response of the bio-indicators was prevented because data from all four sampling periods could only be obtained for 17 of the sites. This was mainly due to varying flow at the identified sites in the different seasons which hindered SASS 5 sampling in many instances. It was therefore deemed preferable to include season as variable in the multiple regression of the combined data set.

Results and discussion

The results are presented and discussed in three sections. Firstly the correlations between the different bio-indicators will be investigated. This was done to establish whether there is any value in using more than one bioindicator. High correlation between the indicators will show that they respond in a similar fashion to environmental variables, showing that little additional information can be obtained from the extra effort, time and money spent in acquiring the data for the additional indicator. This section will also be used to determine whether bio-indicators sampled from the same biotope are more alike in response to water quality and habitat quality than bio-indicators from other biotopes.

The second section examines the correlation of the biological indices to specific components of water quality in order to ascertain whether the tested bio-indicators respond differently

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to water quality variables. Whereas the previous section focused on whether the bio-indicators are correlated with one another, the focus in this section is therefore rather which water quality and habitat variables influence specific bio-indicators.

Section three entails exploration of the response of bio-indicators to water quality and habitat data by means of multiple regressions. This enables quantification of the influence of water quality and habitat on the different bio-indicators.

1. Correlation between different bio-indicators

Table 1 shows the correlation of the different bio-indicators between one another. The correlations between SASS 5 and the number of (invertebrate) taxa with the diatom-based indices (BDI and SPI) are reasonably high at 62% and 66% respectively. From the three invertebrate indices calculated according to the SASS 5 protocol, the ASPT displays the highest correlation with the diatom indices at approximately 70% correlation. The SASS 5 score exhibited slightly lower correlations with the diatom indices than the ASPT, while the number of taxa displayed the lowest correlation with the diatom-based indices.

An analysis was also performed to compare the macroinvertebrate index scores for the different biotopes mentioned in the SASS 5 protocol. When comparing the scores generated from the individual biotopes (S – stones in current biotope, V – vegetation biotope and GSM – gravel sand and mud biotope) with the diatom-based indices, there was little difference in the correlation values. The stones in current biotope, however, did show a slightly higher correlation with diatom-based indices than the other two biotopes. However, the total SASS scores still showed a higher correlation with the diatom indices than any of the individual biotopes.

Although there is a fairly high correlation between the diatom-based indices and the invertebrate-based indices (62% to 71%; Table 1), there is still a significant amount of difference in the response of the indices to changes in their environment (29% to 38%; Table 1). It is therefore useful to further investigate the relationships of the different indices to individual components of their environment (for instance water quality and habitat). This is explored in the following sections.

2. Correlation of bio-indicators with water quality and habitat variables

The correlation of biological indicators with various water quality variables and habitat (IHAS) is presented in Table 2. From the table it is clear that all the bio-indicators (invertebrate-based as well as diatom-based) correlate well with water quality and habitat variables. The water quality variables that show the lower correlation with the bio-indicators (diatom-based and SASS 5) are pH, dissolved oxygen and water temperature. In general, it is also of interest that diatom indices display a stronger correlation

TABLE 1
Significant correlation between different bioindicators. Correlations are significant at $p < 0.05$. N=102

	SPI	BDI
SASS 5	0.66	0.62
ASPT	0.71	0.69
No. of Taxa	0.65	0.63
SASS (S)	0.63	0.59
ASPT (S)	0.79	0.76
No. Taxa (S)	0.60	0.57
SASS (V)	0.56	0.56
ASPT (V)	0.73	0.72
No. Taxa (V)	0.48	0.49
SASS (GSM)	0.57	0.54
ASPT (GSM)	0.76	0.73
No. Taxa (GSM)	0.53	0.52

(S) Stone biotope; (V) Vegetation biotope; (GSM) Gravel, Sand and Mud biotope

TABLE 2
Correlation of bioindicators with water quality and habitat variables. Shaded correlations are significant at $p < 0.05$. N=102

	SASS 5	ASPT	No. of Taxa	SASS (S)	ASPT (S)	No. Taxa (S)	SASS (V)	ASPT (V)	No. Taxa (V)	SASS (GSM)	ASPT (GSM)	No. Taxa (GSM)	SPI	BDI
Ca (mg/ℓ Ca)	-0.28	-0.41	-0.28	-0.26	-0.47	-0.25	-0.28	-0.33	-0.28	-0.23	-0.44	-0.23	-0.46	-0.57
Cl (mg/ℓ Cl)	-0.51	-0.53	-0.53	-0.47	-0.58	-0.46	-0.46	-0.54	-0.43	-0.45	-0.60	-0.45	-0.73	-0.75
EC (mS/cm)	-0.43	-0.54	-0.42	-0.40	-0.61	-0.38	-0.39	-0.47	-0.37	-0.36	-0.59	-0.33	-0.65	-0.74
F (mg/ℓ F)	-0.25	-0.29	-0.17	-0.26	-0.37	-0.18	-0.19	-0.22	-0.14	-0.16	-0.33	-0.07	-0.34	-0.39
K (mg/ℓ K)	-0.47	-0.51	-0.44	-0.45	-0.55	-0.40	-0.38	-0.49	-0.31	-0.38	-0.54	-0.33	-0.62	-0.63
Mg (mg/ℓ Mg)	-0.39	-0.50	-0.37	-0.38	-0.57	-0.33	-0.36	-0.40	-0.33	-0.32	-0.53	-0.28	-0.54	-0.62
NH_4 (mg/ℓ NH_4-N)	-0.37	-0.43	-0.41	-0.36	-0.51	-0.38	-0.33	-0.45	-0.31	-0.35	-0.49	-0.38	-0.58	-0.59
NO_3+NO_2 (mg/ℓ NO_3+NO_2-N)	-0.37	-0.45	-0.41	-0.33	-0.45	-0.34	-0.34	-0.45	-0.35	-0.33	-0.49	-0.36	-0.47	-0.47
Na (mg/ℓ Na)	-0.54	-0.57	-0.55	-0.51	-0.62	-0.50	-0.48	-0.56	-0.44	-0.46	-0.63	-0.46	-0.77	-0.78
PO_4-P (mg/ℓ PO_4-P)	-0.36	-0.41	-0.40	-0.33	-0.44	-0.35	-0.33	-0.43	-0.32	-0.34	-0.48	-0.38	-0.50	-0.51
SO_4 (mg/ℓ SO_4)	-0.44	-0.47	-0.40	-0.41	-0.51	-0.36	-0.41	-0.42	-0.38	-0.38	-0.49	-0.34	-0.45	-0.50
Si (mg/ℓ Si)	-0.25	-0.36	-0.28	-0.24	-0.44	-0.25	-0.24	-0.35	-0.24	-0.21	-0.42	-0.22	-0.44	-0.47
TAL (mg/ℓ TAL)	-0.34	-0.47	-0.33	-0.33	-0.55	-0.30	-0.32	-0.38	-0.30	-0.28	-0.51	-0.25	-0.53	-0.64
pH	0.09	0.08	0.08	0.06	0.12	0.02	0.06	0.18	0.02	0.13	0.15	0.12	0.09	-0.06
Do (mg/ℓ DO)	0.15	0.21	0.13	0.10	0.33	0.05	0.17	0.25	0.14	0.17	0.28	0.17	0.30	0.28
Temp (°C)	0.11	0.09	0.16	0.12	-0.08	0.19	0.13	0.02	0.18	0.15	0.01	0.18	-0.13	-0.11
Turbidity (NTU)	-0.36	-0.25	-0.36	-0.37	-0.29	-0.35	-0.32	-0.27	-0.29	-0.27	-0.29	-0.25	-0.36	-0.32
IHAS (%)	0.60	0.40	0.61	0.61	0.40	0.59	0.57	0.44	0.53	0.50	0.39	0.50	0.31	0.27

to almost all water quality variables than do the invertebrate indices. The water quality variables which display the highest correlation to the diatom-based indices are variables reflecting the salinity of the water like Na, Cl and EC. Note that in Part I (De la Rey, 2008), Na and Cl were also significant contributors in the regressions of water quality with SPI and BDI, but not in the regressions of water quality with species diversity and evenness. This is probably due to the weak regression results (low R^2 values) found for species diversity and evenness. The highest correlation of SASS with water quality variables is also with the ionic components of water quality such as Cl, EC, SO_4 and Na. This may be because the sampling area is dominated by agricultural activities which may lead to changes in salinity and this may in turn be the dominating determinant of water quality.

SASS indices display a higher correlation to the IHAS index than the diatom-based indices. This correlation is as high as 60% in the case of the SASS 5 score, while the correlation of IHAS and diatom indices is about 30%. It is interesting to note that the ASPT showed slightly better correlations with water quality variables and a lower correlation with IHAS. Table 1 also shows that ASPT is more closely correlated with the diatom-based indices than the SASS5 score or the Number of Taxa. This shows that the ASPT score is more likely to be influenced by water quality than habitat availability. This finding corresponds with the findings of Dallas (1997) who states that the ASPT score is relatively constant between biotopes, suggesting that sites that have different biotopes and habitats available for habitation by aquatic fauna can be compared on the basis of ASPT so that the extent of the impairment of the water quality can be established.

In their evaluation of the relationship of IHAS with SASS, Ollis et al. (2006) suggested that the results obtained showed weak correlation between the two indices. This statement was mainly based on the results obtained from SASS 4 and IHAS scores. The study did however show strong correlations (up to 60%) when Total IHAS scores for Mpumalanga and the Western Cape were correlated with SASS 5 scores. This is approximately the same correlation found in the current study (Table 2) which also used SASS 5 as apposed to SASS 4. This correlation however does not indicate cause and effect but merely correlation (Hair et al., 1998). The results from the multiple regression are more indicative of the effect of habitat (as represented by IHAS) on SASS. This evaluation is dealt with in the next section.

TABLE 3
Results for regression performed for bioindicators with (1) habitat and water quality (2) water quality and (3) habitat

Dependent variable	Independent variables used	R^2	Significant contributors
SASS 5	WQ & IHAS	0.651	IHAS, Na, SO_4, Cl, pH, PO_4, Mg, F, K
	WQ	0.491	Na, SO_4, pH, Temp, Cl, Turb
	IHAS	0.365	IHAS
ASPT	WQ & IHAS	0.623	IHAS, Mg, pH, Temp, K, F
	WQ	0.598	K, Temp, pH, Mg, PO_4, F, SO_4
	IHAS	0.157	IHAS
No of Taxa	WQ & IHAS	0.651	IHAS, Na, Cl, SO_4, F, Mg
	WQ	0.505	Na, Temp, pH, SO_4, Cl, Turb, F
	IHAS	0.367	IHAS
SPI	WQ & IHAS	0.799	Na, Si, pH, PO_4, Ca, Cl
	WQ	0.796	Na, Si, pH, PO_4, Ca, Cl, SO_4
	IHAS	0.093	IHAS
BDI	WQ & IHAS	0.813	Na, Si, NO_3+NO_2, pH, Ca, Cl, PO_4, Mg
	WQ	0.810	Na, Si, NO_3+NO_2, pH, Ca, Cl, PO_4, Mg, F
	IHAS	0.071	IHAS
SASS (S)	WQ & IHAS	0.617	IHAS, Na, Cl
	WQ	0.461	Temp, Turb, pH, SO_4
	IHAS	0.371	IHAS
ASPT (S)	WQ & IHAS	0.732	IHAS, pH, Mg, PO_4, NH_4
	WQ	0.706	Si, pH, Mg, PO_4
	IHAS	0.163	IHAS
No of Taxa (S)	WQ & IHAS	0.578	IHAS, Na, Temp, Cl
	WQ	0.437	Na, Temp, Cl, Turb, SO_4
	IHAS	0.349	IHAS
SASS (V)	WQ & IHAS	0.519	IHAS, SO_4, F
	WQ	0.394	Na, SO_4, Turb, Cl
	IHAS	0.320	IHAS
ASPT (V)	WQ & IHAS	0.587	IHAS, pH, SO_4, F, K
	WQ	0.551	pH, SO_4, F, K, PO_4, Si
	IHAS	0.190	IHAS
No of Taxa (V)	WQ & IHAS	0.429	IHAS, Na
	WQ	0.351	Na, SO_4, Turb
	IHAS	0.281	IHAS
SASS (GSM)	WQ & IHAS	0.470	IHAS, pH, K
	WQ	0.405	Temp, pH, SO_4
	IHAS	0.249	IHAS
ASPT (GSM)	WQ & IHAS	0.673	IHAS, pH, Mg, Temp
	WQ	0.664	Si, pH, Mg, Temp
	IHAS	0.149	IHAS
No of Taxa (GSM)	WQ & IHAS	0.446	IHAS, Na, Temp
	WQ	0.365	Na, Temp, pH, SO_4
	IHAS	0.247	IHAS

Significance chosen at a p-value ≤ 0.05. IHAS denotes habitat scores.
WQ denotes water quality variables.
(S) Stone biotope; (V) Vegetation biotope; (GSM) Gravel, Sand and Mud biotope

3. Response of bio-indicators to water quality and habitat

Table 3 presents the multiple regressions performed for each of the indicators with water quality (indicated with WQ in Table 3), habitat (IHAS) as well as a combined multiple regressions for each indicator with both water quality and habitat as

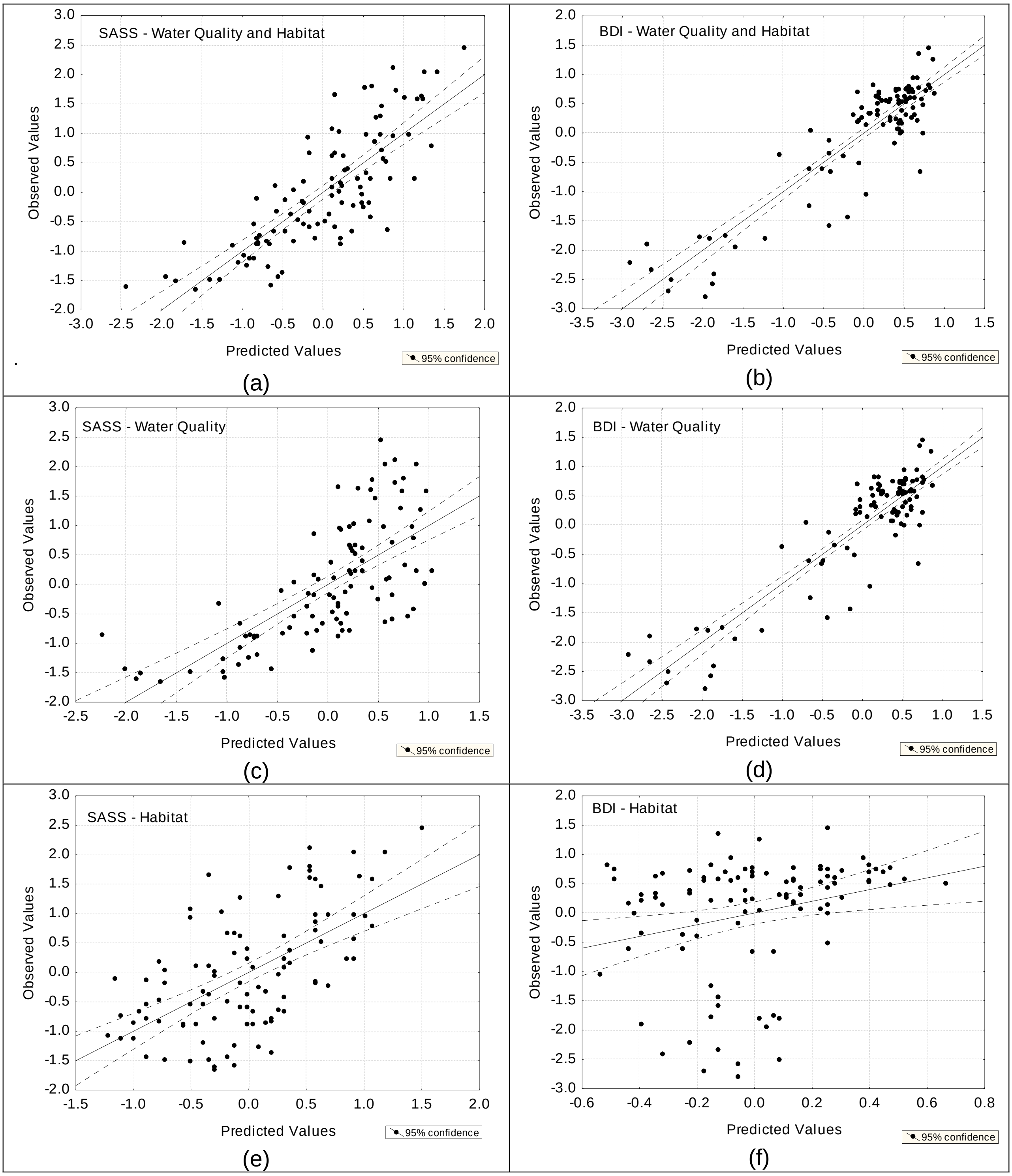

Figure 1

Representation of regression results (predicted against observed graphs) of SASS 5 (left) and BDI (right) using water quality and habitat (top), only water quality (middle) and only habitat (bottom) as independent variables

predictors. Multiple regressions were also performed for the different biotopes (S, V and GSM) that make up the SASS 5, ASPT and number of taxa scores.

From Table 3 one may observe that the habitat score (IHAS) influences SASS 5, ASPT and number of taxa more strongly than the diatom-based indices (SPI and BDI). Only 6 to 8% of the variance explained by the multiple regression for diatom-based indices could be attributed to IHAS, whereas habitat explained 35% of the variance in the SASS 5 and the number of taxa, and 15% to the ASPT score. This is a much lower value than the 60% found in the correlation results (Table 2). The apparently conflicting results can be explained by the fact that, in many instances, lower IHAS scores correspond with sites that also displayed poorer water quality in terms of the measured variables. This will have the effect that the correlation values will be higher but does not necessarily reflect causality. The results from the multiple regression on the other hand do indicate a 'cause and effect' relationship between IHAS and SASS scores. In the current data set therefore, water quality contributes to approximately 50% of the variation in the SASS score, while the habitat as reflected/indicated by IHAS contributes approximately 36%.

These above-mentioned results are illustrated in Fig. 1. From the results indicated in Table 2 as well as Figure 1 it is clear that there is little difference in the predictive power of the linear model for the BDI index if water quality alone is used, or when habitat and water quality are used as independent variables (panels b and d) due to the similarity of the R^2 values and the similarity of the graphs. It is also clear from the graphs that habitat has better predictive power when used for SASS 5 than for BDI (panels e and f).

Table 3 also shows that the chemical variables that influenced the diatom-based indices were Na, Si, pH, PO_4, Cl, Ca, NO_3+NO_2, Mg, F and SO_4. Overall, chemical variables in water influenced the diatom indices more strongly than was the case for the invertebrate indices, while the habitat seemed to exert little influence on the diatom index scores in the combined analysis (R^2= 0.071 to 0.093). There was very little difference in the amount of variation explained by water quality variables for the SPI and BDI respectively. In both cases about 80% of the variation in the diatom index scores could be explained by water quality variables. The main difference between the indices (SPI/BDI) is the number of species accommodated in the system. BDI utilises approximately 209 species while the SPI can accommodate a larger number of taxa (approximately 1 700 species; Coste in Cemagref, 1982). SPI is very sensitive to changes in water quality and provides high correlations with chemistry (e.g. De la Rey et al., 2004) but it has some disadvantages. SPI is regularly updated to take into account taxonomical research results and it is sometimes unclear which version is used. The list of taxa is also dependent on the skills of the operator, on the flora used, and on the time spent on analysis. Since the BDI index employs only 209 important indicator taxa, it facilitates more rapid identification than the SPI. Problems using the BDI may occur in cases where samples contain dominant diatom species that are not used by the index. However, this was not encountered in the current study.

Although the BDI and SPI responded adequately in the current study, these indices need to be adapted for South African conditions by the addition of endemic species. The groundwork for such an adaptation has been laid by a Water Research Commission (WRC) report (Taylor et al., 2007a) that describes about 400 species dominant in South African rivers along with ecological information and gives an account of some endemic species not included in European indices. Another WRC project is currently in progress, the ultimate goal of which will be to formulate a unique diatom index for South Africa and will include the more common diatom species endemic to South Africa (Taylor, 2006).

The important chemical variables that influenced the invertebrate indices were Na, Cl, SO_4, Mg, F and K (Table 3). The temperature of the water also significantly influenced all three of the invertebrate indices.

The ASPT component of the SASS 5 scores (Table 3) showed the strongest response to water quality variables and the weakest response to the habitat scores. Of all the scores generated in the different biotopes, the ASPT in the stones biotope showed the strongest response to water quality and habitat scores in comparison with the other indices and other biotopes. In all biotopes the ASPT was the component of SASS 5 that showed the most reliable response to water quality and habitat variation. This is in agreement with the findings of Dickens and Graham (2002) who stated that the ASPT appears to be a more consistent and repeatable measure of river health.

As can be seen in Table 4, season contributed statistically significantly ($p<0.05$) to the stepwise multiple regressions of SASS, ASPT as well as number of taxa.

The variation of macroinvertebrate indicator scores over season has been noted in several papers (Maloney and Feminella, 2006; Maul et al., 2004; Townsend et al., 1987). Gratwicke (1999) stated that SASS scores improved with the rainy season (January to March) but deteriorated in the dry season. Dallas (2004) found significant differences in ASPT and No. of Taxa between seasons in the Western Cape, with higher ASPT values recorded in winter and spring, while the number of taxa was higher for summer than winter. The same study did not find any significant differences between SASS, ASPT and No. of Taxa values when compared among seasons when samples from Mpumalanga were analysed. For the current study, no statistically significant differences were recorded between samples from different seasons, although SASS and number of taxa scores were higher in spring and summer than for autumn and winter (analysis not shown). The ASPT values were fairly constant between seasons which are also indicated from the multiple regressions in Table 4. The diatom-based indices (Table 4) were not affected significantly by seasonality and water quality remains the only significant factor influencing variation in these indices.

From the presented data it would seem that both indices perform well as bioindicators in all seasons, although the diatom-

TABLE 4
Multiple regression results for the tested bioindicators with water quality, habitat and season* *(season used as a categorical variable)

Dependent variable	Independent variables used	R^2	Significant contributors
SASS 5	WQ & IHAS	0.651	IHAS, Na, SO_4, Cl, pH, PO_4, Mg, F, K
	WQ & IHAS & Season	0.701	IHAS, Season, DO, Temp., pH, Mg
ASPT	WQ & IHAS	0.623	IHAS, Mg, pH, Temp., K, F
	WQ & IHAS & Season	0.627	IHAS, Mg, pH, Cl, Temp., Season, K
No of Taxa	WQ & IHAS	0.651	IHAS, Na, Cl, SO_4, F, Mg
	WQ & IHAS & Season	0.681	IHAS, Na, Season, Cl, Temp., pH, Mg
SPI	WQ & IHAS	0.799	Na, Si, pH, PO_4, Ca, Cl
	WQ & IHAS & Season	0.799	Na, Si, pH, PO_4, Ca, Cl
BDI	WQ & IHAS	0.813	Na, Si, NO_3+NO_2, pH, Ca, Cl, PO_4, Mg
	WQ & IHAS & Season	0.813	Na, Si, NO_3+NO_2, pH, Ca, Cl, PO_4, Mg

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based indices seems to be more stable, in terms of their potential to reflect water quality in rivers, than SASS. This is due to the slight effect of season on SASS scores.

Principle component analysis

A principle component analysis was performed on the data and the results are presented in Fig. 2 and Table 5. The aim of the analysis was to help contextualise the performance of the two types of indices (macroinvertebrate and diatom-based) in terms of the catchment in which the study was performed.

From the PCA it is clear that the main drivers for water quality in the catchment are sodium and chloride (associated with the first ordination axis), while dissolved oxygen and pH were the strongest contributors to the second ordination axis. The two main groups in the figure are associated according to geographical occurrence in the catchments. Group 1 in Fig. 2 includes sites associated with the headwaters of the Groot Marico while group 2 represents sites in the different towns as well as sites lower down in the river system. This observation corresponds with the general hypothesis that rivers show a downstream increase in salinity as discussed for example by Pillsbury (1981) for North American rivers.

It is also clear from Fig. 2 that the various indices also responded negatively to PO_4, NH_4 and NO_2+NO_3 (these elements may indicate organic loading). The IHAS score also responded negatively to these variables and this phenomenon might be explained by increased sedimentation as well as algae growth on rocks that occur in eutrophic river systems. This can have a negative effect on the 'stones in current' and 'stones out of current' biotopes, reducing the surface area of rocks on which macroinvertebrates can colonise, thus reducing the IHAS scores.

Although the different indices seem to react negatively to temperature (Fig. 2), this effect might be due to the fact that the sites showing high levels of PO_4, NH_4 and NO_2+NO_3, represents sites with shallower water levels as the rivers at these sites run through the towns of Mafikeng and Zeerust (see Fig. 1 in Part 1 (De la Rey (2008)). The temperature effect is therefore not to be mistaken for a seasonal effect on the indices. Such an explanation of the data concurs with the results from the regression analysis (Table 4) indicating a low level of influence of season on the various indices.

Interestingly, all of the biological indices respond in a similar fashion to the chemical variables in the water suggesting that both types of indices respond to the main water quality drivers in a given system, corresponding with the results from the correlation analysis in Table 2. However, due to the longer vectors of the diatom-based indices, we can also conclude from the figure they are more strongly influenced by water quality than the macroinvertebrate-based indices. This is in agreement with the results from the multiple regression analysis in the previous section.

This finding may however not necessarily indicate that diatoms are more sensitive to changes in water quality than macroinvertebrates, but may also reflect on the way in which the index is calculated. The diatom based indices as used in the current study are a reflection of the relative abundance of species found at a particular site, while the macroinvertebrate scores mainly utilise presence and absence to calculate the SASS 5 scores. Since the presence or absence of a single individual in a sample may alter the SASS 5 score, this promotes more variability in the scores than would be true for the diatom based indices. The advantage of a presence/absence type index such as SASS 5 is that analysis is more rapidly obtained than is the case for diatom based indices which utilise relative abundance.

Conclusions

Both invertebrate- and diatom-based indices showed significant correlations to water quality variables. The different indices

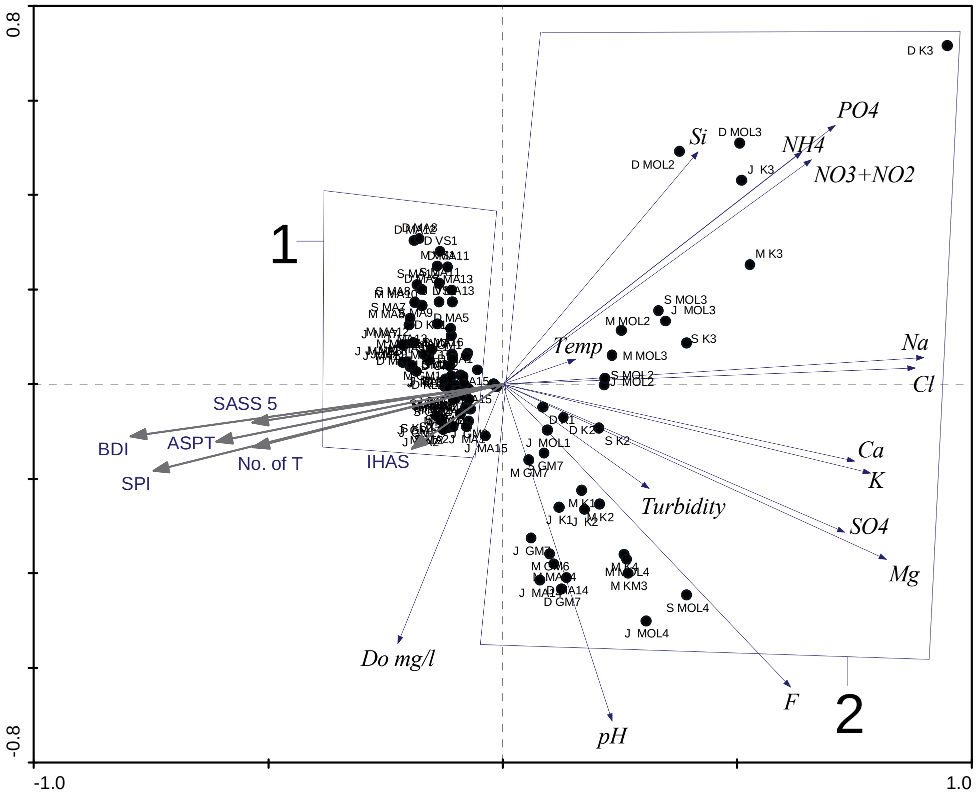

Figure 2
Principal component analysis (PCA) indicating chemical variables and diatom- and macroinvertebrate-based indices as vectors and sites as dots. 1 denotes upstream sites and 2 denotes downstream sites. (Site names are denoted by a letter denoting the month of sampling, followed by the site name as indicated in Fig. 1 in Part 1 (De la Rey, 2008).

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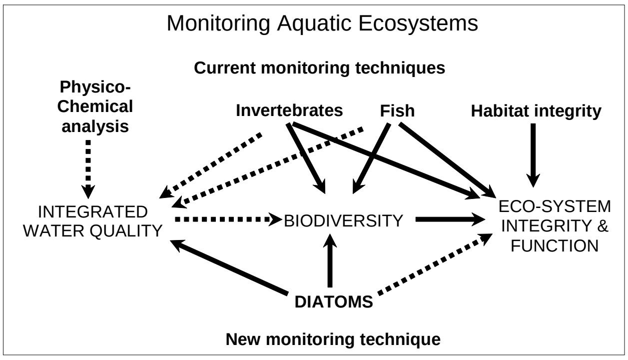

Figure 3
Schematic representation of the relationship between parameters used to monitor the environment and what they indicate. Solid arrows represent a strong relationship while dotted arrows represent a weak relationship (Taylor et al., 2006).

reacted to similar water quality variables, and no conclusions could be made as to which water quality variables most strongly influence diatoms or invertebrates.

The diatom-based indices showed a stronger response to general water quality than did the invertebrate indices and did not respond to changes in season.

The invertebrate indices showed a stronger relationship to changes in habitat scores than did the diatom-based indices. Season also influenced macroinvertebrate indices more than the diatom-based indices, although the total effect of seasonality on the various indices was found to be low.

The ASPT was less influenced by habitat and more by water quality than the other two SASS indices.

This study shows that diatoms can be used to indicate short- to medium-term changes in general water quality that might not be detected when only using invertebrate indices. On the other hand, diatoms are not able to indicate habitat degradation and since this is an important component of the functioning of healthy rivers, invertebrates cannot be excluded from the biomonitoring of rivers and streams.

Figure 3 represents a conceptual model reflecting the positioning of diatoms and SASS 5 as indicators in water resource management.

The concept communicates the relationship between biological indicators and what they may tell us about the environment. Macroinvertebrates, because of habitat affinity, food requirements, reproductive cycles etc. have a stronger relationship to the functioning and the ecological integrity of their direct environment and thus may be used as indicators of these parameters. On the other hand, the diatoms (as micro-organisms and primary producers) are directly influenced by chemical water quality. This is because diatoms need nutrients for growth and reproduction and are physiologically influenced by changes in salinity, pH and other key water quality variables (Taylor et al., 2006).

It is therefore recommended, based on the results of this study that diatoms and SASS 5 can, and should, be used as complementary techniques in the biomonitoring of rivers and streams, in the North-West Province and the entire country.

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TABLE 5
Results for PCA performed for sites in the Marico and Molopo Rivers (Biological and habitat indices were used as supplementary data in the ordination)

Axes	1	2	3	4	Total variance
Species-environment correlations	0.803	0.414	0.368	0.472	
Cumulative percentage variance:					
of species data	40.6	57.8	70.5	78.8	
of species-environment relation	75.4	83.9	88.9	94.2	
Sum of all canonical eigenvalues					0.347

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